

**STUDIES ON EXTRACTION AND ENCAPSULATION OF
ANTHOCYANIN FROM CALYCES OF ROSELLE
(*Hibiscus sabdariffa* L.)**

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**“STUDIES ON EXTRACTION AND ENCAPSULATION OF
ANTHOCYANIN FROM CALYCES OF ROSELLE
(*Hibiscus sabdariffa* L.)”**

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By

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CERTIFICATE

This is to certify that the thesis entitled “**STUDIES ON EXTRACTION AND ENCAPSULATION OF ANTHOCYANIN FROM CALYCES OF ROSELLE (*Hibiscus sabdariffa* L.)**” submitted by **Ms. SHRUTHI, V. H.**, for the degree of **DOCTOR OF PHILOSOPHY in PROCESSING AND FOOD ENGINEERING** to the University of Agricultural Sciences, Raichur, is a record of research work carried out by her during the period of her study in this University, under my guidance and supervision, and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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Affectionately Dedicated
to
My Beloved Parents
“Sri. Hanumanthaiah, P.
and
Smt. Lalitha”

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LIST OF ABBRIVATIONS

AA	:	Antioxidant activity
ABTS	:	2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)
ANOVA	:	Analysis of variance
AOAC	:	Association of official analytical chemists
Avg.	:	Average
a_w	:	Water activity
BHT	:	Butylated hydroxytoluene
CFC	:	Code of Federal Regulations
CI	:	Carr's index
CMC	:	Carboxymethyl cellulose
CV	:	Coefficient of variation
DAD	:	Diode array detection
DD	:	Dehumidified air drying
DE	:	Dextrose equivalence
Df	:	Degrees of freedom
DM	:	Dry matter
DPPH	:	1, 1-diphenyl-2-picrylhydrazyl
DW	:	Dry weight
EMC	:	Equilibrium moisture content
EU	:	European union
Exp	:	Exponential
FAO	:	Food and Agriculture Organization
FCRD	:	Factorial completely randomized design
FRAP	:	Ferric reducing ability of plasma
FW	:	Fresh weight
GA	:	Gum Arabica
GAE	:	Gallic acid equivalence
HDPE	:	High density polyethylene
HPLC	:	High performance liquid chromatography
HR	:	Hausner ratio
IMC	:	Initial moisture content
LC-MS	:	Liquid chromatography mass spectrometry

LN ₂	:	Liquid nitrogen
LN ₂ CP	:	Liquid nitrogen cooled pulverizer
MC	:	Moisture content
MD	:	Maltodextrin
MR	:	Moisture ratio
NP	:	Normal pulverizer
ORAC	:	Oxygen radical absorbance capacity
PET	:	Polyethylene teraphthalate
PRESS	:	Predicted residual error sum of squares
PVC	:	Polyvinyl chloride
RH	:	Relative humidity
RMSE	:	Root mean square error
RSA	:	Radical scavenging activity
SD	:	Shade drying
SEM	:	Scanning electron microscope
SSE	:	Sum square error
STD	:	Solar tunnel drying
Std.Dev.	:	Standard deviation
TAC	:	Total anthocyanin content
TEAC	:	Trolox equivalent antioxidant capacity
Temp.	:	Temperature
TFA	:	Trifluoroacetic acid
TFC	:	Total flavonoid content
TPC	:	Total phenolic content
TSS	:	Total soluble solids
US	:	United States
UV	:	Ultraviolet
UV-VIS	:	Ultraviolet visible spectroscopy
WCP	:	Water cooled pulverizer
WP	:	Whey protein

LIST OF SYMBOLS

%	:	per cent
$\mu\text{.ml}^{-1}$	:	micron per milliliter
μg	:	microgram
$\mu\text{g.ml}^{-1}$	:	microgram per milliliter
μm	:	micrometer
μM	:	micro molar
$\mu\text{mol TE.g}^{-1}$	:	micromol trolox equivalents per gram
a^*	:	redness to greenness
b^*	:	yellowness to blueness
Cm	:	centimeter
dm.min^{-1}	:	dry matter per minute
<i>et al.</i>	:	and others
G	:	gram
g.cc^{-1}	:	gram per cubic centimeter
g.g^{-1}	:	gram per gram
g.L^{-1}	:	gram per litre
H	:	hour
<i>i.e.</i>	:	that is
K	:	Kelvin
kg.h^{-1}	:	kilo gram per hour
kJ.kg^{-1}	:	kilo Joules per kilo gram
kJ.gmol^{-1}	:	kilo Joules per gram mole
kJ.mol^{-1}	:	kilo Joules per mole
L^*	:	whiteness to darkness
M	:	Molar
M	:	meter
MPa	:	Mega Pascal
Mg	:	milligram
$\text{mg Cya3G).100 g}^{-1}$	:	milligram of cyanidin 3-glucoside per 100 gram
mg D3S.100 g^{-1}	:	milligram of delphinidin 3-sambubioside per 100 gram
mg GAE. g^{-1}	:	milligram gallic acid equivalent per gram
mg RE.g^{-1}	:	milligram rutin equivalent per gram

mg.100g^{-1}	:	milligram per 100 gram
mg.g^{-1}	:	milligram per gram
mg.kg^{-1}	:	milligram per kilogram
mg.l^{-1}	:	milligrams per liter
mg.ml^{-1}	:	milligrams per milliliter
Min	:	minutes
Ml	:	milli litre
ml.100 g^{-1}	:	millilitre per 100 gram
Mm	:	millimeter
mM	:	milli molar
m.s^{-1}	:	meter per second
$\text{m}^2.\text{s}^{-1}$	:	meter square per second
mm^2	:	millimeter square
mol.L^{-1}	:	mole per litre
Nm	:	nanometer
$^{\circ}\text{Brix}$	:	degree Brix
$^{\circ}\text{C}$	:	degree centigrade
Pa s	:	Pascal second
Ppm	:	parts per million
R^2	:	coefficient of determination,
S	:	second
<i>viz.</i>	:	namely
v/v	:	volume per volume
v/w	:	volume per weight
w/v	:	weight per volume
% d.b.	:	percent dry basis
% w.b.	:	percent wet basis
χ^2	:	chi-square

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*Introduction*_____

I. INTRODUCTION

Food colourants are either natural or synthetic depending on source. Natural colourants are extracted from renewable sources such as plant materials, insects, algae, etc., while the synthetic colourants are manufactured chemically and are the most commonly used dyes in the food, pharmaceutical and cosmetic industries. Due to this limitation and worldwide tendency towards the consumption of natural products, the interest in natural colourants has increased significantly. Of special interest to the food industry is the limited availability of red pigments, therefore research into natural sources of red pigments has increased recently (Arab *et al.*, 2011).

Colour is one of the most important quality attributes affecting the consumer's acceptance of food since it gives the first impression of food quality. Many convenience foods such as confectionery products, gelatin desserts, snacks, cakes, puddings, ice creams and beverages would be colourless and would thus appear undesirable without the inclusion of colourants (Hirunpanich *et al.*, 2006). In this respect, Roselle calyces appear to be good and promising sources of water soluble red colourants that could be utilized as natural food colourants (Arab *et al.*, 2011).

Hibiscus subdariffa Linn. is a shrub belonging to the family – *Malvaceae*. It is thought to be a native to Asia (India to Malaysia) or Tropical Africa. The plant is widely grown in tropics like Caribbean, Central America, India, Africa, Brazil, Australia, Hawaii, Florida and Philippines as a home garden crop. In addition to Roselle, in English-speaking regions it is called as Rozelle, Sorrel, Red sorrel, Jamanica sorrel, Indian sorrel, Guinea sorrel, Sour-sour, Queensland jelly plant, Jelly okra, Lemon bush and Florida cranberry. In Indian languages it is called as Lal-ambari, Patwa (Hindi), Lal-ambadi (Marathi), Gongura, Yerra gogu (Telugu), Pulichchai kerai (Tamil), Pulachakiri, Pundibija (Kannada), Polechi, Pulichchai (Malayalam) and Chukiar (Assam) (Mahadevan *et al.*, 2009). Two botanical types of Roselle are recognized, *Hibiscus sabdariffa* var *altissima* and *Hibiscus sabdariffa* var *subdariffa* (Eltayeib and Hamade, 2014).

The plant is about 3.5 m tall and has a deep penetrating taproot. It has a smooth or nearly smooth, cylindrical, typically dark green to red stems. Leaves are alternate, 7.5-12.5 cm long, green with reddish veins and long or short petioles. Leaves of young

seedlings and upper leaves of older plants are simple; lower leaves are deeply 3 to 5 or even 7-lobed and the margins are toothed. Flowers, borne singly in the leaf axils are up to 12.5 cm wide, yellow or buff with a rose or maroon eye and turn pink as they wither at the end of the day. The typically red calyx, consists of 5 large sepals with a collar (epicalyx) of 8-12 slim, pointed bracts (or bracteole) around the base, they begin to enlarge at the end of the day, 3.2-5.7 cm long and fully enclose the fruit. The fruit is a velvety capsule, 1.25-2 cm long, which is green when immature, 5-valved, with each valve containing 3-4 seeds. The capsule turns brown and splits open when mature and dry. Seeds are kidney-shaped, light-brown, 3-5 mm long and covered with minute, stout and stellate hairs (Mahadevan *et al.*, 2009).

Roselle is more than just a pretty fruit: it's been used in dishes, beverages and traditional medicine for centuries. Julia Morton attests in her book, "Fruits of Warm Climates" that Roselle originated in the regions between and including India and Malaysia; shortly thereafter, she states it was brought to Africa. According to the Food and Agriculture Organization of the United Nations, the world's largest producers of Roselle are China and Thailand. Interestingly, the FAO ranks Roselle from Sudan as of the best quality, but poor packaging and distribution inhibit fruits from this part of the world from gaining global popularity. China and Thailand are the largest producers and control much of the world supply. Thailand invested heavily in Roselle production, and their product is of superior quality (Anon., 2013).

The Roselle calyces production, in developing countries, is of great importance since its production represents a very important income for people from rural communities. Roselle is a robust and thriving industry in India: In fact, the country is the fourth largest exporter of Roselle plant parts to Germany and the fifth largest exporter of plant parts to the US for the nation's thriving tea companies. Roselle tolerates a remarkable variety of climates and soil conditions. These factors make them a suitable crop found in many states including West Bengal, Assam, Bihar, Orissa, Uttar Pradesh, Tripura, Andhra Pradesh, Karnataka, Tamil Nadu and Meghalaya (Anon., 2013).

In several countries, Roselle is also considered to be one of the most famous folk medicinal plants. Where, many chemical components present in Roselle have potential health benefits and support the ethno medicinal use of Roselle in promoting cardiovascular health and preventing hypertension, pyrexia and liver disorders, microorganism

growth limitation, as well as a diuretic, digestive and sedative. The red varieties of Roselle have antioxidant and cyclooxygenase inhibitory activity. Also, Roselle enters in pharmaceutical and cosmetic industries (Ansary *et al.*, 2016).

Roselle has been used by people for preparing soft drinks and in traditional medicine. There are several reports on the benefits of Roselle calyces, as they are an excellent source of natural antioxidants, providing even higher levels than traditional sources such as raspberries and blueberries (Juliani *et al.*, 2009). It has been observed that its components, such as vitamins (C and E), polyphenols acids and flavonoids, mainly anthocyanins, have functional properties. Several studies have shown that compounds found in aqueous and ethanol Roselle calyces extracts might have antioxidant properties. These compounds could work in several ways in humans; for instance, they could have anticancer characteristics. They may also reduce chronic diseases such as diabetes mellitus, dyslipidemias, hypertension and cardiovascular diseases. Some of these compounds (flavonoids and anthocyanins) are natural which have no toxic or mutagenic effects (Ortega and Beltran, 2014).

The flowers of Roselle are generally small. Both the leaves and the fleshy base of the flower (the calyx) are employed in the preparation of soups and sauces. The vegetable is a popular diet during the rainy season. Roselle calyx is a cheap source of vegetable protein, fat and minerals. Regular consumption of Roselle may reduce nutritional deficiency problems such as night blindness, scurvy and rickets (Babalola *et al.*, 2001; Ashaye, 2013). The types can be distinguished by three different colour groups: green, red and dark red. Calyces of the red and dark red coloured types are extracted and sweetened to produce a refreshing drink while calyces and leaves of the green type are used for making vegetable stew. Chemical composition of Roselle calyces is shown in Table 1 (Babalola *et al.*, 2001).

Table 1: Chemical composition of Roselle calyces

Element	Calyx types		
	Green	Red	Dark red
Crude protein (%)	17.9	17.4	8.6
Ether extract (%)	3.2	2.1	2.9
Crude fibre (%)	11.2	8.5	9.8
Ash (%)	6.6	6.5	6.8
Ascorbic acid (mg/100g)	86.5	63.5	54.8
Moisture (FW) %	88.3	86.5	85.3
Calcium (mg/100g)	1209	1583	1602
Magnesium (mg/100g)	235	316	340
Potassium (mg/100g)	1850	2060	2320
Sodium (mg/100g)	9.5	5.5	6.5
Iron (mg/100g)	32.8	37.8	34.6
Zinc (mg/100g)	5.8	6.5	6.3

Source: Babalola *et al.* (2001)

Roselle calyx is usually harvested at high moisture content (85%, w.b.). Therefore, drying is an important post-harvest treatment prior to reduce the moisture content and to increase the shelf life. Drying is a process comprising simultaneous heat and mass transfer (Fajar *et al.*, 2012). Dehydration is a preservation method that involves the removal of biologically active water in order to reduce microorganism growth (Hahn *et al.*, 2011). With a longer product shelf life, dried products can be sold all year-round. The drying process eliminates the water or humidity content of the calyces but must maintain the nutritional properties, specifically the ascorbic acid content (Meza *et al.*, 2008). Traditionally, calyces are spread over open area floors and dried naturally by the incidence of solar radiation. Roselle is stored when it is completely dry, after three or four days depending on ambient temperature and relative humidity conditions (Hahn *et al.*, 2011).

Milling is reduction in the particle size. A reduction in the particle size may also cause change in the fibre matrix structure, resulting in an increase in the surface area (Sankalpa *et al.*, 2016). In the ambient grinding process, heat is produced when energy is used to fracture a particle into a smaller size. This generates heat which is detrimental to the product and results in loss of quality *i.e.*, high temperature reduces the volatile components and heat sensitive constituents. Low temperature grinding is a method of powdering at sub zero temperature with liquid nitrogen. This process does not damage or alter the chemical composition of the material (Singh and Goswami, 1999). Therefore, in this investigation it was considered to see the effect of different milling methods like milling using normal pulverizer, water cooled pulverizer, liquid nitrogen (LN₂) cooled pulverizer to analyze the quality of Roselle powder.

Extraction and purification of bioactive compounds from natural sources have become very important for the utilization of phytochemicals in the preparation of dietary supplements or nutraceuticals, functional food ingredients, food additives, pharmaceutical and cosmetic products. The polar character of anthocyanins makes them soluble in several types of polar solvents such as methanol, ethanol, acetone, and water. Solvent extraction of anthocyanins is the initial step in the determination of total and individual anthocyanins prior to quantification, purification, separation, and characterization (Rivas, 2003). The extraction of anthocyanins is commonly carried out under cold conditions using methanol or ethanol containing a small amount of acid in order to obtain the flavylium cation form, which is red and stable in a highly acid medium (Du and Francis, 1973; Amor and Allaf, 2009). The aqueous extract of *H. sabdariffa* calyces has a very rich in red pigment due to the presence of anthocyanins and the colour properties has been the subject of intense scientific investigations (Ali *et al.*, 2005; Salazar *et al.*, 2012; Aishah *et al.*, 2013).

Encapsulation facilitates light and heat labile molecules to maintain their stability and improve their shelf life and activity. It is a rapidly expanding technology, highly specialized, with affordable cost. Among the diverse encapsulation techniques available, only few were evaluated for anthocyanin encapsulation, these include: spray drying using different coating materials, gelation using polymers as sodium alginate, pectin, curdlan and glucan and lyophilization (Santos *et al.*, 2013). The spray drying minimizes the loss of nutrients during processing and storage, the powder obtained is soluble and convenient to carry, it requires less storage space and most common method to transform a wide

range of liquid food products into powder. Some of the most used materials include pectin, whey protein, carrageenan, carboxymethyl cellulose (CMC), gelatin and xanthan gum amongst others, trying to protect the core of the particles against adverse environmental conditions, providing at the same time, an easy handling and release rate control (Bandera *et al.*, 2015).

Anthocyanins are an important group of water soluble plant pigments commonly found in various fruits and vegetables. The interest arises due to the pigments wide range of attractive colour spectrum from shiny orange, pink, red, purple and blue that has great potential for use as natural food colourant to replace synthetic dyes. However, the pigment is not stable due to processing and storage conditions. Several factors influencing the pigment stability have been investigated such as species, environmental and agronomic conditions; extraction and processing parameters such as pH, storage temperature, concentration, chemical structure, light, oxygen, proteins, ascorbic acid, sugars, sulfites, enzymes and metallic ions. Among these factors, pH is one of the major factors significantly influenced the pigment colour variations and stability. In general, anthocyanins are more stable in acidic media at low pH values than in alkaline solutions (Aishah *et al.*, 2013).

Anthocyanins present in Roselle are delphinidin 3-sambubioside, cyaniding 3-sambubioside, delphinidin 3-glucoside and cyanidin 3-glucoside. They contribute benefits for health as a good source of antioxidants as well as a natural food colourant (Kilima *et al.*, 2014). Anthocyanins are approved as food colourants in the USA under the category of fruit (21 CFR 73.250) or vegetable (21 CFR 73.260) juice colour. The EU classifies anthocyanins as 'natural colourants' under classification number E163 (Duangmal *et al.*, 2008). Roselle contains 0.68-1.07 mg.g⁻¹ of total anthocyanin which is almost equal to total anthocyanin content of red rose (0.89-1.60 mg.g⁻¹) (Juliani *et al.*, 2009).

Drying is an important post-harvest treatment to reduce the moisture content and to increase the shelf life. Since the anthocyanins are heat sensitive materials, drying under lower temperature could be beneficial. Therefore drying using shade drying and dehumidified air dryer may reduce loss of anthocyanins. The literature is almost silent in milling of dried calyces of Roselle before extraction. By keeping this in view low temperature milling methods like water cooled pulverizer and liquid nitrogen cooled

pulverizer have been selected to retain the heat sensitive anthocyanin content in the ground powder. In preparing anthocyanin-rich phenolic extracts from plant materials, an acidified organic solvent, most commonly methanol or ethanol is used. This solvent system denatures the cell membranes, simultaneously dissolves the anthocyanins and stabilizes them. Extraction of anthocyanin was carried out using ethanol and citric acid under cold conditions to obtain maximum yield and minimum quality losses. Encapsulation facilitates light and heat-labile molecules to maintain their stability and improves their shelf life and activity. The spray drying technique was used to transform a Roselle extract into encapsulated powder. Colour stability of anthocyanin depends on a combination of various factors including: structure of anthocyanin, pH, temperature, oxygen, light and water activity. Among these factors pH and temperature are most responsible for stability of anthocyanin. Therefore, different pH and temperatures were studied for stability of Roselle anthocyanin.

Keeping in view of the above facts, a research topic entitled “Studies on extraction and encapsulation of anthocyanin from calyces of Roselle (*Hibiscus sabdariffa* L.)” was undertaken with the following objectives:-

- To standardize the drying and milling methods for calyces of Roselle
- To optimize the extraction and encapsulation processes for anthocyanin from calyces of Roselle
- To study the stability of extracted anthocyanin under different pH and temperature conditions

*Review of Literature*_____

II. REVIEW OF LITERATURE

A comprehensive review is mandatory in any research endeavour. This requires thorough efforts on the part of investigator to select relevant subject matter, to organize and to report it systematically. This chapter deals with brief account of literature, which has direct and indirect bearing on the specific objectives of the investigation.

In the present investigation, an attempt has been made to summarize the detailed “Studies on extraction and encapsulation of anthocyanin from calyces of Roselle (*Hibiscus sabdariffa* L.)”. The literature pertaining to present investigation (objective wise) is reviewed under the following sub headings:

2.1 Drying and milling methods

Doymaz *et al.* (2006) investigated the drying characteristics of dill and parsley leaves. The drying of dill and parsley leaves was investigated in a laboratory dryer, the effect of temperature on drying rate of samples at constant air velocity (1.1 m.s^{-1}) and various temperatures (50, 60 and 70 °C). Four thin-layer drying models, namely, Lewis, Handerson and Pabis, Page, and Midilli and Kucuk, were fitted to drying data. The Midilli and Kucuk model was found to satisfactorily describe the air-drying curves of dill and parsley leaves. The effective diffusivity values changed from 6.693×10^{-10} to $1.434 \times 10^{-9} \text{ m}^2.\text{s}^{-1}$ and 9.0×10^{-10} to $2.337 \times 10^{-9} \text{ m}^2.\text{s}^{-1}$ for dill and parsley leaves, respectively, within the given temperature range. Calculated values of the effective diffusivity showed Arrhenius-type temperature dependence. The activation energy values were found to be 35.05 and 43.92 kJ.mol^{-1} for dill and parsley leaves, respectively. The colour of samples were determined after drying process using a Hunter lab in terms of Hunter L^* , a^* and b^* values. From the results of colour quality, drying at temperature of 60 °C was found to be the optimum temperature for dill and parsley leaves.

Singh and Goswami (2007) conducted a study on the effects of cryogenic grinding of cloves to observe influence of parameters, viz., grinding temperature, rotor speed, sieve size and feed rate on specific energy consumption and volatile oil content. It was observed that the clove could be successfully ground at temperatures below -50 °C without any deposition over the sieve surface. An increase in temperature in the cryogenic range (-110 to -50 °C) had no significant effect on volatile oil content, whereas temperatures in the range of 55 to 85 °C significantly reduced the volatile oil content

from 11.0 to 9.3 ml.100g⁻¹. Thus, cryogenic grinding resulted in 29.5% more volatile oil in comparison to that of ambient grinding. The specific energy consumption increased quadratically from 62 to 85 kJ.kg⁻¹ with the increase in feed rate from 1.50 to 6.00 kg.h⁻¹ with constant grinding temperature of -110 °C, rotor speed of 69.00 m.s⁻¹ and sieve opening size of 0.5 mm.

Ramachandra and Rao (2009) carried out investigations on the desiccant dehumidified air drying characteristics of Aloe vera (*Aloe barbadensis* Miller) gel at temperature range of 40 to 70 °C, relative humidity of 15 to 30% and air velocity of 0.5 to 2.0 m.s⁻¹. The drying time was ranged from 330 to 900 minutes. The specific energy consumption during drying was found in the range of 201.60 to 415.17 MJ. kg⁻¹. The diffusivity co-efficient was increased with the temperature from 4.93 to 16.38×10⁻¹⁰m.s⁻¹. Response surface methodology (RSM) was applied to optimize the desiccant dehumidified air drying process to produce best quality Aloe vera gel powder. The optimized process parameters were; temperature 64 °C, relative humidity 18% and air velocity 0.8 m.s⁻¹.

Zaiton *et al.* (2009) investigated the quality of Roselle tea (*Hibiscus sabdariffa* L.) extracted from two locally grown varieties. Roselle calyces were dried immediately after harvesting (AH) or frozen (AF) for two weeks prior to drying using a food dehydrator at 40, 50 and 60 °C overnight. Roselle tea were packed in sachet and stored at room temperature for 12 months. Quality of tea measured were antioxidant activities by 2, 2-diphenyl-1-picrylhydrazyl (DPPH), total phenolics by Folin-Ciocalteau method and total anthocyanins by the pH-differential method. Colour measurements were measured by UV-VIS spectrophotometer. DPPH and pH of tea was not affected by drying conditions and storage. Total phenolic content was affected by drying temperatures, variety and condition of calyces before drying. Drying at 60 °C caused significant reduction (p<0.001) in anthocyanin content for both Roselle teas. Significant losses in colour and anthocyanin content were observed in dried Roselle calyces during storage.

Meghwal and Goswami (2010) conducted experiment on ambient and cryogenic grinding and to test the novelty of cryogenic grinding and pin point the drawbacks of ambient grinding in case of spices. Comparative study had shown that ambient grinding need more power (8.92%) and specific energy (14.5%) than cryogenic grinding. Particle size analysis had shown that cryogenic grinding produced coarser particles. Comparative

study of energy law constant showed that ambient was more power consumptive. The higher amount of volatile oil ($2.15 \text{ ml.100g}^{-1}$) content was found in cryogenic grinding and also powder of freshness and lower whiteness (40%) and higher yellowness (14%) indices found for cryogenic grinding.

Hahn *et al.* (2011) studied the optimization of Roselle drying time and drying quality. A polyethylene plastic covered tunnel was constructed for drying of Roselle calyces. Three different drying techniques were evaluated and compared to search for optimum drying conditions. The first one used controlled fans for heating the air inside the tunnel to its maximum capacity; the second one recirculated air and passed it through a silica gel desiccant filter for drying the air, while the last method used a hybrid solar-biogas combination for drying the calyces with hot air. Calyx colour images, water loss and calyx crispness of dried products were evaluated along the tunnel. Roselle drying using fan control took 27 hours, optimizing energy consumption and increasing air temperature within the tunnel. An air recirculating system using silica gel for removing air moisture decreased the drying time to 8 hours and obtained crisper calyces. Moisture retained by the silica gel desiccant material was automatically removed by heat regeneration. Calyces were dried in 4.5 hours at 70°C with a hybrid solar biogas system. One kg of biogas was burned per kg of dried product.

Suliman *et al.* (2011) studied the chemical composition of dry red and white Roselle calyces. It was found that, the red and white karkade calyces were contained 11 and 9.3% (w.b.) moisture content, respectively. Chemical composition was found 0.16 and 0.12% (fat), 13.2 and 12% (fiber), 7.88 and 7.35% (protein), 10.6 and 9.5% (ash) and 57.16 and 61.55% (carbohydrates), respectively. The red and white karkade calyces were also contained 11 and $15.5 \text{ mg.100 g}^{-1}$ of vitamin C, 60 and 50 mg.100 g^{-1} of calcium, 25 and 20 mg.100 g^{-1} of iron and 9 and 11 mg.100 g^{-1} titratable acidity, respectively.

Daniel *et al.* (2012) studied the effect of fixed bed drying on the retention of phenolic compounds, anthocyanins and antioxidant activity of Roselle (*Hibiscus sabdariffa* L.). The experimental variables were temperature (40, 60 and 80°C), bed ratio (1, 2 and 3), and superficial air velocity (0.75 , 1.5 and 2.25 m.s^{-1}). Drying curves showed both a constant drying period and a decreasing falling rate period. To determine kinetic parameters, the drying data were fitted to a first order chemical model, based on the moisture content ratio versus the drying time. The 'k' value ranged from 9.0×10^{-4} to

$2.7 \times 10^{-3} \text{ s}^{-1}$. The different drying conditions showed significant differences in the monomeric anthocyanin content (188.13 ± 6.78 - $230.23 \pm 5.30 \text{ mg D3S.100 g}^{-1}$ residue d.b.), the total phenol content (435.03 ± 16.8 - $637.65 \pm 12.8 \text{ mg GAE.100 g}^{-1}$ residue d.b.) and the antioxidant activity (181.13 ± 2.27 - $288.44 \pm 18.22 \text{ } \mu\text{mol TE.g}^{-1}$ of dry extract) ($p < 0.05$). The results confirmed that lixiviated Roselle calyces can compete with other traditional products, such as grapes or bilberries, in terms of their antioxidant activity and their low cost associated with waste materials.

Dattaprasad *et al.* (2012) investigated the effect of milling methods on quality parameter of Byadagi chilli. Dried chillies were milled by spice pulverizer, low temperature pulverizer and cryogenic grinder. The quality parameter viz., colour, ascorbic acid, capsaicin content and pH of chilli powder obtained from cryogenic grinder was significantly different from spice pulverizer and low temperature pulverizer. The colour value of cryogrinder chilli powder were observed to be 40.15 (L^*), 33.66 (a^*) and 36.80 (b^*) and was found much better than low temperature pulverizer 39.34 (L^*), 32.03 (a^*) and 37.28 (b^*) and spice pulverizer 35.84 (L^*), 30.09 (a^*) and 31.39 (b^*), respectively. The ascorbic acid ($18.57 \text{ mg.100g}^{-1}$) and capsaicin content (0.017%) was found to be retained more in cryogenic grinder compared to other methods.

Fajar *et al.* (2012) determined the most appropriate thin layer drying model and the effective moisture diffusivity of Roselle (*Hibiscus sabdariffa*). Roselle with an Initial Moisture Content (IMC) of 85% (w.b.) was dried in a conventional tray dryer at temperatures of 40, 50 and 60 °C. The drying data were fitted to eleven thin layer models and a thin layer model for the Roselle calyx was developed by regressing the coefficients of the best fit model. The Newton model was most adequate model for describing the thin layer drying kinetics of the Roselle calyx. The drying constant was found to vary linearly with temperature. Also, effective diffusivity was evaluated by using Fick's second law, which varied from 1.405×10^{-10} to $2.283 \times 10^{-10} \text{ m}^2.\text{s}^{-1}$. The dependence of moisture diffusivity on temperature was described by Arrhenius type equation. The diffusivity constant D_0 activation energy E_a could be, respectively, estimated as $4.5 \times 10^{-7} \text{ m}^2.\text{s}^{-1}$ and $21.02 \text{ kJ.gmol}^{-1}$.

Ji *et al.* (2012) investigated the effects of different drying methods (sun drying, hot-air drying, freeze drying and microwave-vacuum drying) on the antioxidant properties and phenolic content in *Robinia pseudoacacia* L. flowers. Simultaneously, the

correlation between phenolic content and antioxidant capacity was discussed. Results showed that antioxidant properties and phenolic content in dried RPF were affected by the drying methods to different extents. Freeze drying sample possessed the highest antioxidant activity including DPPH radical scavenging, reducing power and hydroxyl radical scavenging ability, and the total phenolic content was 47.30 mg gallic acid equivalents (GAE) .g⁻¹ DW. Microwave-vacuum drying samples had the best iron-chelating ability and its phenolic content (45.52 mg GAE.g⁻¹ DW) was comparable to that of the freeze drying samples ($P > 0.05$). Sun drying was the worst in antioxidant activities and phenolic content was only 29.15 mg GAE.g⁻¹ DW. Significant positive correlation was found between the total phenolic content and antioxidant activity ($p < 0.01$). the results confirmed that, the microwave-vacuum drying was a potential drying technique for *Robinia pseudoacacia* L. flowers.

Wankade *et al.* (2012) determined the drying characteristics of okra using hot air dryer, solar dryer and open sun drying methods. For the hot air drying, the test samples were dried in a laboratory scale hot air dryer at a constant air velocity of 1 m.s⁻¹ and air temperature in the range of 40-90 °C. The three drying methods used greatly affected the drying characteristics okra. The solar dryer was found to be more efficient than the open sun drying. The drying characteristic and time required for drying of okra was studied and final dry weight of the okra slices were estimated. It was found that okra samples, dried by hot air drying were reported to take minimum time for drying with maximum removal of moisture. The initial first hour of hot air drying and sun drying and initial 6 hours under open sun drying resulted in maximum removal of moisture from okra. The okra slices dried in hot air dryer at 40 °C gives better results in maintaining better appearance, colour and texture.

Ashaye (2013) studied on moisture sorption isotherm and nutritional properties of dried Roselle calyces. Freshly harvested Roselle calyces of dark and light red varieties were sundried and oven dried and evaluated for proximate composition and moisture sorption studies. pH of sundried Roselle calyces were higher than oven dried Roselle calyces. Also, oven dried dark red Roselle calyces was significantly higher in crude protein, fat, dry matter, moisture and ash. Vitamin C content of oven dried light red Roselle calyces was significantly lower than Vitamin C of other Roselle calyces at $p < 0.05$. Roselle calyces exhibited the typical three stage sigmoidal curve found in most

foods (Braunaeur-Emett-Teller - BET Type II). There was also a concomitant increase in the equilibrium moisture content (EMC) as relative humidity increased in all Roselle calyces irrespective of the temperature regimes. Sun dried Roselle calyces gave the highest rate of water absorption unlike oven dried Roselle calyces. In conclusion oven drying is the best method to dry freshly harvested Roselle calyces, oven dried dark red Roselle calyces was high in nutrient composition and all Roselle calyces exhibited the typical three stage sigmoidal curve found in most foods (Braunaeur-Emett-Teller - BET Type II).

Langova *et al.* (2013) carried out water sorption tests of Roselle (*Hibiscus sabdariffa* L.) under laboratory conditions together with mathematical analyses of the moisture sorption isotherms (MSIs). Moisture equilibrium data for adsorption and desorption of water from Roselle powder were investigated at near ambient air temperatures in the range of 5 and 35 °C and water activity (a_w) ranging from 0.11 to 0.97. The manometric method has been used for water sorption tests. Models for MSIs are exponential equations. Coefficients of determination are 0.998 and 0.996 (for adsorption and desorption at 5 °C, respectively), 0.998 and 0.999 (for adsorption and desorption at 20 °C, respectively), and 0.998 and 0.999 (for adsorption and desorption at 35 °C, respectively). The equilibrium moisture content (EMC) of Roselle samples increased with an increase of a_w at a constant temperature both for adsorption and desorption. Adsorption curve equates to desorption curve at higher temperatures of tests carried out. Critical values of EMC of samples tested corresponding to the a_w equal to 0.6 were between 13.401% and 15.934% moisture content (w.b.) for moisture adsorption and desorption, respectively.

Manjula *et al.* (2013) investigated the drying characteristics of *Byadagi* chilli (*Capsicum annuum* Linn.) using solar tunnel dryer. Freshly harvested *Byadagi* chilli fruits were treated with dipsol, potassium nitrate and citric acid and dried in solar tunnel dryer (STD) and open yard sun drying (OYSD). A comparative study was conducted to evaluate two drying methods with respect to temperature and time combinations. It took 39 h to bring down the moisture content of chillies from 339.14% d.b. to 10.00% d.b. in STD as against 57 h under OYSD. The per cent time saved for drying chilli by using improved method of drying (STD) was found to be 31.57% in comparison with open yard

sun drying. Drying took place in the falling rate period and the Newton model was found to be the best fit to describe the drying behavior of *Byadagi chilli*.

Pirbalouti *et al.* (2013) assessed the effects of drying methods on qualitative and quantitative of the essential oil of Bakhtiari savory (*Satureja bachtiarica* Bunge.). The aerial parts of Bakhtiari savory were dried using different drying methods, *i.e.*, sun-drying, shade-drying, oven-drying at 45 °C and 65 °C and freeze-drying. The essential oils of fresh and dried samples were obtained by hydro-distillation, and analyzed using gas chromatography-mass spectrometry (GC-MS). The highest essential oil yields (v/w on dry weight basis) were obtained by oven-drying at 45 °C (2.3%) followed by freeze-drying (2.1%), oven-drying at 65 °C (2.0%), shade-drying (1.7%), sun-drying (1.6%) and fresh sample (1.2%). Twenty-seven components were determined in essential oils of *S. bachtiarica*, which were mostly oxygenated monoterpenes and hydrocarbons monoterpenes. The main components in essential oils of fresh and dried Bakhtiari savory aerial parts were carvacrol (31.2-42.2%), γ -terpinene (10.9-18.3%), thymol (11.7-19.4%) and *p*-cymene (8.2-14.1%). Drying of aerial parts of Bakhtiari savory in the oven at 45 °C was the most suitable drying method considering short drying time and high-oil yield.

Barhe and Tchouya (2014) conducted comparative study of the anti-oxidant activity of the total polyphenols extracted from *Hibiscus Sabdariffa* L., *Glycine max* L. Merr., yellow tea and red wine through reaction with DPPH free radicals. The method applied is free radical scavenging using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The antioxidant properties were identified and measured by the determining the anti-radical activity reducing index, expressed in percentage % RSA (Radical Scavenger Activity), and by the determination of the Colouring Intensity (IC₅₀). All results are compared to those of ascorbic acid as reference antioxidant. The results indicate the following order for the antioxidant power of the extracts tested. % RSA (Tea) > % RSA (Glycine max) % > RSA (Red Wine) % > RSA (*Hibiscus sabdariffa*), and Colouring intensities (IC₅₀) ranging from 4.62 μ M (ascorbic acid) to 1.10 μ M (*Hibiscus sabdariffa*) correlated with their chemical structure and the content of phenolic compounds.

Gouda *et al.* (2014) studied the drying behaviour of different onion varieties namely, *Arka kalyan*, *Bijapur white* and *Arka pragati* which were dried under open yard sun drying, solar tunnel drying and dehumidified air drying methods. The onions were pre-treated with 0.2% potassium metabisulphite for 15 minutes before drying. The

samples of *Arka kalyan*, *Bijapur white* and *Arka pragati* onions required 17 h to dry under open sun, 15 h in solar tunnel drier and 12 h in dehumidified air drier to bring down initial moisture content ranging from 774.13-891.08% (d.b.) to final moisture content of 4.34-5.10% (d.b.). Thin layer drying models namely, Page, Midilli-Kucuk and Logarithmic models were applied to the experimental moisture loss data with respect time to predict the drying pattern of onions. The higher coefficient of determination (0.9991) with the lowest root mean square error (0.010) and sum of square error (1.001×10^{-3}), indicated Logarithmic model a better fit to the experimental data compared to other models.

Manjula and Ramachandra (2014) investigated the effect of drying methods and pre-treatments on the quality of dried Byadagi chilli (*Capsicum annuum* Linn.) were investigated. The temperature of open yard sun drying around 37 °C and solar tunnel drying around 60 °C was used. The quality parameters viz., moisture content, colour (L^* , a^* , b^* values), ascorbic acid content, capsaicin content, titrable acidity and aflatoxin content. The solar tunnel-dried (STD) sample gave more bright-red colour and contained higher ascorbic acid content than the open yard sun-dried (OYSD) samples ($p < 0.05$). The aflatoxin content was monitored using Enzyme Linked Immuno Sorbent Assay (ELISA). The Byadagi chilli dried in solar tunnel dryer showed less aflatoxin content than open yard sun drying. In addition, the unique visible attributes in the STD and OYSD samples were bright and dull, respectively.

Gouda *et al.* (2015) investigated the drying characteristics of Bijapur white onion (*Allium cepa* L.) using solar tunnel dryer. Fresh Bijapur white onion (*Allium cepa* L.) were treated with 10 per cent NaCl for 1 h, 0.2 per cent KMS for 15 min and 10 per cent NaCl + 0.2 per cent KMS for 15 min and dried in solar tunnel dryer (STD) and open yard sun drying (OYSD). A comparative study was conducted to evaluate two drying methods with respect to temperature and time combinations. The sample of Bijapur white onion required 18 to 21 h to dry under open yard sun drying and 16 to 18 h in solar tunnel drier to bring down initial moisture content ranging from 599.30-774.13 per cent (d.b.) to final moisture content of 4.56-5.25 per cent (d.b.). Drying took place in the falling rate period and the Midilli and Kucuk model was found to be the best fit to describe the drying behaviour of Bijapur white onion.

Kouakou *et al.* (2015) studied on identification of anthocyanin from calyx and callus of *Hibiscus sabdariffa* and comparison of their antioxidant properties. HPLC experiments were conducted using a reversed-phase C18 column. Antioxidant activity of anthocyanin was analyzed by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH). The HPLC studies using mobile phase system for each fraction showed various peaks in each extract. Six anthocyanins were determined in callus with the major compounds were cyaniding-3-O-sambubioside and delphinidin-3-O-glucoside. In calyx, four anthocyanins were determined with the major compounds were delphinidin-3-O-sambubioside and cyanidin-3-O-sambubioside. Antioxidant activity is higher with anthocyanin from callus than that of callus.

Pattali *et al.* (2015) studied the effects of different drying methods in terms of drying behaviour, drying rate and mathematical modelling of drying process for whole leaf Aloe vera. Open yard sun drying, hot air drying and dehumidified air drying were taken as different drying methods. The whole leaf Aloe vera slices took 20 h to dry under open yard sun drying, 16 h in case of hot air drying, 11 h in dehumidified air drying having initial moisture content ranging from 3115.43% (d.b.) to a final moisture content of 8.6% (d.b.). The different thin layer drying models were applied on the experimental moisture loss data with respect to time to predict the drying pattern properly. On the basis of coefficient of determination (0.9764) and standard error (0.0552), the Page model showed better fit.

Sankalpa *et al.* (2016) investigated the effect of four drying methods (open yard sun drying, solar tunnel drying, hot air drying and dehumidified air drying) and three grinding methods (hammer mill grinding at ambient temperature, with water cooling and with liquid nitrogen cooling) on functional properties of sweet orange peel powder. Among all combinations of treatments, dehumidified air drying and hammer mill with LN₂ grinded powder showed the highest specific volume, lowest bulk density and apparent density. It results with good hydration properties with highest swelling capacity, water retention capacity, water holding capacity and oil holding capacity.

Sankalpa *et al.* (2016a) investigated the effect of four drying (open yard sun drying, solar tunnel drying, hot air drying and dehumidified air drying) and three grinding methods (hammer mill grinding at ambient temperature, hammer mill grinding with water cooling and hammer mill grinding with liquid nitrogen cooling) on composition of sweet

orange peel powder. Among all the combination of treatment low temperature drying and grinding *i.e.* dehumidified air drying and hammer mill grinding result in good quality of powder, with highest protein, ash and crude fibre content of 8.92%, 0.99% and 51.54%, respectively.

2.2 Extraction and encapsulation processes

Hassan and Hobani (1998) determined the flow properties of water extract of Roselle calyces using a concentric cylinders rotational viscometer for extract concentration range of 5-65% TS, temperature range of 20-60 °C and shear rate range of 2.5-979 s⁻¹. Measured shear stress was within the range 0.5 to 49.7 Pa, corresponding to an apparent viscosity range of 0.0015-1.55 Pa s. Within the tested ranges of concentration, temperature, and shear rate, the extract exhibited a pseudoplastic behaviour; and was adequately described by the simple power law model. The flow behaviour index was consistently below unity (0.76-0.97), with the consistency index varying between 0.007 and 1.6 Pa s. Activation energy of flow varied from 18.74 to 42.80 (kJ.kmol⁻¹) for the extract concentration range of 5-65% TS, at a shear rate of 100 s⁻¹.

Tsai *et al.* (2002) investigated the anthocyanin and antioxidant capacity in Roselle (*Hibiscus sabdariffa* L.) extract. Roselle extract was prepared by extracting dried Roselle petals in boiling water. The relation between the anthocyanin colour and antioxidant capacity was elucidated by comparing absorbance at 520 nm, with ferric reducing ability of plasma (FRAP), oxygen radical absorbance capacity (ORAC) and total antioxidant status (TAS) antioxidant assays. The results showed that the antioxidant capacity of Roselle extract increased when extraction time or weight of petals increased. The FRAP assay showed a linear relationship with anthocyanin as determined at 520 nm. Comparisons between FRAP and ORAC or FRAP and TAS assays gave a linear relation. These results suggest that anthocyanin is the major source of antioxidant capacity in Roselle extract. Further purification using Amberlite XAD-2 and HPLC indicated that anthocyanin and a brown pigment in the extract account for about 51 and 24% of the antioxidant capacity, respectively. Under different processing temperatures and storage periods, anthocyanin content declines. However, other phenolic compounds increase and overall there is only a relatively small decrease in total phenolic compounds and antioxidant activity.

Chumsri *et al.* (2008) studied the optimum conditions for the extraction and concentration of Roselle (*Hibiscus sabdariffa* Linn.) extract. The study found that the L^* , a^* and b^* values of fresh Roselle calyces were 0.09 ± 0.01 , 0.02 ± 0.01 and 0.05 ± 0.01 . The pH, total acidity and total soluble solids content were 2.16 ± 0.05 , $4.20 \pm 0.01\%$ as malic acid and 5.83 ± 0.04 °Brix, respectively. The yields of fresh and dried Roselle calyces production were $47.45 \pm 0.71\%$ and $9.58 \pm 0.77\%$. The optimum conditions for fresh Roselle calyces, fresh calyces to water ratio was 1:2, with the extraction temperature of 50 °C for 30 min. For dried Roselle calyces, the optimum conditions were 1:10 ratio of dried calyces to water and the extraction temperature of 50 °C for 30 min. The method of evaporation under the vacuum of 44 cm Hg at 70 °C was an appropriate selected method for both concentrated fresh and dried Roselle extracts. The pH, total acidity and total soluble solids contents of concentrated fresh Roselle extract were 2.77 ± 0.02 , $12.73 \pm 0.09\%$ as malic acid and 25.07 ± 0.10 °Brix, respectively. The total anthocyanin, total phenolic contents and EC50 (DPPH radical scavenging assay) were 37.67 ± 0.02 mg.100 g⁻¹ fresh Roselle calyces, 31.26 ± 0.75 mg GAE.g⁻¹ and 39.37 ± 0.61 mg/ml (n=9). The pH, total acidity and total soluble solids contents of concentrated dried Roselle extract were 2.89 ± 0.05 , $11.96 \pm 0.34\%$ as malic acid and 25.07 ± 0.10 °Brix. The total anthocyanin, total phenolic contents and EC50 were 340.97 ± 0.15 mg.100 g⁻¹ dried Roselle calyces, 31.18 ± 0.62 mg GAE.g⁻¹ and 47.53 ± 0.85 mg.ml⁻¹ (n=9), respectively.

Selim *et al.* (2008) studied on extraction, encapsulation and utilization of red pigments from Roselle (*Hibiscus sabdariffa* l.) as natural food colourants. The stability of the encapsulated pigments was investigated during storage under different water activities. The results showed that ethanol acidified with 1.5 N HCl (85:15) had the greatest efficiency in extracting Roselle pigments followed by 2% citric acid solution while distilled water was the less effective. The results indicated that at low pH values (1.5, 2, and 3); the pigment extracts exhibited their greatest stabilities during the entire holding time. Data showed that Roselle pigment extracts heated for 30 min at temperatures of 60, 70, 80, 90, and 100 °C, retained 99.87, 99.24, 94.49, 86.35, and 78.59% of their anthocyanins contents, respectively. As heating time was extended to 60 min, the retention values decreased to 96.99, 86.75, 82.10, 76.72, and 57.69%, respectively. The effect of three different encapsulating agents *i.e.* Maltodextrin D.E. 10, Maltodextrin DE 20 and gum Arabic on pigments stability was investigated.

Maltodextrin DE 20 was found as the most effective carrier in stabilizing the pigments under all storage conditions examined. The half-life periods for anthocyanins encapsulated in maltodextrin DE 20 were 433.1, 238.96, 80.58, and 94.93 days for storage water activities of 0.43, 0.53, 0.64 and 0.75, respectively.

Farimin and Nordin (2009) conducted a study on production of spray-dried Roselle-pineapple powder using spray drier. Roselle calyces and pineapples were extracted under optimum condition. Three different maltodextrin DE 10 concentrations (3%, 5% and 10%) were added (w/w) as the encapsulating agent prior to spray drying. Inlet temperatures were varied at 140 °C, 160 °C and 180 °C, respectively and the outlet temperature was set at 80 °C. The spray-dried Roselle-pineapple powder were analysed for moisture content, dissolution, water activity and hygroscopicity. The results indicated that maltodextrin concentration and inlet temperature had significant effects on the percentage of yield. Extracts with 10% maltodextrin gave the highest percentage of yield and produced less sticky film at the wall of the dryer chamber. At 180 °C, juice with 10% of maltodextrin produced the finest powder. When inlet temperature and the percentage of maltodextrin increased, the moisture content, dissolution and water activity decreased and its hygroscopicity increased.

Esa *et al.* (2010) evaluated the antioxidant activity, free radical-scavenging and total phenolic content in other parts of the Roselle plant. Roselle seed extracts were found to have the highest antioxidant activity and strongest radical-scavenging activity of all plants tested. Methanol extracts showed a positive correlation between phenolic content and antioxidant activity, as measured by β -carotene bleaching assay and DPPH radical-scavenging activity. The antioxidant efficacy of Roselle seeds in a whole food system was investigated by testing the effect on lipid oxidation in cooked beef patties stored at 4 °C for 14 days. Results showed that patties treated with Roselle seeds had reduced lipid oxidation compared to patties treated with BHT. This study suggests that Roselle seeds have the potential to be used as food antioxidants.

Gartaula and Karki (2010) optimized the extraction of anthocyanins from Roselle (*Hibiscus sabdariffa* var. *sabdariffa*) in aqueous medium. The dried Roselle calyces were studied for the extraction of anthocyanin in aqueous solution. The total anthocyanin content in dried Roselle powder was found to be 310.48 mg.100 g⁻¹ of dry matter. The extraction temperature at 70 °C gave the highest yield (66.69-70.61%), while at 90 °C the

degradation of anthocyanins occurred. The solid-liquid ratio revealed no significant effect ($p>0.05$), whereas the particle size showed a significant effect ($p<0.05$) on the extraction of anthocyanin. The yields of anthocyanin extraction for 300 and 600 μm particle powders were significantly higher ($p<0.5$) than that of 1180 μm powder. Reduction in the particle size inversely increased the yield of anthocyanins. In conclusion, the extraction of anthocyanin from dried Roselle calyces with an average particle size of 600 μm using a solid-liquid ratio of 1:50 in aqueous medium with pH 7.0 at 70 °C for 20 min showed the maximum yield (69.84%).

Arab *et al.* (2011) studied the physico-chemical properties of natural pigments (anthocyanin) extracted from Roselle calyces (*Hibiscus subdariffa*). Physico-chemical properties of Roselle calyces (*Hibiscus subdariffa*) indicated that moisture content, protein, fat, fiber and ash were 12.81%, 7.51%, 0.46%, 11.17% and 11.24%, respectively. Besides, contained ascorbic acid (140.13 $\text{mg}\cdot 100\text{ g}^{-1}$), total anthocyanins (622.91 $\text{mg}\cdot 100\text{ g}^{-1}$) and total phenolics (37.42 $\text{mg}\cdot \text{g}^{-1}$ dry weight). The DPPH scavenging capacity obtained from raw dried Roselle was 36.53 μmL^{-1} . The extraction of natural pigments (anthocyanins) from Roselle calyces by different solvents (ethanol acidified with 1.5N/L HCl (85:15, v/v), ethanol acidified with 1% citric acid, citric acid solution of 2% concentration and distilled water) were applied and pigments were analyzed for colour, pH, total acidity, total soluble solids, total anthocyanins, total phenolic and antioxidants activity. Water acidified with citric acid 2% indicating anthocyanins yield of 1063 $\text{mg}\cdot 100\text{ g}^{-1}$ might be the best choice and the more preferable solvent compared with ethanol acidified with HCl which showed the highest yield *i.e.* 1386 $\text{mg}\cdot 100\text{ g}^{-1}$ dry weight. The results from this study showed that the greater the Roselle extracted by 2% citric acid solution the more the red colour intensity observed (a^* 5.25).

Liaqid *et al.* (2011) studied the microwave assisted extraction of anthocyanins from grape skins. A new method for the analysis of anthocyanins in grapes based on a systematic study of the extractability of eleven anthocyanins from grapes has been developed. The stability of anthocyanins under the extraction conditions was checked using a standardised extract from grape skins. Temperatures from 50 °C up to 150 °C were evaluated. A fractional factorial experimental design was developed to analyse the influence on the extraction process of six different extraction variables: solvent (mixtures of methanol and water), stirring, extraction temperature, extraction time, microwave

power and extraction volume. The extraction solvent was the most important variable for the recovery of most anthocyanins from grapes. Finally, the influence of the extraction time was also studied. With this new method, anthocyanins can be extracted from grapes in 5 min, using 100 °C as extraction temperature and 40% methanol in water as the extraction solvent.

Tee *et al.* (2012) optimized the spray drying process parameters of *Piper betle* L. (Sirih) leaves extract coated with maltodextrin. The process parameters of spray drying were inlet hot air temperature, pump flow rate and aspirator rate. Maltodextrin acted as carrier in the spray drying process. The properties of dried powder were investigated in terms of bioactive compound, hydroxychavicol (HC) content, particle size distribution, moisture content, powder yield and hygroscopicity. The experimental run and optimization work were designed using Box-Behnken method of Response Surface Methodology. The optimum operation conditions for the highest HC content with the lowest moisture content; the smallest particle size; highest powder yield and lowest hygroscopicity were obtained at inlet drying temperature of 159.52 °C; feed flow rate of 10.5 ml.min⁻¹ and aspirator rate of 98.33%. The optimal properties of spray-dried powder obtained from this study were 229.29 ppm of hydroxychavicol, 5.48 µm in size; 6.99% in moisture content; 10.53 g of powder yield and 28.88% of hygroscopicity.

Attia *et al.* (2013) studied the characterization of red pigments extracted from red beet (*Beta vulgaris* L.) and its potential uses as antioxidant and natural food colourants. Red pigments extracted from red beet (*Beta vulgaris* L.) were identified for major constituents by using HPLC, these pigments were used as alternative natural red colourants in some processed foods, *i.e.*, jelly and ice sherbets. Also, the effect of using red beet extract as natural antioxidant on corn oil was studied. The results showed that red beet had 380 mg betalain /100g on fresh weight. The major constituents of red beet pigments were betalain and isobetalain by analyzing with HPLC. The best carriers for red beet pigments were dextrin followed by soluble starch, lactose and glucose, respectively. On the other hand, colour and higher pigment stability of betalain pigments extracted from red beet were at pH ranged between 3.0 to 7.0 and temperature ranged between 40 to 50 °C. Meanwhile, the degradation of red pigments were 82.0% of the total pigments after 180 min at 90 °C. Antioxidant activity of red beet extracts were assessed by measuring peroxide value in corn oil during 7 days at 60 °C corn oil contained 1000 ppm

was able to lower the peroxide value than using 200 ppm synthetic antioxidant butylated hydroxyl toluene (BHT). Analysis of variance for sensory evaluation of prepared jelly and ice sherbets indicated that, jelly samples containing 0.30% and ice sherbets containing 0.20% red beet pigments were given the highest scores of colour, taste and overall acceptability similar with synthetic colour carmine.

Cabrera *et al.* (2013) analysed the antimicrobial activity of calyx extracts from five *Hibiscus sabdariffa* varieties against *Salmonella typhimurium* and *Salmonella choleraesuis*. Calyx extracts produced with water, ethanol and methanol were applied to *Salmonella* cultures. All extracts exhibited antimicrobial activity against both *Salmonella* serotypes. The antimicrobial effect was highest in the variety named as “Alma blanca” (in which anthocyanin production has been almost completely suppressed). Extract pH had a significant ($p < 0.05$) impact on extract antimicrobial effect. The variety had influence in the antimicrobial activity of *H. sabdariffa* calices.

Esam (2013) investigated on rheological properties of Roselle as influence of temperature (20-60 °C), shear rate (5-200 s⁻¹), shearing time (15, 30 and 45 s) and time (storing at 4 °C) on the apparent viscosity and shear stress. An increase in temperature decreased the viscosity and shear stress. However, the effect of temperature was more pronounced at higher shear rates. Studying the effect of shearing time showed almost no change in viscosity and shear stress at all shearing times applied. No change in viscosity and shear stress was observed when the effect of time was studied (storing at 4 °C) until week 25 wherein a sudden increase in viscosity and shear stress was noticed.

Oancea *et al.* (2013) investigated the conventional and ultrasound-assisted extraction procedures in order to isolate an enriched crude anthocyanin extract from blackberry (Thorn free cultivar) and sweet cherry (Black gold cultivar). Hydro-ethanolic solution and acidified ethanol were used to conventionally extract anthocyanins by a discontinuous process at 4 °C for 2/24 h. Addition of hydrochloric acid in ethanol of different concentrations proved to be more efficient in both type of samples. In the ultrasound-assisted extraction, the highest recovered anthocyanin content in blackberry (107.81 mg.100 g⁻¹ FM) was obtained with a 10/1 solvent/solid ratio (v/w) at 30 °C for 5 min, while a 15/1 solvent/solid ratio (v/w) at 30 °C for 20 min lead to an increased antioxidant capacity as determined by ferric reducing antioxidant power in the extract using 0.1% HCl in 80% ethanol. The optimum conditions obtained for ultrasound-

assisted extraction from sweet cherry in 0.1% HCl in 60% ethanol at 30 °C include a 15/1 solid/solvent ratio (w/v) and 5 minutes for the maximum yield (36.05 mg.100⁻¹ FM). The final crude anthocyanin extracts may find useful application as dietary supplements, or may be further purified for application as food ingredients.

Santos *et al.* (2013) evaluated the encapsulation of anthocyanin extract obtained from jaboticaba (*Myrciaria cauliflora*) skins in Polyethyleneglycol (PEG) using supercritical CO₂ as solvent and ethanol as co-solvent. For comparison, a conventional method, ionic gelification, was employed to produce encapsulated particles by entrapment in Ca-alginate beads. The encapsulation efficiency of the extract in Ca-alginate beads was higher (98.67%) than those obtained by Rapid Expansion of Supercritical Solution (RESS) process (79.78%). Encapsulated particles made by RESS at different pressures, and temperatures, retained the extracts' biological activity. The best operating RESS process condition for anthocyanin extract encapsulation was determined as 313.15 K and 20 MPa. The degradation studies indicated that both encapsulated systems were more stable to light and temperature than the free extract. Ca-alginate encapsulated Anthocyanin extract release in acid buffer solution was incomplete and slower than when the extract was encapsulated by PEG. Both systems increased the stability of anthocyanin, although presenting different characteristics.

Cruz *et al.* (2013) evaluated the release of phenolic compounds from Roselle extract encapsulated in binary and ternary mixtures of whey protein, carboxymethyl cellulose (CMC) and pectin. Films were formulated according to a simplex centroid experimental design and total polyphenols and antioxidant activity were quantified in the release medium as time dependent variables. Films weight gain was measured and swelling behavior was calculated. The data obtained correlated well with a first-order kinetic model ($R^2 > 0.9$). While CMC avoids the release of polyphenols and the expression of the antioxidant activity, whey protein and to a lesser extent pectin enable the release of these compounds faster and in larger amounts. Higher antioxidant activities were observed at higher concentrations of pectin and whey protein in the mixtures (80 g.100 g⁻¹) combined with lower concentrations of CMC (10 g.100 g⁻¹). The results obtained showed that the release of Roselle's polyphenols can be predicted by controlling the composition of the mixtures.

Silva *et al.* (2013) studied the parameter optimization for spray-drying microencapsulation of jaboticaba (*Myrciaria jaboticaba*) peel extracts using simultaneous analysis of responses. The 30% maltodextrin (control), 25% arabic gum + 5% maltodextrin and 25% Capsul™ + 5% maltodextrin carriers were used at air-drying inlet temperatures of 140, 160 and 180 °C. The following responses were evaluated: anthocyanin retention (AR), moisture content (MC), total solids (TS), hygroscopicity (H), overall colour difference and antioxidant activity. Scanning electron microscopy (SEM) analysis was also performed. The AR, MC, H and overall colour difference parameters were selected for simultaneous optimization by the desirability approach. The results showed that the highest desirability (0.7-0.8) was achieved when 30% maltodextrin was used at 180 °C. According to the SEM analysis, the use of maltodextrin and gum arabic allowed for the formation of more homogeneous particles, which is recommended in spray drying microencapsulation.

Arueya and Akomolafe (2014) investigated the stability studies of microencapsulated anthocyanins of Roselle (*Hibiscus sabdariffa* .L) in native starch and its potential application in jam production. Various solvent extracted, microencapsulated in native corn starch and freeze dried samples [ethanol acidified with 1% citric acid (FDMEE), 2% citric acid solution (FDMCE) as well as distilled water (FDMWE)] were prepared using standard techniques. These were kept under room temperature conditions for eight weeks and physic-chemical evaluations conducted periodically. Some of these were utilized (1% inclusion) in the production of jam and evaluated by a 30 man sensory panel. Colour intensity indicated by Chroma Values (a^*) of microencapsulated samples were: FDMEE (2.20 ± 0.01), FDMCE (3.05 ± 0.02) and FDMWE (1.98 ± 0.00). Analysis of total soluble solids ($^{\circ}\text{Brix}$), titratable acidity (% citric acid) and anthocyanins ($\text{mg} \cdot 100 \text{ g}^{-1}$) in this order gave the following FDMEE (5.1 ± 0.00 , 15.35 ± 0.2 , 968.53 ± 0.26); FDMCE (5.5 ± 0.00 , 18.41 ± 0.16 , 1217.13 ± 0.26); FDMWE (4.8 ± 0.00 , 13.93 ± 0.14 , 882.51 ± 0.44). These values were significantly different from their non-encapsulated equivalents ($p < 0.05$). Sensory scores for jam containing microencapsulated extract compared favourably with the non-encapsulated inclusive samples. Freeze dried microencapsulated anthocyanins in native corn starch is colour stable and valuable in the production of acceptable jam.

Ortega and Beltran (2014) studied the effect of average particle size (APS), type of solvent, and extraction times (ET) on the physico-chemical (moisture, pH, total soluble solids (TSS), titratable acidity, colour, water activity (a_w), density) and phytochemical (total anthocyanins and phenols content) properties in *Hibiscus sabdariffa* (Roselle) calyces. The phytochemical properties evaluation was performed using a factorial design $2 \times 3 \times 3$: two APS (median diameters, d_{50} , of 0.55 ± 0.016 (fine powder) and 0.97 ± 0.034 (ground powder) mm), three solvents (water, 2% citric acid and 50% ethanol) and three ET (30, 45 and 60 min). All extractions were performed at 50 °C. The APS was determined by sieve analysis using Tyler sieves of different number of mesh. Regarding physico-chemical properties, no significant differences ($p > 0.05$) were observed in moisture content, pH, and titratable acidity; however, the 0.55 mm fine powder (FP) of *Hibiscus* calyces had lower a_w (0.37 ± 0.01) and higher TSS ($5.53 \pm 0.05\%$) than the 0.97 mm ground powder (GP). The extracts obtained from GP showed a deeper red colour than those of FP. The best combination of independent variables, in order to obtain the highest concentration of anthocyanins (451.4 ± 28.1 mg.100 g⁻¹ d.s.) and total phenols (2016.2 ± 159.8 mg.100 g⁻¹ d.s.) were APS of 0.55 mm, 50% ethanol and ET of 30 min.

Jafarian *et al.* (2014) determined the total phenolic content and antioxidant activity of Roselle (*Hibiscus Sabdariffa* L.) calyces extracts. Liquid extracts of dried Roselle (*Hibiscus Sabdariffa* L.) calyces obtained using water, ethanol (50%), methanol (50%), ethanol-methanol (50-50%, v/v) to evaluate selected phenolic compounds and antioxidant activity using the β -carotene bleaching method, free-radical scavenging by DPPH. The phenolic compounds contents ranged from 24.36 (in water) to 44.43 (in ethanol-methanol) mg of gallic acid 100 g⁻¹ of dried calyces and the antioxidant activity ranged from 39.45% (in water) to 60.43% (in ethanol-methanol). Free radical scavenging capacity of extracts by DPPH were obtained in range of 43.85% (in water) to 91.77% (in ethanol-methanol). The antioxidant properties of Roselle calyces make the extracts ideal for use in foods as a natural extract, concentrate or powder.

Pacome *et al.* (2014) studied the phytochemical and antioxidant activity of Roselle (*Hibiscus sabdariffa* L.) petal extracts. The results showed that alkaloids, anthocyanins, flavonoids, saponins, steroids, sterols and tannins are present in petals of the *H. sabdariffa*. Anthocyanin content was highest while the contents of phenols and flavonoids were lowest. HPLC analysis revealed two phenolic acids, 16 flavonoids and

four anthocyanins in petal of *H. sabdariffa*. The major compounds were gossypetin, hibiscetin, quercetin and sabdaretin (flavonoids) while delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside were the major anthocyanins. The antioxidant activity of the investigated extract has a scavenging ability of DPPH radical scavenging activity (around 97%). The IC_{50} values of *H. sabdariffa* extract was 0.24 mg.ml^{-1} while that of ascorbic acid used as the reference control was 0.35 mg/ml . This indicated that the compounds constituting mainly petals of *H. sabdariffa*, such as anthocyanins, flavonoids and phenolic acid contribute to the antioxidative activity. The researchers concluded that, the petal extract of *H. sabdariffa* is a potential source of natural antioxidants and this justify its uses in folkloric medicines.

Rajha *et al.* (2014) studied the optimization process of phenolic compounds solid liquid extraction from grape byproducts at high temperatures and short incubation times. The effect of five experimental parameters (solidliquid ratio, particle size, time, temperature and solvent mixture) mostly believed to affect the extraction process was undertaken. A first response surface methodology (RSM) experimental design was used to optimize the solid-liquid ratio and milling time parameters. A second design was used for the optimization of the quantitative and qualitative parameters. The quantitative parameters studied are: total phenolic compounds, flavonoid content, total monomeric anthocyanin composition and tannin concentration. The qualitative parameters analyzed are: antiradical activity and antioxidant capacity. The second design was based on the use of time, temperature and solvent mixture as optimization parameters. The assays were first conducted separately revealing the best experimental conditions for the maximization of each response variable alone. A simultaneous RSM of all the responses taken together was then conducted, showing the optimal extraction conditions to be: 93 minutes at 94°C and in 66% ethanol/water solvent. The maximal response values obtained for each parameter are: total phenolic compounds yield ($5.5 \text{ g GAE.100g}^{-1} \text{ DM}$), flavonoid content ($5.4 \text{ g GAE.100 g}^{-1} \text{ DM}$), total monomeric anthocyanin yield ($70.3 \text{ mg.100 g}^{-1} \text{ DM}$), tannin concentration (12.3 g.L^{-1}), antiradical activity (67.3%) and total antioxidant capacity ($393 \text{ mg AAE.l}^{-1}$). All of the optimal values were acquired at 3 ml.g^{-1} solid-liquid ratio and 6.8 min milling time. The obtained extracts could be used as natural bioactive compounds in several industrial applications.

Sindi *et al.* (2014) studied the comparative chemical and biochemical analysis of extracts of *Hibiscus sabdariffa*. The extracts have attracted attention because of potentially useful bioactivity. However, there have been no systematic studies of extraction efficiencies of *H. sabdariffa*. The nature of extracts used in different studies has varied considerably, making comparisons difficult. Therefore, a systematic study of extracts of *H. sabdariffa* made with different solvents was carried out using water, methanol, ethyl acetate and hexane in the presence/absence of formic acid, using different extraction times and temperatures. The extracts were analysed for total polyphenol content, antioxidant capacity using DPPH, FRAP and TEAC assays, and specific anthocyanins were determined using HPLC and LC–MS. The results showed the highest antioxidant capacities were obtained by extracting using water, with or without formic acid, for 10 min at 100 °C. These extracts provided the highest concentrations of cyanidin 3-sambubioside and delphinidin 3-sambubioside.

Sirag *et al.* (2014) investigated the total phenolic content and antioxidant capacity of Roselle (*Hibiscus sabdariffa*) calyx ethanolic extract. The total phenolic content was estimated by Folin Ciocalteu method using Gallic acid as standard while the antioxidant capacity was determined based on the plant extract to scavenge DPPH radical. The total phenolic content was found to be 41.07 mg GAE.g⁻¹. The extract exhibited a notable dose dependent inhibition of the DPPH activity. At a concentration 250 µg.ml⁻¹, *Hibiscus sabdariffa* calyx extract scavenged 86% of DPPH radical whereas 125 and 50 µg.ml⁻¹ caused 53% and 23% DPPH inhibition, respectively and a very mild inhibition was produced at a concentration 5 µg.ml⁻¹. It can be concluded that *Hibiscus sabdariffa* calyx could be a potential source of antioxidant principles.

Chen *et al.* (2015) used the response surface methodology (RSM) to optimise experimental conditions for ultrasonic-assisted extraction (UAE) of functional components from sugar beet molasses. The central composite design (CCD) was used for the optimisation of extraction parameters in terms of total phenolic contents, antioxidant activities and anthocyanins. Result suggested the optimal conditions obtained by RSM for UAE from sugar beet molasses were as follows: HCl concentration 1.55–1.72 mol.L⁻¹, ethanol concentration 57–63% (v/v), extraction temperature 41–48 °C, and extraction time 66–73 min. In the optimal conditions, the experimental total phenolic contents were 17.36 mg GAE.100 mL⁻¹, antioxidant activity was 16.66 mg TE.g⁻¹, and total

anthocyanins were $31.81 \text{ mg} \cdot 100 \text{ g}^{-1}$ of the sugar beet molasses extract, which were well matched the predicted values. Teen compounds, *i.e.* gallic acid, vanillin, hydroxybenzoic acid, syringic acid, cyanidin-3-O-rutinoside, cyanidin-3-O-glucoside, catechin, delphinidin-3-O-rutinoside, delphinidin-3-O-glucuronide and ferulic acid were determined by HPLC-DAD-MS/MS in sugar beet molasses.

Diessana *et al.* (2015) investigated the influence of particle size and calyx/water ratio on the anthocyanins extraction and biochemical composition during extraction using bright red and dark red *Hibiscus sabdariffa* calyces. Results showed that the pH of the bright red calyces was 2.24 ± 0.01 corresponding to $12.66 \pm 0.15 \text{ g} \cdot 100 \text{ g}^{-1}$ dry weight (DW) titratable acidity equivalent of malic acid. The pH of the dark red calyces was 2.15 ± 0.01 corresponding to $15.44 \pm 0.15 \text{ g} \cdot 100 \text{ g}^{-1}$ DW titratable acidity equivalent malic acid. Total sugars content was 3.24 ± 0.04 and $3.13 \pm 0.06 \text{ g} \cdot 100 \text{ g}^{-1}$ DW for bright red and dark red, respectively. Proteins content ranged from 4.56 ± 0.04 to $6.96 \pm 0.17 \text{ g} \cdot 100 \text{ g}^{-1}$ DW. The level of the total phenols ranged from 3.82 ± 0.33 to $5.22 \pm 0.08 \text{ g} \cdot 100 \text{ g}^{-1}$ DW. Average anthocyanins content was 2.61 ± 0.11 and $2.98 \pm 0.02 \text{ g} \cdot 100 \text{ g}^{-1}$ DW for dark red and bright red calyces, respectively. Calyces were found to show an antioxidant capacity of up to $300 \text{ } \mu\text{mol Trolox equivalent} \cdot 100 \text{ g}^{-1}$. For all calyx/water ratios, the anthocyanins content increased with extraction time till 60 min and remained almost stable thereafter. The optimization of the aqueous extraction was obtained at the ratio 1/10 (calyx/water) for the production of Roselle concentrated extract, using calyces size less than $250 \text{ } \mu\text{m}$, at $30 \text{ } ^\circ\text{C}$, for one hour percolation.

Rajabi *et al.* (2015) investigated the retention of saffron bioactive components by spray drying encapsulation using maltodextrin, gum Arabic and gelatin as wall materials. Emulsions with a constant ratio of saffron extract/wall material of 1:20 and two levels of total solids (TS of 30 and 40%), were prepared using a homogenizer and then spray dried. Powders were characterized in terms of powder yield, encapsulation efficiency, and retention of saffron active components, microstructure and moisture content. Retention of picrocrocin, safranal and crocin after spray drying was analyzed by measuring absorbance at 257, 330 and 440 nm, respectively. It was observed that a mixture with 40% TS consisting of maltodextrin, gum Arabic and gelatin in the weight ratio of 0.94:0.05:0.01 retained the highest amount of picrocrocin, safranal and crocin, by retention values of 90.06, 80.37, and 91.03%, respectively. Both encapsulation efficiency

and powder yield were positively influenced by total solids content, which could be related to the emulsion viscosity and droplet size.

Youssef *et al.* (2015) studied the effect of engineering treatments on extraction of Roselle juice (karkdah). The adsorption kinetics and isotherms was also studied to determine the parameters to be optimized to recover the anthocyanins from aqueous extracts of the Roselle. The results revealed that, the best combination of independent variables, in order to obtain the highest of anthocyanins ($1250 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ d.s.}$), Vitamin C ($145 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ d.s.}$) and total phenols ($4163 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ d.s.}$) were obtained by extracting using acidified water with 2% citric acid for 210 min as retention time at 25 degrees °C, using the highest solid-to-solvent ratio (1/25). The deepest red-purple (Hue = 14.04 and purity (C) 51.39) coloured solution was observed under this condition. The best DPPH scavenging activity expressed as EC_{50} was obtained ($40.50 \mu\text{.ml}^{-1}$).

Zaidel *et al.* (2015) investigate the efficiency of encapsulation of anthocyanins from red dragon fruit (*Hylocereus polyrhizus*) using microwave-assisted technique with different drying times and thermal stability of the encapsulated anthocyanins. Encapsulated anthocyanins were produced in form of powder using maltodextrin as the wall material. The encapsulation drying time were varied from 1 min to 3 min with fixed power, 330 W. The encapsulated anthocyanins were analyzed in effect of thermal heating (60°C and 80°C). The results indicated that, at different encapsulation drying time, 2 min capsule showed the highest yield of encapsulation where 3 min capsule showed the highest encapsulation efficiency followed by 2 min capsule with 4% differences. From this study the encapsulation at 2 min drying time showed the most feasible encapsulation process. Thermal study at 60°C and 80°C showed that anthocyanins degraded faster at higher temperature, 80°C for all capsule types.

Tawiah *et al.* (2016) extracted natural pigment (anthocyanins) from Roselle calyces (*Hibiscus subdariffa*) using different solvents (ethanol acidified with 1.5N/L HCl (85:15, v/v), ethanol acidified with 1% formic acid, acidified ethanol with Tween-80 surfactant, water acidified with 1% HCl) and then isolated the main anthocyanins by aqueous macroporous resin adsorption method (AMRAM). The extracted pigment (anthocyanins) was used to dye wool fabric and its colouristic properties were investigated. Acidified ethanol doped with Tween-80 surfactant showed the highest extraction efficiency at pH 5 due to denaturing of cell membranes under acidic condition,

which together with the Tween-80 surfactant dissolves and stabilizes the anthocyanins. It was also observed that the presence of Tween-80 increased the extraction efficiency from 987.38 to 1175.45 mg.100 g⁻¹ powdered calyces compared to other solvents. HPLC-MS result proved the isolation of the two major anthocyanins components at molecular weights 594 and 448, respectively. The K/S value of the dyed wool fabrics changed with different treatment reagents. The mordant (Potassium antimony (III) tartrate hydrate) helped improve the colour strength at optimal dyeing conditions of 100 °C for 1 h at pH 6. The dyed fabric had good washing, rubbing and light fastness using the standard test methods for textiles.

2.3 Stability of anthocyanin

Gradinaru *et al.* (2003) investigated on thermal stability of anthocyanins isolated from the dry calyces of *Hibiscus sabdariffa* L. (Roselle) in aqueous solutions (55-98 °C), either as free or copigmented anthocyanins with chlorogenic acid, and in the dry state as free anthocyanins or co-lyophilized with an amorphous polysaccharide (pullulan) and stored in different relative humidity environments (water activities 0.33, 0.53, 0.75 and 0.84) at 40 °C. The rate constants for degradation were obtained from first-order reaction kinetic plots. The degradation kinetics of individual anthocyanin components in solution, as assessed by HPLC, followed an Arrhenius-type response with respect to temperature; activation energies, E_a , varied between 13.3 and 15.1 kcal.mol⁻¹. Copigmentation of anthocyanins with chlorogenic acid did not seem to improve their stability in solution. In the dry state, the degradation rate constants increased with the water activity, particularly above 0.53. In the freeze-dried pullulan-anthocyanin mixtures, the polysaccharide matrix delayed colour degradation compared to the free anthocyanin preparations by 1.5-1.8 times.

Janna *et al.* (2006) analysed the colour pigment, anthocyanin in malabar melastome flower (*Melastoma malabathricum*) and its stability in extracted form. From the observations, it showed that S3 flower at developmental stage contained the highest anthocyanin concentration. Simple linear regression method was used to find the significant level correlating the anthocyanin concentration and storage time. Throughout the experiments, the extracted anthocyanin contents were found quite stable. The graphs showed a small degradation rate in the anthocyanin total concentration, except for the extracts that were exposed to the light where the degradation level reached more than

50%. At different pH values, the anthocyanin concentration decreased and the colour faded at higher pH. Extracts that were stored at high temperatures (31 °C) showed higher degradation levels compared to the one kept at lower temperatures (25 °C). From the study, it was concluded that acidic solution (pH 0.5 and 1.0) kept in the dark and at low temperature (4 °C) were found as the suitable storage condition for coloured anthocyanin pigments.

Laleh *et al.* (2006) investigated the effect of light, temperature, pH and species on stability of anthocyanin pigments in four *Berberis* species. The anthocyanin pigment was extracted from the four different *Berberis* plant (*B. khorasanica*, *B. integerrima*, *B. orthobotrys*, and *B. vulgaris*) using the soaking and wetting in Ethanol (1% acidified). The extracted anthocyanin pigments then were exposed to number of environmental conditions, which could destabilize the anthocyanin molecules. These environmental conditions were included three different pHs (0, 1.5 and 3), various temperatures (5 °C, 15 °C, 25 °C and 35 °C) and presence or absence of light. The results of the study showed that increasing in pH, temperature or exposure to light is able to spoil the anthocyanin molecule. Another factor affecting the tolerance of anthocyanin towards the environmental condition is the role of different species. Among the various *Berberis* species anthocyanin pigment in *B. khorasanica*, showed the greatest resistance to destruction by environmental conditions followed by *B. vulgaris*, *B. orthobotrys*, and *B. integerrima*.

Ruangeri *et al.* (2008) investigated the changes in quality and bioactive properties of concentrated Roselle (*Hibiscus sabdariffa* Linn.) extract. It was found that quality changes and bioactive properties of concentrated Roselle extract before and after pasteurization included pH of 2.79 ± 0.00 and 2.80 ± 0.01 , total acidity (as malic acid) of 2.24 ± 0.00 and 2.24 ± 0.03 %, total soluble solids of 44.50 ± 0.00 and 44.56 ± 0.01 °Brix, total anthocyanin contents of 382.66 ± 2.49 and 370.72 ± 1.71 mg.l⁻¹ concentrated Roselle extract, total phenolic contents of 6.06 ± 0.18 and 5.82 ± 0.01 mg.g⁻¹ concentrated Roselle extract, respectively. The antioxidant activities using DPPH assay with BHT standard, expressed as EC₅₀ (Efficient Concentration) were 230.01 ± 2.40 and 235.34 ± 0.79 µg.ml⁻¹, respectively. In addition, concentrated Roselle extract had nutrition facts (percent Thai RDI as over 6 years of age) for 1 serving size (70 ml) providing 140 kilocalories, 11% carbohydrate, 50% Vitamin E (α-Tocopherol equivalent), 15% Vitamin A (Retinol

equivalent) and 15% Vitamin B1. After 90 days storage at 4 °C and 27 °C, concentrated Roselle extract in glass container had pH of 2.80 ± 0.01 and 2.80 ± 0.01 , total acidity of 2.23 ± 0.04 and $2.25 \pm 0.04\%$, total soluble solids of 44.60 ± 0.28 and $44.64 \pm 0.11^\circ\text{Brix}$, total anthocyanin contents of 298.25 ± 0.77 and $100.86 \pm 1.54 \text{ mg.l}^{-1}$ concentrated Roselle extract, total phenolic contents of 3.39 ± 0.41 and $2.58 \pm 0.43 \text{ mg.g}^{-1}$ concentrated Roselle extract and EC50 of 359.96 ± 0.89 and $390.63 \pm 4.75 \text{ }\mu\text{g.ml}^{-1}$, respectively. Both pasteurization and storage conditions significantly affected the bioactive properties of concentrated Roselle extract.

Stanciu *et al.* (2010) studied the stability of the anthocyanins extracts obtained from black grapes skins of *Vitis Vinifera*. In order to obtain the extract with the highest concentration of anthocyanins and the one most stable under different varying factors such as: concentration of anthocyanins, light exposure, temperature and oxygen, different extraction solvents were used. The quantity of anthocyanins in the extracts and stability of anthocyanins extracts were established applying the pH differential method. The results show that black grapes skins have rich anthocyanins content ($497.747\text{--}842.180 \text{ mg.100 g}^{-1}$ in fresh products). The stability of anthocyanins extracts have been significantly affected by temperature (8.45%–17.41% degradation) and exposure to light (37.91%–89.48% degradation).

Tonon *et al.* (2010) studied the anthocyanin stability and antioxidant activity of spray dried acai (*Euterpe oleracea* Mart.) juice produced with different carrier agents. Anthocyanin stability and antioxidant activity of powdered acai juice was evaluated throughout 120 days. Powders were produced by spray drying using four types of carrier agents: maltodextrin 10DE, maltodextrin 20DE, gum arabica and tapioca starch. Samples were stored at different temperatures (25 and 35 °C) and water activities (0.328 and 0.529), in order to verify the effect of these conditions on anthocyanin degradation and antioxidant activity reduction. Anthocyanin degradation exhibited two first-order kinetics: the first one, with higher reaction rate constant, up to 45–60 days of storage, and the second one, after this period, with lower degradation rate. Both temperature and water activity negatively affected anthocyanin stability. Antioxidant activity also decreased with increasing water activity, but was higher for the powders stored at 35 °C. Maltodextrin 10DE was the carrier agent that showed the best pigment protection and the highest antioxidant activity, for all the conditions studied.

Wang *et al.* (2010) studied the stability and antioxidant activity of anthocyanins from blueberry. The results indicate that anthocyanins from blueberry were stable against the low pH (≤ 5.0), NaCl (0.125-0.500 mol.L⁻¹), sucrose (0.584-2.336 mol.L⁻¹) and preservative (sodium benzoate, 0.035-0.140 mol.L⁻¹), but were sensitive to alkaline conditions (≥ 7.0), high temperature (≥ 80 °C), light (natural light), oxidizing agent (H₂O₂, 0.5-2.0%) and reducing agent (Na₂SO₃, 0.005-0.040 mol.L⁻¹). At concentrations of 25 and 50 mg.mL⁻¹, anthocyanins from blueberry could protect ECV-304 cells against oxidative damage induced by H₂O₂. These results suggest that anthocyanins from blueberry can be regarded as a potential colourant for some acidic (pH ≤ 5.0) food products and could be used as health food to prevent diseases arising from oxidative processes.

Clemente and Galli (2011) studied the stability of the anthocyanins extracted from residues of the wine industry. Anthocyanins are highly important due to their antioxidant capacity. They are the most important among the phenolic compounds and one of the main natural dyes used in the food industry. In this research, residue of processed grapes was used to investigate the presence of anthocyanins, the possibility of their extraction from the residue, and their stability. The extraction solution consisted of 70 mL of ethanol 70% and 30 mL of HCl 0.1% at pH 2.0. The results found for the processed grapes residue was 26.20 mg.100 g⁻¹. In order to evaluate stability, caffeic acid was added at 0.5:1 w/v; 0.8:1 w/v; and 1:1 w/v concentrations. Anthocyanins extract reached the greatest stability at 0.5:1 w/v concentration, with 82.47% colour retention and a half-life period of 15 days. Therefore, the use of this organic acid as a stabilizer for anthocyanins is feasible.

Preethi and Balakrishnamurthy (2011) worked on the assessment of banana cultivars for pigment extraction from bracts, its suitability and stability as food colourant. Bracts from six commercial banana cultivars viz., Grand Naine, Ney Poovan, Poovan, Karpuravalli, Red Banana and Virupakshi were used to extract and estimate the anthocyanin content, assess their antimicrobial properties and their suitability as food colourant. The results revealed that Red Banana recorded the highest anthocyanin, phenolic and flavonoid content (89.73 mg.100g⁻¹ bracts, 238.93 mg pyrocatechol.100ml⁻¹ and 333.37mg quercetin.100 ml⁻¹, 333.37mg quercetin.100 ml⁻¹, respectively). The anthocyanin extract from banana bracts exhibited antimicrobial activity against bacteria

and fungi. Banana bract extract in amla squash showed the highest stability under refrigerated condition (8 °C) for 28 days, as observed from the lowest degradation rate (12.91%).

Reshmi *et al.* (2012) studied the effect of light, temperature, pH on stability of betacyanin pigments in *Basella alba* fruit. The study was aimed at exploring the feasibility of using the fruit as natural colourant using acidified methanol extraction. The extracted betacyanin pigments then were exposed to number of environmental conditions, which could destabilize the betacyanin molecules. These environmental conditions were included fourteen different pHs, various temperatures and presence or absence of light. The temperature stability of the betacyanin extract was calculated by reaction velocity constants (k) as well as the half-life time ($t_{1/2}$). The results of the study showed that increasing in pH, temperature or exposure to light is able to spoil the betacyanin molecule. Copigmentation of betacyanin resulting in increase in both hypochromic effects and bathochromic shifts. *Basella alba* betacyanin extract was more stable at pH 4.1 and 6.0, temperature at 10 °C, 20 °C and 30 °C both in the presence and absence of light. The results shows that betacyanin obtained from *Basella alba* fruit has a high potential to be used as a natural food colourant

Sharifi and Hassani (2012) studied the extraction methods and stability of colour extracted from barberry pigments. The anthocyanins of barberry were first extracted by means of a reflux system. Then, the concentration of the main anthocyanin in barberry, that is, Cyanidin-3-glycoside was measured by the pH differential method. The results showed that the maximum amount of anthocyanin was extractable at 50 °C using a mixture of ethanol and methanol as a solvent. In order to test stability of the colour, researchers stored it in chromatic bottles for three months. The maximum and minimum amounts of destruction of anthocyanins were observed at 40 °C and -18 °C, respectively. The most stable colour during the storage period was the one stored at -18 °C. It can be used in products such as confectionary cream, ice cream and those food products that are not exposed to heat.

Aishah *et al.* (2013) determined the stability of the anthocyanins in freeze-dried *Hibiscus sabdariffa*, *Melastoma malabathricum* and *Ipomoea batatas* in various acidic pH (pH 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5). Total monomeric anthocyanin, degradation index, colour density and percent polymeric colour were analyzed to determine anthocyanins

degradation and their colour variations. Among the samples, *H.sabdariffa* contain the highest monomeric anthocyanins (163.3 mg.L^{-1}) followed by *M. malabathricum* (49.9 mg.L^{-1}) and the lowest is *I.batatas* (13.8 mg.L^{-1}). Monomeric anthocyanins from *I.batatas* were found to be very stable and not affected by changes in pH than in *H. sabdariffa* and *M. malabathricum*. However, degradation index (DI) of *H. sabdariffa* was the lowest with value of 0.365 ± 0.049 at pH 3.5. The lowest percentage of polymeric colour (4.94 ± 0.64) was also shown by *H. sabdariffa* at pH 2.5 and maintained a deep red colour with increasing pH indicating higher colour stability compared to *M. malabathricum* and *I. batatas*. Overall, natural pigment in *H. sabdariffa* was found to be the most stable in both monomeric anthocyanin content and chromaticity properties. These results provided information that will further proven the potential usage of *H. sabdariffa*, *M. malabathricum* and *I. batatas* as natural colouring agents to replace the synthetic colourant in food and beverage industries.

Bhardwaj and Sharma (2013) carried out the spectrophotometric analysis of different flowers of *Tagetus erecta* colourant at different pH values. The result shows that *Tagetus erecta* extract (colourant) was stable at the experimental pH range of 2.6-9.4 (light yellow coloured flowers) and 2.5-8.4 (orange coloured flowers). This was indicated by the similar peaks of absorption maxima at the different pH values. Beer's law is valid over the concentration range 50-400 ppm. The complex has absorption maximum at 470 and 480 nm, respectively. *Tagetus erecta* extract, with further studies could be used as a colourant in pharmaceutical formulations.

Vargas *et al.* (2013) studied the extraction and stability of anthocyanins present in the skin of the dragon fruit (*Hylocereus undatus*). The extraction of anthocyanins present in the skin of the dragon fruit was performed using trifluoroacetic acid (TFA) plus a mixture of methanol, acetic acid and water; the anthocyanins were then purified with a LC-18 cartridge, using methanol acidified with TFA as eluent, reaching concentrations of $44.3865 \pm 1.3125 \text{ mg.100g}^{-1}$ of sample. The extracts were put through stability tests under different storage conditions, modifying the pH of the extracts (pH of 1, 4 and 6), the temperature (4 °C, 25 °C and 68 °C) and the absence and presence of light for a time period of 4 days; the tests indicated that anthocyanins remain more stable at a temperature of 4 °C with a pH of 4 in the absence of light, retaining up to 80% of the pigment. Three anthocyanins were partially identified in the extracts by high performance

liquid chromatography (HPLC); they were: cyaniding-3-O-glucoside, cyaniding-3, 5-O-glucoside and pelargonidin-3, 5-O-glucoside.

Kuhestanya and Khodabandehlo (2014) examined the effect of saffron petals synthetic colour stability during storage. For this purpose, firstly extracted by ultrasonic extract of saffron petals by solvent (ethanol acidic) to ratio of about 50 with three factors of time (5, 10, 15) and three intensities (20, 60 and 100%) were the best conditions to extract the sound intensity of 100% and took 15 minutes to determine and then stability of ascorbic acid, 10%, 20% and 30% at 25 and 35 °C and 45 °C were measured within 5 days. The results are treated as destruction effect of ascorbic acid on the pigment of saffron petals and the destruction increases at higher temperatures and it is worth noting that this course destruction in the presence of ascorbic acid at the temperature of 45 °C has reached its maximum.

Kungsuwan *et al.* (2014) studied the effects of pH and anthocyanin concentration on colour and antioxidant activity of *Clitoria ternatea* flower extract. Possibility of the applications, changes in colour, antioxidant activity and colour acceptance of crude anthocyanin extracts had been evaluated over a pH range of 3 to 9 and different anthocyanin concentration from 10 to 20 mg.100 mL⁻¹ of the extract using response surface methodology (RSM). As a result, pH was a major factor influencing both colour and antioxidant activity, while anthocyanin concentration showed only a minor influence. Although colour acceptance was not significant, the change in colour of extracts was apparent; for instance, the colour of the extract was red in acidic solution, blue in neutral solution and green in basic solution. Furthermore, the antioxidant activity showed high activity at acidic pH and gradually decreased as pH increased to basic value. Consequently, the optimum point yielding highest antioxidant activity in the study was at pH 3 and 20 mg.100 mL⁻¹ anthocyanin concentration.

Piyarat *et al.* (2014) determined the stabilities in micellar solutions of extracts from Roselle calyx (*Hibiscus sabdariffa* Linn.) and lac resin (*Laccifer lacca* Kerr.) providing red colourants. The main constituents of these extracts are two anthocyanins, delphinidin-3-sambubioside and cyanidin-3-sambubioside, and anthraquinones in the form of laccaic acids. The per cent yields of Roselle and lac from the extraction process were 31.96±0.32 and 2.79±0.23, respectively. Roselle colourant faded in the absence and presence of sodium dodecylsulfate (SDS) and Tween-80 micelles. The order of stabilities

of Roselle colourant in solvent systems was buffer ~ Tween80 > SDS. Lac colourant is quite stable in the absence and presence of the surfactants and no significant difference in the per cent colour remaining in the absence and presence of the surfactants was observed.

Arslan (2015) studied the effects of degradation preventive agents on storage stability of anthocyanins in sour cherry concentrate. The effects of sugar (sucrose, 25%), gallic acid (700 mg.kg⁻¹) and ascorbic acid (700 mg.kg⁻¹) were used in sour cherry concentrate in order to prevent the degradation of main anthocyanin compounds (cyanidin-3-glucosylrutinoside (Cy-3GR), cyanidin-3-rutinoside (Cy-3R) and cyanidin-3-glucoside (Cy-3G)) which are natural bioactive pigments responsible for red, blue and purple colour of many fruits and vegetables. Thermal degradation of anthocyanins was evaluated by determination of anthocyanin content and calculation of the reaction rate constant, half-life of degradation, activation energy. Anthocyanin content decreased at all of the storage temperatures, as an example; there were 75, 51 and 55% reductions in Cy-3G contents of control samples (with no preventive agent) stored at 45, 24 and 4 °C, respectively. The values of half-life time were above 200 days in most cases at all storage temperatures for sugar treated samples. Cy-3-GR (activation energy values 35.6-84.4 kJ.mol⁻¹) was found to be the most unstable among the other anthocyanins. The most contributing agent on anthocyanin stability was sugar, whereas ascorbic acid exhibited the lowest effect in terms of preventing anthocyanin degradation.

Ramirez *et al.* (2015) studied the effect of stevia and citric acid on the stability of phenolic compounds and in vitro antioxidant and antidiabetic capacity of a Roselle (*Hibiscus sabdariffa* L.) beverage. The optimum extraction conditions of Roselle polyphenolic compounds was of 95 °C/60 min, which was obtained by a second order experimental design. The incorporation of stevia increased the stability of colour and some polyphenols, such as quercetin, gallic acid and rosmarinic acid, during storage. In addition, stevia decreased the loss of ABTS, DPPH scavenging activity and α -amylase inhibitory capacity, whereas the incorporation of citric acid showed no effect. These results may contribute to the improvement of technological processes for the elaboration of hypocaloric and functional beverages.

*Materials and Methods*_____

III. MATERIALS AND METHODS

This chapter deals with the raw materials used in the experimentation, methods employed for drying, milling, extraction, encapsulation and stability studies of anthocyanin and methodology adopted for determination of physico-chemical composition of fresh, dried calyces of Roselle, extracted colour and encapsulated anthocyanin.

The experiments were conducted in the Department of Processing and Food Engineering, College of Agricultural Engineering, Raichur, Karnataka, India. Freshly harvested Roselle samples were procured from M/s “Herbs n Health”, Gadag, Karnataka, India. The chemicals and reagents were procured from M/s Sigma Aldrich Chemicals, Bengaluru.

3.1 Experimental setup for drying and milling of calyces of Roselle

Fresh calyces were selected uniformly according to harvest, colour and freshness. The calyces of Roselle were separated from seed capsules manually by hand picking. The fresh Roselle and fresh calyces of Roselle were depicted in Plate 1 and Plate 2. After separation of seed capsule from Roselle, the whole calyces were used for the study of drying and milling of fresh calyces of Roselle. Fresh whole calyces were subjected to drying using three different drying methods. *i.e.*, shade drying, solar tunnel drying and dehumidified air drying at 40 ± 1 °C and $15\pm1\%$ RH. The dried calyces of Roselle were then subjected to three types of milling namely normal pulveriser, water cooled pulveriser and liquid nitrogen (LN₂) cooled pulveriser. After the milling, powder was passed through 0.5 mm sieve to get uniform particle size. Finally calyces of Roselle powder was packaged in HDPE with aluminium foil packaging material and stored at 4 °C until further processing. The process flow chart for production of calyces of Roselle powder is shown in Fig. 1.



Plate 1. Fresh Roselle



Plate 2. Fresh calyces of Roselle

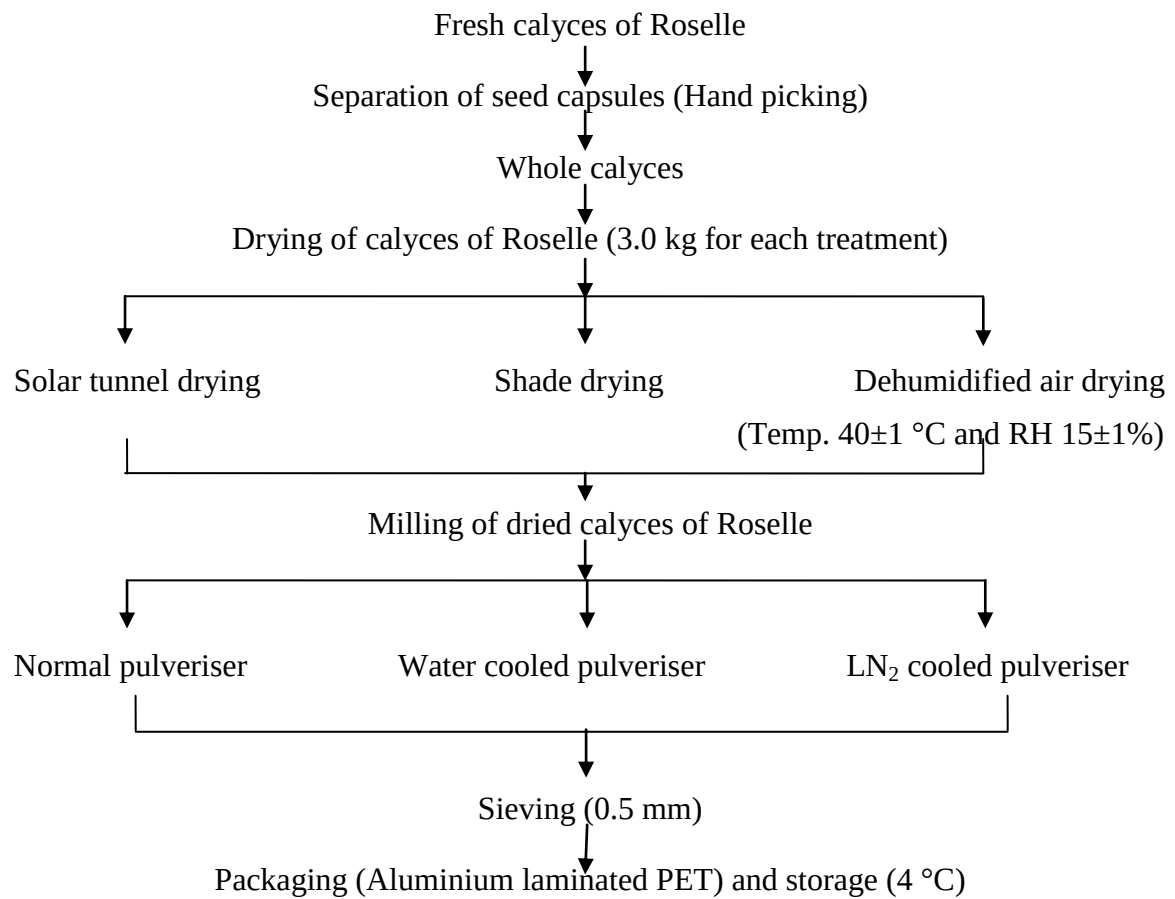


Fig. 1. Process flow chart for drying and milling of calyces of Roselle

3.1.1 Drying methods

Drying, as a preservation method, is a very important aspect of food processing. Drying can be defined as a simultaneous heat and mass transfer operation in which the water activity of the material is lowered by evaporation of water into an unsaturated gas stream. The main function of drying is to lower the water activity of the product and consequently, to inhibit the growth of microorganisms and decrease chemical reactions in order to prolong the shelf-life of the product at room temperature. It also results in less space needed for storage and lighter weight for transportation (Inchuen *et al.*, 2010). The drying methods employed for the present investigation were;

- i. Solar tunnel drying (STD)
- ii. Shade drying (SD)
- iii. Dehumidified air drying (DD)

3.1.1.1 Solar tunnel drying (STD)

The solar tunnel dryer of one tonne capacity designed and developed in the Department of Processing and Food Engineering, College of Agricultural Engineering, UAS, Raichur was used for drying of fresh calyces of Roselle (Plate 3). The solar tunnel dryer was positioned in East-West direction, which had length of 9.0 m, breadth of 3.0 m and height of 3.2 m. The dryer was of tunnel shape made of semi cylindrical metallic (galvanized pipe) structure covered with UV stabilized transparent thermic polyethylene sheet of 200 micron. The drying tunnel had a sliding door of 1.5 m width and 2.0 m height for easy loading and unloading. An exhaust pipe (PVC) of 110 mm diameter with two outlets (chimney) on either side of the drying chamber was provided at the top of the tunnel. Several holes of 12 mm diameter were drilled throughout the length on the lower side of the exhaust pipe. Two ventilators of 1.0×0.3 m size were fitted at the bottom on the rear side of the dryer to remove the moist air from the dryer by natural convection. To enhance the air circulation, one exhaust fan (400 mm size) was provided on the top of the front side of the dryer for obtaining a higher drying rate. Two 3 tier supporting structures (trolleys) on either side were fitted using 30×30 mm MS angle iron to hold 48 trays inside the dryer.



Plate 3. Drying of calyces of Roselle in solar tunnel dryer

3.1.1.2 Shade drying (SD)

Shade drying is carried out for products which can lose their colour and/or turn brown if put in direct sunlight. Products which have naturally vivid colours like herbs, green and red sweet peppers, chilies, green beans and okra give a more attractive end-product when they are dried in the shade (Dauthy, 1995).

The principles for the shade drying are the same as for sun drying. The material to be dried requires full air circulation. Therefore, shade drying is carried out under a roof or thatch which has open sides; it cannot be done either inside conventional buildings with side walls or in compounds sheltered from wind. Under dry conditions when there is a good circulation of air, shade drying takes little more time than is normally required for drying in full sunlight. Shade drying was carried out under natural air flow and at ambient temperature for fresh calyces of Roselle (Plate 4).

3.1.1.3 Dehumidified air dryer (DD)

The dehumidified air dryer of 600 kg capacity (make: Bry Air Asia; model: FSD-600) was used for drying of fresh calyces of Roselle which is shown in Plate 3. In this method of drying, the ambient air was passed through a desiccated medium or source of silica gel. The desiccator absorbed moisture from the air, as a result, the relative humidity of air was reduced and at the same time there was an increase in its temperature. The drying took place when such air came into contact with wet products. Dehumidified air dryer essentially consisted of two parts: the first was a chamber fitted with perforated trays where the product to be dried was placed and on the top housed the second part of the dehumidifying dryer, which continuously fed dry air into the chamber. The removal of moisture was based on the principle of physical adsorption. In dehumidified air dryer, the fresh calyces of Roselle were uniformly spread over the entire drying surface of the dryer. Drying temperature of 40 ± 1 °C and relative humidity of $15 \pm 1\%$ were adopted during the present investigation. The detailed specifications of dehumidified air dryer is presented in Appendix A1.



Plate 4. Drying of calyces of Roselle in shade



Plate 5. Drying of calyces of Roselle in dehumidified air dryer

3.2 Drying kinetics of calyces of Roselle

The drying kinetics of calyces of Roselle was studied by analysing the reduction in moisture content, drying rate and moisture ratio during drying period. The weight of dried sample was taken at an interval of 30 min until completion of drying process in STD, SD, and DD. Reduction in moisture content v/s drying time curves were plotted for shade drying and solar tunnel drying. The temperature and relative humidity of the air inside and outside STD were recorded during the drying period.

3.2.1 Moisture content

The moisture content of fresh calyces of Roselle was determined by following AOAC (2000, 14:004) method. A brief description of the method is as follows. Three grams of Roselle samples were kept in a pre-dried moisture box. The mass of the sample was recorded as W_1 . The box was placed in the hot air oven maintained at 100 ± 2 °C for 5 h. After drying, the box was kept in the desiccator and then weighed. The mass of the dried sample was recorded as W_2 . All measurements were replicated thrice and the average moisture content was calculated. The moisture content of the sample was calculated by using the following equation;

$$\text{Moisture content (\% d.b.)} = \frac{W_1 - W_2}{W_2} \times 100 \quad \dots (3.1)$$

where,

W_1 = Initial weight of sample, g

W_2 = Final weight of sample, g

3.2.2 Drying rate (Rd)

The drying rate of calyces of Roselle for different drying methods was calculated from the following equation (Wankhade *et al.*, 2012).

$$R_d = \frac{M_i - M_d}{t} \quad \dots (3.2)$$

where,

M_i = Mass of sample before drying, g

M_d = Mass of sample after drying, g

t = Drying time, min

3.2.3 Moisture ratio (MR)

The moisture ratio of calyces of Roselle for different drying methods was calculated by using the following equation (Daniel *et al.*, 2012).

$$MR = \frac{M_t - M_e}{M_o - M_e} \quad \dots (3.3)$$

where,

M_t = Moisture content at any time, t (% d.b.)

M_e = Equilibrium moisture content (% d.b.)

M_o = Initial moisture content (% d.b.)

3.3 Mathematical modelling for drying of calyces of Roselle

Six thin layer drying models viz., Newton, Page, Modified Page, Henderson and Pebis, Modified Handerson and Pebis and Logarithmic (Fajar *et al.*, 2012) were selected and are as follows:

i. Newton : $MR = \exp(-kt)$... (3.4)

ii. Page : $MR = \exp(-kt^n)$... (3.5)

iii. Modified Page : $MR = \exp(-kt)^n$... (3.6)

iv. Henderson and Pabis : $MR = a \cdot \exp(-kt)$... (3.7)

v. Modified Henderson and Pabis : $MR = a \cdot \exp(-kt) + b \cdot \exp(-gt) + c \cdot \exp(-ht)$... (3.8)

vi. Logarithmic : $MR = a \cdot \exp(-kt) + c$... (3.9)

where,

MR = moisture ratio

t = drying time, h

k, n, a, b, c, g and h = drying constants

The parameters of all the models were estimated by using ‘MATLAB’ version 7.0 software. The fit quality of the proposed models on the experimental data was evaluated using linear regression analysis using curve fitting tool in MATLAB. The statistical parameters, standard square error (SSE) and root mean square error (RMSE) were calculated employing the following equations.

$$RMSE = \sqrt{\frac{\sum_{i=0}^N (MR_o - MR_p)^2}{df}} \quad \dots (3.10)$$

$$SSE = \frac{1}{N} \sum_{i=1}^N (MR_o - MR_p)^2 \quad \dots (3.11)$$

$$\chi^2 = \frac{\sum_{i=1}^N (MR_o - MR_p)^2}{N - z} \quad \dots (3.12)$$

where,

MR_o = Observed moisture ratio

MR_p = Predicted moisture ratio

df = Degrees of freedom

N = No. of data points

z = No. of constants

3.4 Milling of dried calyces of Roselle

Grinding reduces the size of solid materials by mechanical action, dividing them into smaller particles. The force applied may be compression, impact or shear and both the magnitude of the force and the time of application affect the extent of grinding achieved. For efficient grinding, the energy applied to the material should exceed the minimum energy needed to rupture the material. Excess energy is lost as heat and this loss should be kept as low as practicable (Earle, 2013). The grinding equipment used in the investigation were, hammer mill/ normal pulverizer, water cooled pulverizer and liquid nitrogen (LN₂) cooled pulverizer.

3.4.1 Normal pulverizer (NP)

Dried calyces of Roselle was ground in impact type normal pulverizer (Spectra Cryogenic systems) for the ambient grinding process. In this normal pulverizer, swinging hammer heads were attached to a rotor that rotated at high speed inside a hardened casing. Dried calyces of Roselle were put into the hopper of the normal pulverizer. Dried calyces of Roselle were ground with the help of rotor and fine ground sample of Roselle powder was collected from the outlet of the pulverizer. The normal pulverizer used for milling of dried calyces of Roselle is shown in Plate 6.

3.4.2 Water cooled pulverizer (WCP)

Water cooled pulverizer (Sri Ram Machinery Works; SRM-108) was used for grinding of dried calyces of Roselle. Capacity of the water cooled pulverizer was 25 kg.h⁻¹. The flow of material from the feeding hopper was regulated by means of a vertical adjustable slide to suit the load. Rotor with six beaters rotated in anticlockwise direction. The swinging beaters passed just beneath the ratchet teeth liner fitted inside the top half of the crushing chamber, leaving a gap of 3 to 4 mm between the liner teeth and the tips of the rotating beaters. Dried calyces of Roselle were fed through the hopper of the grinder and these calyces were ground with the help of rotor inside the grinder. Temperature was reduced in the grinder with the help of water circulation through it. After the grinding, the material was passed through the screen fitted inside the lower discharge end of the grinding chamber. In this process, water was used as the cooling medium for grinding. The water cooled pulverizer used for grinding of dried calyces of Roselle is shown in Plate 7.

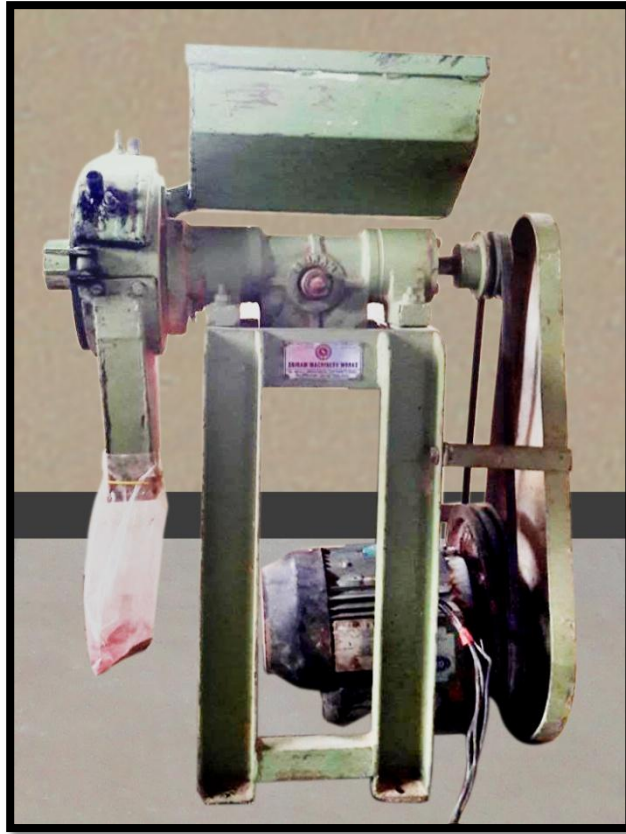


Plate 6. Normal pulverizer

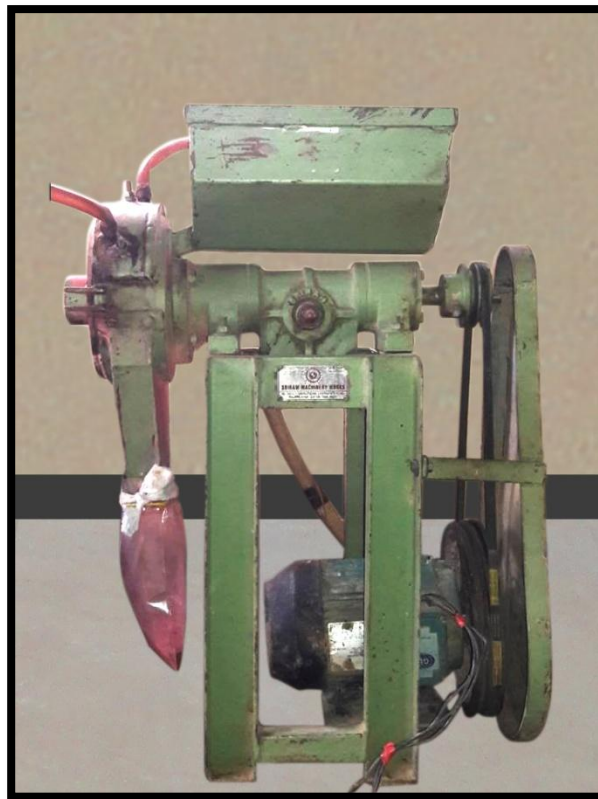


Plate 7. Water cooled pulverizer

3.4.3 Liquid nitrogen (LN₂) cooled pulverizer (LN₂CP)

The modified low temperature grinder was designed and developed by NAIP consortia at University of Agricultural Sciences, Raichur and was fabricated at M/s. Spectra Cryogenic Systems, Kota, Rajasthan (Plate 8). This was used for grinding of dried calyces of Roselle. Low temperature grinding was done by circulating liquid nitrogen (LN₂) at -196 °C. The capacity of liquid nitrogen grinder was 10 kg.h⁻¹. It consisted of a polycarbonate grinding vial supplied with two stainless steel plugs, immersed in liquid nitrogen and ground with the help of stainless steel hammer mill. Liquid nitrogen was stored in Dewar flask (Chengdu golden phoenix LN₂ container Co. Ltd; YDS-10). It contained a liquid level of 27.5 cm. Dewar flask is a vessel designed to provide very good thermal insulation. The right combination of all these components determined the efficiency and productivity of the system. From the filling hopper, dried calyces of Roselle to be ground were metered at a specific rate into the grinding mill. In the feeder, the liquid nitrogen from a storage container or Dewar flask was injected which combined with the samples leading to cooling and embrittling. The cryo material was transported along with the cold nitrogen to the grinding mill where it was pulverized. The cold dry nitrogen gas kept the collecting container dry and inert preventing the possibility of dust explosions (Sridhar and Manohar, 2004). The detailed specifications of low temperature grinder with LN₂ are presented in Appendix A2.



Plate 8. Liquid nitrogen (LN₂) cooled pulverizer

3.5 Proximate composition of calyces of Roselle

The proximate composition of fresh, dried and milled calyces of Roselle viz., moisture content, crude protein, crude fat, carbohydrate content, crude fibre and total ash were determined by following the standard procedures as explained below.

3.5.1 Moisture content

The moisture content of fresh, dried and milled calyces of Roselle were determined by following AOAC (2000, 14:004) method. A brief description of the method is as follows. Three grams of Roselle samples were kept in a pre-dried moisture box. The mass of the sample was recorded as W_1 . The box was placed in the hot air oven maintained at 100 ± 2 °C for 5 h. After drying, the box was kept in the desiccator and then weighed. The mass of the dried sample was recorded as W_2 . All measurements were replicated thrice and the average moisture content was calculated. The moisture content of the sample was calculated by using the following equation;

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad \dots (3.13)$$

where,

W_1 = Initial weight of sample, g

W_2 = Final weight of sample, g

3.5.2 Crude protein

The nitrogen content in fresh, dried and milled calyces of Roselle was estimated by using Kjeltex instrument (D-40599; Behr Labor Technik GmbH, Germany) as per AOAC (1990, 2.057). The detailed specifications of the Kjeltex distillation unit are given in Appendix A3. Seven grams of K_2SO_4 , 0.8 g of $CuSO_4 \cdot 5H_2O$ and 0.8 g of fresh, dried and milled calyces of Roselle were added into digestion tube and then 12 ml of H_2SO_4 was added slowly. Methyl red indicator and bromocresol green indicator solutions were prepared. The digestion tubes were placed on fume ejection system until digestion was cleared (clear with light blue-green colour) in flask at the temperature of 410 °C and then cooled to room temperature and 80 ml of distilled water was added. Digested samples were distilled with 40% NaOH. The distillate was collected in 30 ml of 4% boric acid

solution and titrated against 0.1N HCl (35.4%) until light pink colour appeared. The per cent protein was computed on total nitrogen basis using the following equation.

$$\text{Nitrogen(\%)} = \frac{\text{Volume of 0.1N HCL(ml)} \times 0.1 \times 14.007}{\text{weight of sample (g)} \times 1000} \times 100 \quad \dots (3.14)$$

$$\text{Per cent protein on total nitrogen basis} = \text{N (\%)} \times \text{Conversion factor} \quad \dots (3.15)$$

Conversion factor = 6.25.

3.5.3 Crude fat

The crude fat content of fresh, dried and milled calyces of Roselle were estimated by Soxhlet extraction method as per AOAC (1990, 7.058) using SOCS-PLUS apparatus (Pelican Equipments; SCS-08). Roselle sample of 5 g (W) was weighed accurately and transferred to a thimble. The empty beaker weight (W_1) was taken and all the eight beakers were loaded into the system. The petroleum ether was filled into the beaker of 90 ml from the top and boiled for about 80-90 min at 80 °C. After the completion of process time, the temperature was doubled to 160 °C for 15-20 min to collect the petroleum ether. All the beakers were removed and placed in a desiccator for about 5 min. The detailed specifications of Soxhlet apparatus are given in Appendix A4. The final weight of the beaker (W_2) was noted down and fat content was estimated by using the following equation;

$$\text{Crude fat (\%)} = \frac{W_2 - W_1}{W} \times 100 \quad \dots (3.16)$$

where,

W_1 = Initial weight of beaker, g

W_2 = Final weight of beaker, g

W = Weight of sample taken, g

3.5.4 Carbohydrates

The carbohydrates estimation was carried out as per AOAC (1990 14:005). Fresh, dried and milled calyces of Roselle were analyzed for carbohydrates. One gram of fresh, dried and milled sample was taken in a boiling tube and then hydrolyzed in a water bath

at 100 °C for 3 h with 5 ml of HCl (2.5 N). The tube was cooled to room temperature. The sample was neutralized with sodium carbonate until effervescence ceased and the volume was made up to 100 ml with distilled water. The sample was centrifuged at 9000 rpm for 10 min and the supernatant was collected. The test sample of 0.1 ml was pipetted out into a test tube. One ml of phenol and then 5 ml of sulphuric acid were added to it. The test tube was placed in a water bath at 25-30 °C for 20 min and then cooled. The cooled solution was transferred into the cuvettes and absorbance was measured at 490 nm in spectrophotometer (Systronics; PC based double beam spectrophotometer 2202). To calculate the concentration of carbohydrate present in the sample, a graph of absorbance versus concentration of carbohydrate was plotted along with a standard curve generated from the analysis of D-glucose.

$$\text{Carbohydrates (\%)} = \frac{X}{0.1} \times 100 \quad \dots (3.17)$$

where,

X = Concentration of D-glucose from standard graph, ppm

3.5.5 Crude fiber

The crude fiber content of fresh, dried and milled calyces of Roselle was determined by sequential acid and alkali hydrolysis method using Fibra-Plus (Pelican Equipments; FES-08S) apparatus as per AOAC (1990, 14:020). Accurately, two grams of sample was weighed and transferred to crucible (W). The sample was boiled in 1.25% sulphuric acid and subsequently boiled in 1.25% sodium hydroxide solution. The sample was dried in hot air oven at 100 °C till all the moisture was evaporated. The weight of the crucible before ashing was noted down (W₁). The obtained dried sample was made into ash in a muffle furnace at 550 °C for 4 h. The detailed specifications of muffle furnace are given in Appendix A5. After ashing, the crucibles were cooled in a desiccator and reweighed (W₂). The residue obtained after subtraction of the ash was regarded as fiber. The crude fiber was obtained by using the following equation.

$$\text{Crude fibre (\%)} = \frac{W_1 - W_2}{W} \times 100 \quad \dots (3.18)$$

where,

W_1 = Weight of sample before ashing, g

W_2 = Weight of sample after ashing, g

W = Weight of sample, g

3.5.6 Total ash

The total ash content of fresh, dried and milled calyces of Roselle was determined as per AOAC (1990, 14:006) using muffle furnace (MAC; MSW-251). Accurately, 2 g of sample was weighed into a crucible (which was previously heated to about 550 °C and then cooled). The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 4 h at 550 °C. It was then cooled in a desiccator and weighed. The percentage of ash was calculated by using the following expression;

$$\text{Total ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100 \quad \dots (3.19)$$

3.6 Physico-chemical properties of calyces of Roselle

The physico-chemical properties of food ultimately determine their perceived quality, sensory attributes and behaviour during production, storage and consumption. Determination of physico-chemical characteristics viz., colour, density, pH, water activity, titratable acidity, ascorbic acid, solubility index and particle size of Roselle are explained below.

3.6.1 Colour

Colour is an important attribute because it is usually the first property consumer observes. It is one of the most important quality acceptances for products, which reflect sensation to the human eye. The dried products are usually darker in colour, but darker colour does not mean better quality. Too dark may imply that the product is over dried. The advantage is that this parameter can be visually determined for assessing product quality (Satishkumar *et al.*, 2015).

Hunter's lab colourimeter (Premier colourscan, Colour Flex EZ; Mumbai, India) was used for the measurement of colour of fresh, dried and milled calyces of Roselle samples (Plate 9). The detailed specifications of Hunter's lab colourimeter are given in Appendix A6. The colour was measured by using CIELAB scale at 10° observer with D₆₅ illuminant. It works on the principle of focussing the light and measuring the energy reflected from the sample across the entire visible spectrum. It provides reading in terms of L^* , a^* and b^* where, luminance (L^*) forms the vertical axis, which indicates whiteness (+) to darkness (-). Similarly a^* indicates redness (+) to greenness (-) and b^* indicates yellowness (+) to blueness (-) (Manjula and Ramachandra, 2014).

The instrument was calibrated using black tile and white tile provided with the instrument. The fresh, dried and milled samples were filled in the sample cup. The deviation of the colour of the sample to the standard was also observed and recorded in the computer interface. The measurements were replicated thrice.

3.6.2 Bulk density

Bulk density includes loose bulk density and tapped bulk density. Loose bulk density is defined as the state obtained by pouring a powder sample into a vessel without any consolidation and tapped bulk density is defined as the state obtained when the vessel containing the powder sample is to be repeatedly dropped a specified distance at a constant drop rate until the apparent volume of sample in the vessel becomes almost constant.

The bulk density of Roselle powder obtained from different treatments were measured according to the procedure described by Caparino *et al.* (2012) and Lebrun *et al.* (2012). Approximately, 1 g of Roselle powder was freely poured into a 5 ml glass graduated cylinder (readable at 1ml) without tapping and disturbance and this was measured as loose bulk density of Roselle powder and the same sample was repeatedly tapped manually by lifting and dropping the cylinder under its own weight at a vertical distance of 14±2 mm until negligible difference in volumes between succeeding measurements was observed, and this was measured as tapped bulk density of Roselle powder.

Determination of loose bulk density and tapped bulk density calyces of Roselle powder samples was carried out at room temperature thrice and the average value was



Plate 9. Hunter's lab colourimeter

considered as loose bulk density and tapped bulk density of calyces of Roselle powder. The loose and tapped bulk density of calyces of Roselle powder was computed using the following expression;

$$\text{Loose bulk density (g.cc}^{-1}\text{)} = \frac{\text{Weight of powder (g)}}{\text{Bulk powder volume (cc)}} \quad \dots (3.20)$$

$$\text{Tapped bulk density (g.cc}^{-1}\text{)} = \frac{\text{Weight of powder (g)}}{\text{Tapped powder volume (cc)}} \quad \dots (3.21)$$

3.6.3 Flowability and cohesiveness of calyces of Roselle powder

The flowability of powders and their flow behaviour under pressure, temperature and humidity are important in handling and processing operations, such as storage in hoppers and silos, transportation, formulation and mixing, compression and packaging (Teunou *et al.*, 1999). The calyces of Roselle powders were evaluated for their flowability and cohesiveness in terms of Carr's Index (CI) and Hausner Ratio (HR), respectively. Both CI and HR were calculated from the loose and tapped bulk densities of Roselle powder, according to formula given by Olayemi *et al.* (2008). Table (2) illustrates the specifications for compressibility (Carr's) index and Hausner ratio (Lebrun *et al.*, 2012).

3.6.3.1 Carr's index (CI)

The compressibility index of the calyces of Roselle powder was determined by Carr's Index (Compressibility Index). It is a simple test to evaluate the Carr's index from loose bulk density and tapped bulk density of Roselle powder. The formula for Carr's index is as below;

$$\text{Carr's Index (\%)} = \frac{\text{Tapped bulk density (g.cc}^{-1}\text{)} - \text{Loose bulk density (g.cc}^{-1}\text{)}}{\text{Tapped bulk density (g.cc}^{-1}\text{)}} \times 100 \quad \dots (3.22)$$

3.6.3.2 Hausner ratio (HR)

The Hausner ratio is a number that is correlated to the flowability of calyces of Roselle powder. It was calculated by using the following expression;

$$\text{Hausner ratio} = \frac{\text{Tapped bulk density (g.cc}^{-1}\text{)}}{\text{Loose bulk density (g.cc}^{-1}\text{)}} \quad \dots (3.23)$$

Table 2. Specifications for Carr's index and Hausner ratio

Sl. No	Flowability	Carr's index (%)	Hausner ratio
1	Excellent	0-10	1.00-1.11
2	Good	11-15	1.12-1.18
3	Fair	16-20	1.19-1.25
4	Possible	21-25	1.26-1.34
5	Poor	26-30	1.35-1.45
6	Very poor	32-37	1.46-1.59
7	Very very poor	>38	>1.60

Source: Lebrun *et al.* (2012)

3.6.4 pH

The pH of fresh, dried and milled calyces of Roselle were measured by using digital pH meter (Systronics; 361). One gram powdered sample was weighed and it was dissolved in a 10 ml of distilled water in a beaker, then the electrode of the pH meter was dipped in the sample under test. The enter key was pressed to show the pH value and temperature of sample simultaneously. For next sample measurement, electrode was removed and washed properly with distilled water, then the above procedure was followed and reading was taken as the pH of the next sample. All the readings were taken in triplicate (Arab *et al.*, 2011).

3.6.5 Water activity

The water activity of fresh, dried and milled calyces of Roselle were measured by Rotronic hygrolab water activity analyzer (a_w -HP23). The detailed technical specifications of the Rotronic hygrolab water activity meter are given in Appendix A7. Before measuring the water activity of Roselle samples, the instrument was tested for its accuracy by measuring the water activity of the distilled water. If the water activity for distilled water recorded one, then the instrument was said to be calibrated. The sample under test was kept in sample cup (2 g of sample) which was provided with water activity meter. The sensor was placed on the sample cup by firmly closing in such a way that the air should not enter into the sample cup. The reading was directly displayed on the water activity meter and was taken as water activity of Roselle samples (Manjula and Ramachandra, 2014).

3.6.6 Titratable acidity

About 25 ml of filtrate was transferred into 125 ml conical flask. A 25 ml burette was filled with 0.1N NaOH and adjusted to zero. About two to three drops of phenolphthalein indicator was added to the conical flask containing the filtrate. The filtrate was titrated with 0.1N NaOH until indicator changed to pink and the volume was recorded. Titratable acidity was calculated as % citric acid (AOAC, 2000).

$$\% \text{ Titratable acidity} = A \times N \times 0.064 \times 100 \quad \dots (3.24)$$

where,

A = Average titre value

N = Normality of NaOH (0.1)

0.064 = Equivalent factor of citric acid

3.6.7 Ascorbic acid

Ascorbic acid was determined using the procedure described by Ashaye (2013). Standard indophenol's solution was prepared by dissolving 0.05 g of 2,6-dichloro indophenol in water diluted to 100 ml and filtered. To standardize, 0.053 g of ascorbic acid was dissolved in 90 ml of 20% metaphosphoric acid and diluted with water to 100 ml. Ten ml of this solution was pipetted into a small conical flask and titrated with indophenol's solution until a faint pink colour persists for 15 seconds. Two ml of the extracted juice from the calyces was pipetted into a conical flask and 5 ml of 20% metaphosphoric acid (as stabilizing agent) was added and made up to 10 ml mark with water. It was titrated with the indophenols solution a faint pink colour persists for 15 seconds. The vitamin content in the calyces was calculated using the following formula:

$$\text{Vitamin C (mg.100 g}^{-1}\text{)} = \frac{\text{Titre value} \times 0.212 \times 100}{\text{Weight of sample}} \quad \dots (3.25)$$

3.6.8 Solubility index

Solubility was measured according to the method of Arueya and Akomolafe (2014). Two and half gram of Roselle samples were dispensed into 25 ml of distilled water while taking care to break up any lumps using a glass rod. After 30 min of stirring, the dispersion was rinsed into tarred centrifuge tubes made up to 32.5 ml and then centrifuged at $3000 \times g$ for 10 min. The supernatant was then decanted and the weight of its solid content was determined after it had been evaporated to a constant weight. The Water solubility index (WSI) was then calculated by using following expression;

$$\text{WSI (g.g}^{-1}\text{)} = \frac{\text{Weight of dissolved solids in supernatant (g)}}{\text{Weight of dry Roselle sample (g)}} \quad \dots (3.26)$$

3.6.9 Particle size

Particle size of the milled calyces of Roselle powder and encapsulated Roselle anthocyanin powder was done by the method employed by Tee *et al.* (2012). The Zetasizer nano range (ZETA Sizer, nano383 issue 5.0, Malvern, England) of instruments provide the ability to measure three characteristics of particles or molecules in a liquid medium viz., the three fundamental parameters are particle size, zeta potential and molecular weight. By using Zetasizer system, three parameters can be measured over a wide range of concentrations. Zetasizer (nano series) was used in the study as dynamic light scattering apparatus. The range of particle size that can be determined is: 0.6 nm – 6 µm. The equipment requires the sample to be diluted. Computer system uses the Malvern software for control of the equipment and for analyzing the results and is shown in Plate 8. The detailed technical specifications of the Zetasizer nano series is given in Appendix A8.

The particle size measurements were performed on aqueous suspensions of encapsulated Roselle anthocyanin powder. The aqueous suspensions were prepared as follows: one gram of powder sample dispersed in 10 ml of distilled water. The suspension was stirred by the mechanical stirrer for at least 10 min in order to break powder agglomerates resulting fine, colloidal particles completely dispersed in water. A small portion of the sample was taken into a disposable cuvette and placed in the instrument for measurements and is depicted in Plate 10. Care should be taken to avoid air bubbles in the sample when filling the cuvette.

3.7 Functional properties of calyces of Roselle

The functional properties like total phenolic content, total flavonoids and total anthocyanin content are the most important groups of secondary metabolites and bioactive compounds in plants (Kim *et al.*, 2003). They are also a kind of natural product and antioxidant substance capable of scavenging free superoxide radicals, anti-aging and reducing the risk of cancer (Ghasemzadeh and Ghasemzadeh, 2011). The functional properties of Roselle include total phenolic content, antioxidant activity, total flavonoids and total anthocyanin content are explained below.

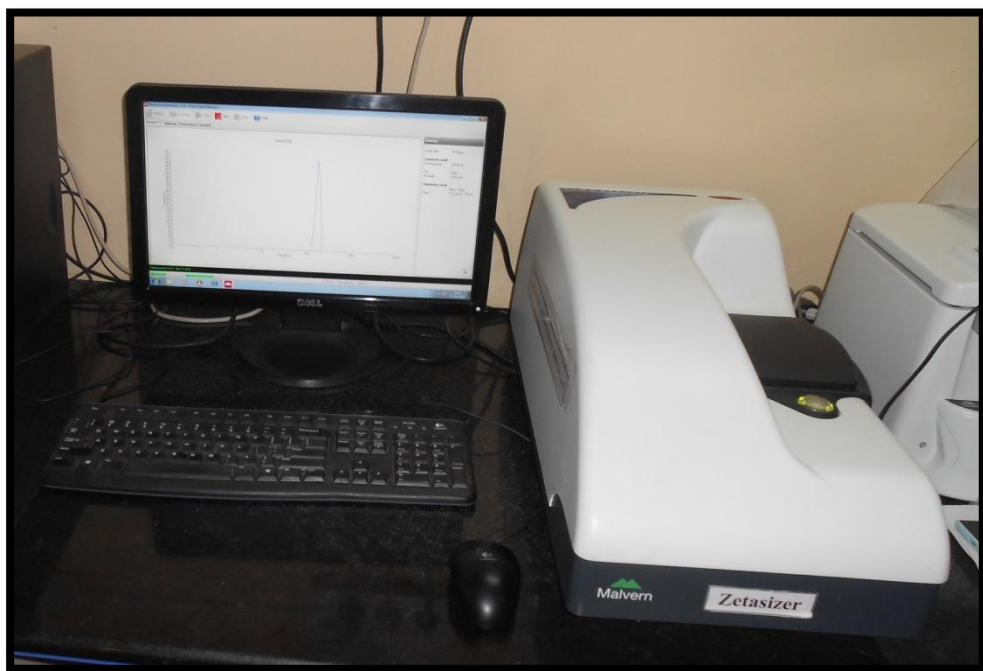


Plate 10. Zeta potential size analyser (Zetasizer)

3.7.1 Total phenolic content

The presence of phenolic compounds in medicinal plants is responsible for the antioxidant and anti-inflammatory activities of these species, allowing them to be used as potential chemopreventives (Sahu and Saxena, 2013).

The total phenol content in the extract of *Hibiscus sabdariffa* calyx was determined with Folin Ciocalteu reagent by the method described by Sirag *et al.* (2014). The crude extract (50 mg) was mixed with Folin Ciocalteu reagent (1ml) and deionized water (7.5 ml). The mixture was kept at room temperature for 5 minutes then 10 ml of 7% sodium carbonate was added to the mixture and incubated for 90 minutes at room temperature. After incubation, the absorbencies against the reagent blank were determined at 760 nm using UV/visible spectrophotometer (Plate 11). The technical specifications of spectrophotometer is given in Appendix A8. The total phenolic content of the plant was expressed as mg.g^{-1} Gallic acid equivalent. All samples were analyzed in triplicates.

3.7.2 Antioxidant activity

The production of free radicals and reactive oxygen species can induce many kinds of diseases. Nevertheless, natural antioxidants protect against these oxidative stresses and associated diseases. They have played an important role in the health care industry (Lopez *et al.*, 2007). Studies demonstrated that the calyx of Roselle (Mourtzinis *et al.*, 2008) was rich in phenolic compounds and anthocyanins. It was reported that those compounds could be considered as a great source of natural antioxidants (Yang *et al.*, 2012).

Antioxidant activity of extracts measured included radical-scavenging activity. The free radical scavenging capacity of the extracts was determined using the 1,1-diphenyl-1-picrylhydrazyl (DPPH) assay (Pacome *et al.*, 2014). The spray dried extract was mixed with methanol to prepare the stock solution (1 mg.ml^{-1}). Freshly prepared DPPH solution ($5.9 \text{ mg.100 ml}^{-1}$ methanol) was taken in test tubes and extracts were added followed by serial dilutions to every test tube such that the final volume was 2 ml, and discolouration was measured at 517 nm after incubation for 30 min in the dark at ambient temperature. Measurements were performed at least in triplicate.

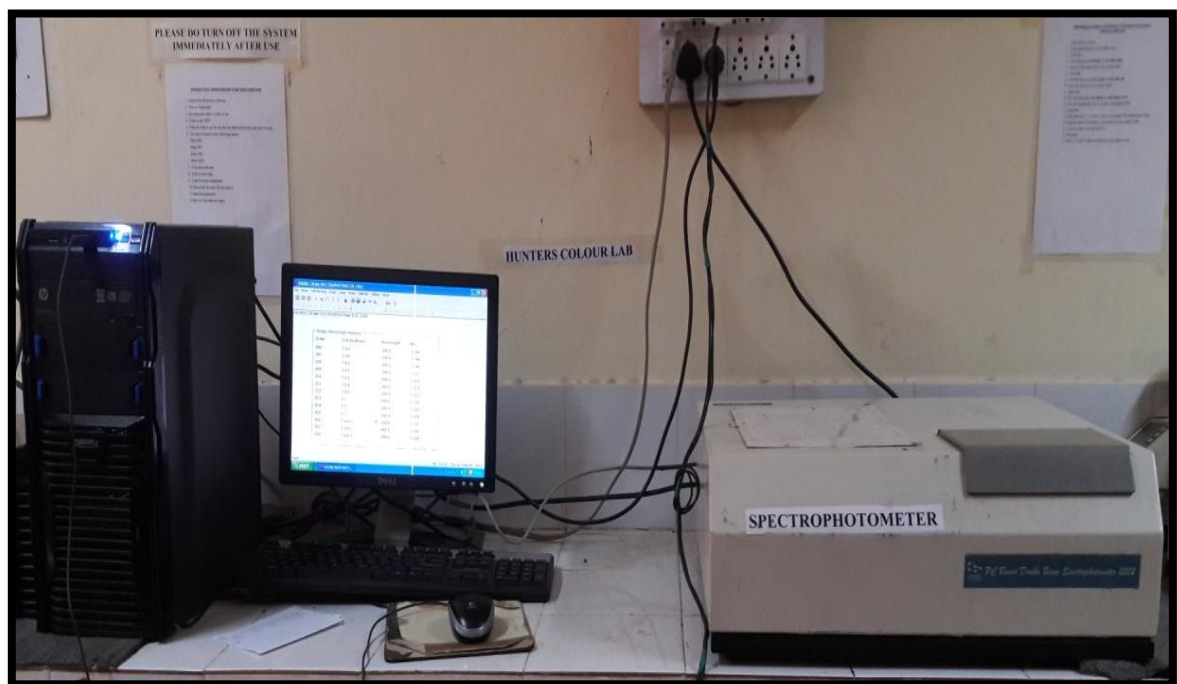


Plate 11. Spectrophotometer

Ascorbic acid, a well known antioxidant, was used as a reference standard and dissolved in methanol to bring the stock solution to the same concentration (1 mg.ml⁻¹). The control sample was prepared, which contained the same volume without any extract and methanol was used as the blank. The antioxidant activity based on inhibition ratio (per cent) was calculated according to the following equation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad \dots (3.27)$$

The actual decrease in absorption induced by the tested compounds was compared with the positive control.

3.7.3 Total flavonoids

Flavonoids are large family of polyphenolic components synthesized by plants. It was found that flavonoids functioned to reduce blood lipid and glucose and to enhance human immunity. Flavonoids are natural antioxidants capable of scavenging free superoxide radical, antiaging and reducing the risk of cancer (Ghasemzadeh and Ghasemzadeh, 2011).

Two and half grams of sample was placed in a Soxhlet extractor and refluxed with methanol for more than 12 h at 85 °C. The extract was evaporated to dryness in a rotary vacuum evaporator at less than 40 °C and then dissolved with methanol. Exactly, 0.3 ml of 5% NaNO₂ was added to a 1 ml extract in a 10 ml volumetric flask and the mixture was kept for 6 min at room temperature. Addition of 0.3 ml of 10% Al(NO₃)₃ to the mixture, which was incubated for 6 min again, was followed by addition of 4 ml of 1 N NaOH and of methanol, up to volume. After incubating for 15 min at room temperature for colour development, absorbance at 510 nm was measured. Total flavonoid content was expressed as rutin equivalents (Wang *et al.*, 2007).

3.7.4 Total anthocyanin content

Anthocyanins, the biggest group of water soluble natural pigments, are a sub-class of flavonoids. They give attractive colours of flowers, fruits (especially berries) and vegetables, as well as their products. Except as colourants, anthocyanins have multiple biological roles, e.g. antioxidant activity, anti-inflammatory action, inhibition of blood

platelet aggregation and antimicrobial activity, treatment of diabetic retinopathy and prevention of cholesterol induced atherosclerosis (Arslan, 2015).

Total anthocyanin content (TAC) of extract was determined using the method described by (Pacome *et al.*, 2014). Ten mg of extract was mixed in 5 ml of methanol acidified with trifluoroacetic acid 0.1% (v/v). Aliquots of the extracts were taken in a 10 ml glass tube and adjusted to a volume of 3 ml with methanol acidified with trifluoroacetic acid (TFA) and the absorbance was measured at 530 nm using a UV/Vis spectrophotometer against the blank sample containing the mixture methanol/TFA 0.1% without the sample extract, TAC was estimated as Cyanidin 3-O-glucoside at 530 nm using a molar extinction coefficient of 26,900 L/mol/cm) and molar mass (449 g.mol⁻¹) (Chaovanalikit and Wrolstad, 2004) and was expressed as mg Cyanidin-3-glucoside (mg Cya3G)/100 g of spray dried extract).

3.8 Experimental design for drying and milling of calyces of Roselle

All the experiments in the study were conducted in triplicate and mean values are reported. 3² factorial design was used to analyse the data. After proper analysis, data were accommodated in the tables as per the needs of objectives for interpretation of results. Statistical significance of the terms in the regression equation was examined by analysis of variance (ANOVA) for each response. The p-values were used as a tool to check the significance of each of the coefficients, which, in turn were necessary to understand the pattern of the mutual interactions between the test variables. The smaller the magnitude of the P, the more significant is the corresponding coefficient. Values of P less than 0.05 indicate that, the model terms are significant. The adequacy of regression model was checked by R² and Fisher's F-test (Montgomery, 2001).

The significance of all terms in the polynomial model was judged statistically by computing the F-values at a probability (P) of 0.01 or 0.05. A 2FI (2 factor interaction) model was employed to fit the data and adequacy of the model was tested considering R². The mathematical model is reliable with an R² value closer to 1 (The closer the R² value to 1, the better is the model fit to the experimental data). Coefficient of determination, R² is defined as the ratio of the explained variation to the total variation and is a measure of the degree of fit (Haber and Runyon, 1977).

It is also the proportion of the variability in the response variables, which is accounted for the quadratic analysis (Mc-Laren *et al.*, 1977). For any term in the models, a large F-value and a small P-value would indicate a more significant effect on respective response variables (Yuan *et al.*, 2008). The models provide several comparative measures for model selection. R^2 statistics, which give a correlation between the experimental response and the predicted response and it should be high for a particular model to be significant. Adjusted R^2 , which gives a similar correlation after ignoring the insignificant model terms, should have good agreement with predicted R^2 for the model to be fit (Gonnissen *et al.*, 2008).

The experimental design was done with the aid of the Design Expert software version 7.7.0 (Statease Inc., Minneapolis, USA) to identify optimum levels of two independent variables viz., drying methods and milling methods. Twenty three responses were selected as dependent variables such as moisture content, crude protein, crude fat, carbohydrate, crude fibre, total ash, L^* , a^* , b^* , loose bulk density, tapped bulk density, Carr's index, Hausner ratio, pH, water activity, titratable acidity, ascorbic acid, solubility index, particle size, total phenolic content, antioxidant activity, total flavonoids and total anthocyanin content were examined for dried and milled calyces of Roselle.

The experiments were designed according to the general factorial design. The order of experiments was fully randomised. The test of statistical significance was performed on the total error criteria, with a confidence level of 95%. The significant terms in the model were found by analysis of variance (ANOVA) for each response. The adequacy of the model was checked by calculating R^2 and adjusted R^2 value. The treatment combinations for drying and milling of calyces of Roselle are given in Table 3.

Table 3. Treatment combinations for drying and milling of calyces of Roselle

Sl. No.	Treatments	Drying method	Milling method
1	DM1	STD	NP
2	DM2	STD	WCP
3	DM3	STD	LN ₂ CP
4	DM4	SD	NP
5	DM5	SD	WCP
6	DM6	SD	LN ₂ CP
7	DM7	DD	NP
8	DM8	DD	WCP
9	DM9	DD	LN ₂ CP

SD = Shade drying; STD = Solar tunnel drying; DD = Dehumidified air drying

NP = Normal pulveriser; WCP = Water cooled pulveriser;

LN₂CP = Liquid nitrogen cooled pulveriser

3.9 Optimization of process parameters for drying and milling of calyces of Roselle

The numerical optimization technique was used for the simultaneous optimization of the multiple responses using Design Expert software version 7.7.0. The desired goals for each variable and response were chosen. The values of all the responses at operating conditions were converted to a desirability function. The desirability values of the minimum and maximum were configured as 0 and 1, respectively. All of the independent variables were kept within range, while the responses were either maximized or minimized. Numerical optimization was applied for dried and milled fresh calyces of Roselle on the basis of proximate composition, physico-chemical properties and functional properties of Roselle.

The quadratic response surface analysis was based on multiple linear regressions taking into account linear, quadratic and interaction effects according to the equation below:

$$Y = b_0 + \sum a_i x_i + \sum a_{ij} x_i x_j + \sum a_{ii} x_i^2 \quad \dots (3.28)$$

Where Y is the response value predicted by the model; b_0 is offset value, a_i , a_{ij} and a_{ii} are main (linear), interaction and quadratic coefficients, respectively. The adequacy of the models was determined using model analysis; lack-of-fit test and coefficient of determination (R^2) analysis. For model to be suited, R^2 should be at least 0.80 for a good fitness of a response model (Mirhosseini *et al.*, 2009).

3.9.1 Verification of predicted and actual responses

To verify the predicted and actual responses of dried and milled fresh calyces of Roselle, the mean relative per cent deviation modulus was calculated by using the following relation:

$$\text{Per cent relative deviation} = \frac{100}{N} \sum_{i=1}^N \frac{|e_i - p_i|}{p_i} \quad \dots (3.29)$$

where,

N = Total number of observations

e_i = Experimental value

p_i = Predicted value

3.10 Experimental setup for the extraction of anthocyanin from calyces of Roselle

Anthocyanins are water soluble plant pigments, abundantly consumed by humans, which belong to the class of flavonoids and which display a wide range of beneficial properties based on their free-radical scavenging and antioxidant capacities (Oancea *et al.*, 2013). Hence the study was undertaken to extract crude anthocyanin from calyces of Roselle and the detailed procedure is followed here under.

3.10.1 Solvent extraction using ethanol (Control)

One hundred grams of coarsely powdered calyces of *Hibiscus sabdariffa* were extracted by maceration using ethanol (70%) in a conical flask for 72 h in dark, filtered and evaporated by a rotary vacuum flash evaporator at 40 °C. The resulting solution was stored in a refrigerator (4±2 °C) until use (Sirag *et al.*, 2014).

3.10.2 Solvent extraction using ethanol (50%) and citric acid (50%)

Roselle samples were extracted with solvent according to Arab *et al.* (2011). Fifty gram of ground dried calyces were thoroughly mixed with a suitable amount of solvent in volumetric flask and incubated overnight in the refrigerator at 4 °C. The soluble extract was filtered using Whatman No. 1 filter paper to obtain Roselle pigment extract. Extract was then concentrated using rotary flash evaporator at 40 °C (Plate 12). The detailed technical specifications of the rotary vacuum flash evaporator are given in Appendix A10. Extract was collected in plastic vials and stored at 4 °C till the further analysis. The process flow chart for anthocyanin extraction from calyces of Roselle is given in Fig. 2.

3.11 Physico-chemical analysis of extracted anthocyanin from calyces of Roselle

The determination of physico-chemical characteristics viz., extraction yield, pH, total soluble solids and colour of extracted anthocyanin from calyces of Roselle are explained below.

3.11.1 Extraction yield (%)

The extraction yield was calculated by using the following equation (Ho *et al.*, 2011).

$$\text{Yield (\%)} = \frac{\text{Weight of extract (g)}}{\text{Weight of sample (g)}} \times 100 \quad \dots (3.30)$$

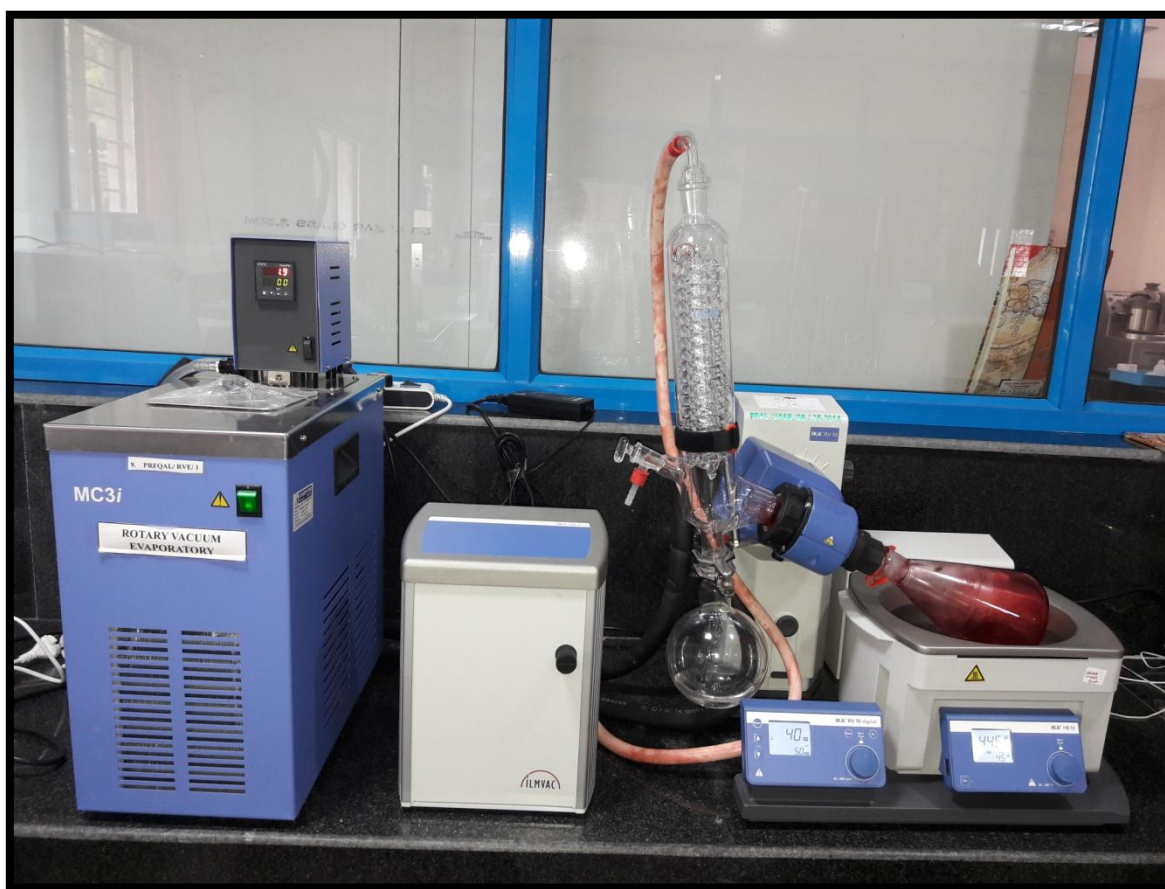


Plate 12. Rotary vacuum flash evaporator used for evaporation of solvent

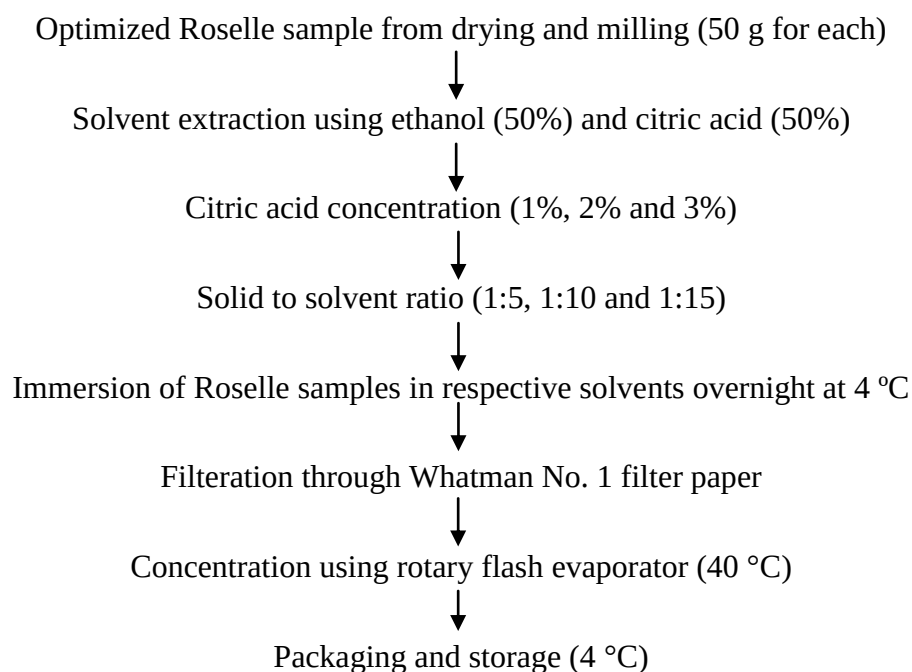


Fig. 2. Process flow chart for extraction of anthocyanin from calyces of Roselle

3.11.2 pH

The pH of extracted samples were measured by using digital pH meter (Systronics; 361) and the detailed procedure is explained in Section 3.6.4.

3.11.3 Total soluble solids

The total soluble solids (TSS) (°Brix) of extracted samples were determined using hand refractometer according to AOAC (2000) at room temperature (25±1 °C) expressed as °Brix (0 - 90). The samples were analysed in triplicate.

3.11.4 Colour

Colour values of L^* , a^* and b^* were measured by using Hunters lab colourimeter and the detailed procedure is explained in Section 3.6.1.

3.11.5 Titratable acidity

Titrateable acidity was calculated as % citric acid according to AOAC (2000) method and the detailed procedure is explained in Section 3.6.6.

3.12 Functional properties of extracted anthocyanin from calyces of Roselle

The functional properties of extracted anthocyanin from calyces of Roselle include total phenolic content, antioxidant activity, total flavonoids and total anthocyanin content are explained below.

3.12.1 Total phenolic content

The total phenolic content of extracted samples was determined with Folin Ciocalteu reagent by the method described by Sirag *et al.* (2014) and the detailed procedure is explained in Section 3.7.1.

3.12.2 Antioxidant activity

Antioxidant activity of extracts measured included radical scavenging activity. The free radical scavenging capacity of the extracts was determined using the 1,1-diphenyl-1-picrylhydrazyl (DPPH) assay by the method described by Pacome *et al.* (2014) and the detailed procedure is explained in Section 3.7.2.

3.12.3 Total flavonoids

Total flavonoids content (TFC) of extract was determined using the method described by Wang *et al.* (2007) and the detailed procedure is explained in Section 3.7.3.

3.12.4 Total anthocyanin content

Total anthocyanin content of extract was determined using the method described by Pacome *et al.* (2014) and the detailed procedure is explained in Section 3.7.4.

3.13 Experimental design for extraction of anthocyanin from calyces of Roselle

All the experiments in the study were conducted in triplicate and the mean values were reported. Factorial completely randomised design (FCRD) was used to analyse the data. After proper analysis, data were accommodated in the tables as per the needs of objectives for interpretation of results. Statistical significance of the terms in the quadratic equation was examined by analysis of variance (ANOVA) for each response. The p-values were used as a tool to check the significance of each of the coefficients, which, in turn were necessary to understand the pattern of the mutual interactions between the test variables. The smaller the magnitude of P, the more significant is the corresponding coefficient. Values of P less than 0.05 indicate the model terms to be significant. The adequacy of regression model was checked by R^2 and Fisher's F-test (Montgomery, 2001).

The significance of all terms in the polynomial were judged statistically by computing the F-values at a probability (P) of 0.01 or 0.05. A complete second order quadratic model was employed to fit the data and adequacy of the model was tested considering R^2 (the coefficient of multiple determination, a measure of the amount of variation around the mean explained by the model). Coefficient of determination, R^2 is defined as the ratio of the explained variation to the total variation and is a measure of the degree of fit (Haber and Runyon, 1977).

It is also the proportion of the variability in the response variables, which is accounted for the quadratic analysis (Mc-Laren *et al.*, 1977). For any term in the models, a large F-value and a small P-value would indicate a more significant effect on respective response variables (Yuan *et al.*, 2008). The quadratic coefficients were then used to make statistical calculation to generate three-dimensional plots from the quadratic model. The models provide several comparative measures for model selection. R^2 statistics, which

give a correlation between the experimental response and the predicted response and it, should be high for a particular model to be significant. Adjusted R^2 , which gives a similar correlation after ignoring the insignificant model terms, should have good agreement with predicted R^2 for the model to be fit (Gonnissen *et al.*, 2008).

The experimental design was done with the aid of the Design Expert software version 7.7.0 (Statease Inc., Minneapolis, USA) to identify optimum levels of two independent variables viz., citric acid concentration and solvent to solid ratio. Eleven responses were selected as dependent variables such as extraction yield, pH, total soluble solids, L^* , a^* , b^* , titratable acidity, total phenolic content, total flavonoids, antioxidant activity and total anthocyanin content were examined for extracted anthocyanin from calyces of Roselle.

The experiments were designed according to the general factorial design. The order of experiments was fully randomised. The test of statistical significance was performed on the total error criteria, with a confidence level of 95%. The significant terms in the model were found by analysis of variance (ANOVA) for each response. The adequacy of the model was checked by calculating R^2 and adjusted R^2 value. Treatment combinations for extraction of anthocyanin of calyces of Roselle is given in Table 4.

Table 4. Treatment combinations for extraction of anthocyanin from calyces of Roselle

Sl. No.	Treatments	Citric acid concentration	Solid to solvent ratio
1	E1	1%	1:5
2	E2	2%	1:5
3	E3	3%	1:5
4	E4	1%	1:10
5	E5	2%	1:10
6	E6	3%	1:10
7	E7	1%	1:15
8	E8	2%	1:15
9	E9	3%	1:15

3.14 Optimization of solvent extraction of anthocyanin from calyces of Roselle

The numerical optimization technique was used for the simultaneous optimization of the multiple responses using Design Expert software version 7.7.0. The desired goals for each variable and response were chosen. The values of all the responses at operating conditions were converted to a desirability function. The desirability values of the minimum and maximum were configured as 0 and 1, respectively. All of the independent variables were kept within range, while the responses were either maximized or minimized. Numerical optimization was applied for extracted anthocyanin of calyces of Roselle using different citric acid concentration and solvent to solid ratio on the basis of physico-chemical properties and functional properties of anthocyanin of calyces of Roselle.

The quadratic response surface analysis was based on multiple linear regressions taking into account linear, quadratic and interaction effects according to the equation below:

$$Y = b_0 + \sum a_i x_i + \sum a_{ij} x_i x_j + \sum a_{ii} x_i^2 \quad \dots (3.31)$$

where Y is the response value predicted by the model; b_0 is offset value, a_i , a_{ij} and a_{ii} are main (linear), interaction and quadratic coefficients, respectively. The adequacy of the models was determined using model analysis; lack-of-fit test and coefficient of determination (R^2) analysis. For model to be suited, R^2 should be at least 0.80 for a good fitness of a response model (Mirhosseini *et al.*, 2009).

3.14.1 Verification of predicted and actual responses

To verify the predicted and actual responses of extracted anthocyanin of calyces of Roselle, the mean relative per cent deviation modulus was calculated by using the following relation:

$$\text{Per cent relative deviation} = \frac{100}{N} \sum_{i=1}^N \frac{|e_i - p_i|}{p_i} \quad \dots (3.32)$$

where,

N = Total number of observations

e_i = Experimental value

p_i = Predicted value

3.15 Experimental setup for encapsulation of anthocyanin by spray drying

Spray drying is a technique that has been utilized to protect flavour components from destructive changes and to convert flavour into a free flowing form. It is employed to preserve the stability, bioactivity and bioavailability of active components. The process allows sensitive ingredients to be blended or homogenised in a solution which contains macromolecules and emulsifiers to form a stable emulsion. Encapsulating agents are used exclusively or in association with other encapsulating agents to achieve an ideal composition (Brown *et al.*, 2016).

The optimized anthocyanin extract obtained from solvent extraction was considered for the encapsulation. The carrier materials were analytical grade [Gum Arabica, Maltodextrin and Whey protein obtained from M/s Sigma-Aldrich Canada Ltd]. The anthocyanin extract and carrier agents (Gum Arabica, Maltodextrin and Whey protein) were taken in the ratio of 1:2. One drop of Tween-80 was also added to enhance the emulsifying and film forming properties. The resultant solution was then mixed in a shear homogenizer for 5 minutes at 3000 rpm until complete dispersion of colour extract was attained. The mixture were then spray dried in a pilot model of vertical type co-current spray dryer (SM Scientech, Kolkata, India) with a water evaporating capacity of 1000 ml/h equipped with a rotary wheel atomizer operated at 18000 rpm and atomization flow of 2 kg.cm⁻². The feed rate was adjusted to the rate of 7.5 ml.min⁻¹. The inlet air temperature (150, 160 and 170 °C) and the out let temperature of 55±2 °C (Bandera *et al.*, 2015). The hot air supplied at the rate of 110 kg.h⁻¹ provided the latent heat of evaporation and the evaporation of the solvent *i.e.*, water, consequently led to the formation of the microcapsules. The spray dryer used in the present investigation is shown in Plate 13. The microcapsules collected from the collecting chamber were then filled in aluminium foil pouches, sealed air tight and stored at 4 °C. The technical specifications of spray dryer are given in Appendix A11. Process flow chart for microencapsulation of Roselle anthocyanin is given in Fig. 3.



Plate 13. Spray dryer used for encapsulation of anthocyanin from calyces of Roselle

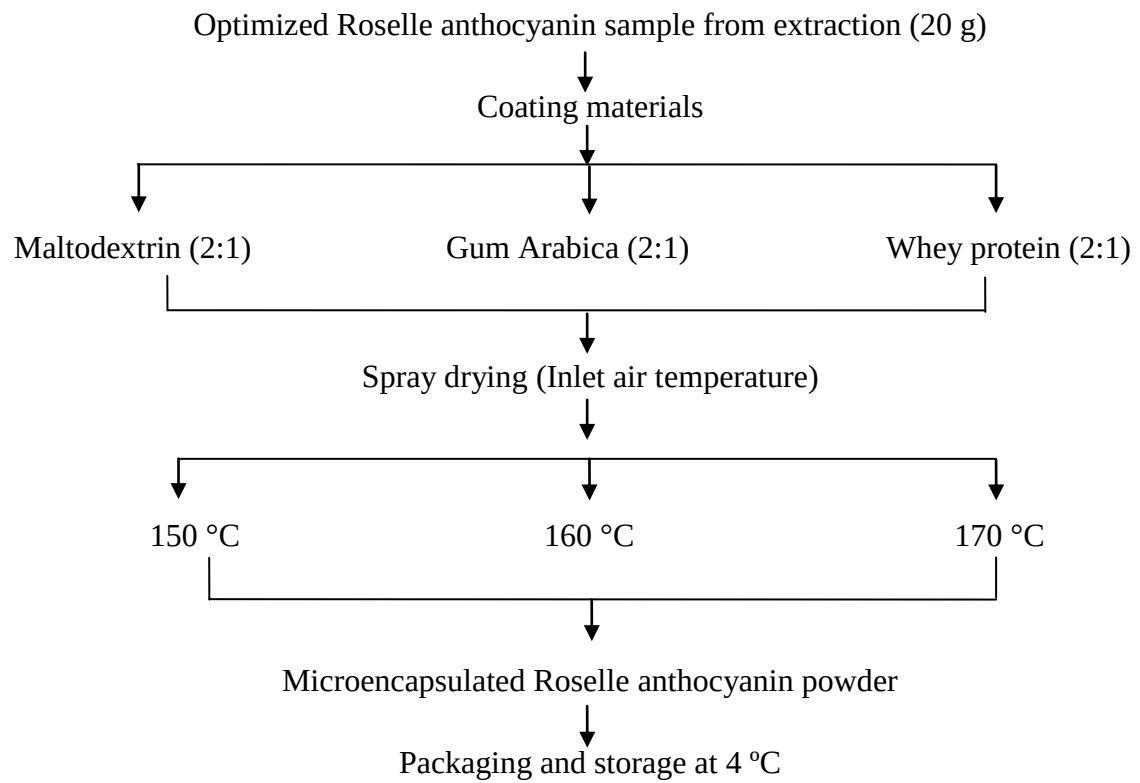


Fig. 3. Process flow chart for microencapsulation of Roselle anthocyanin

3.16 Physico-chemical analysis of microencapsulated Roselle anthocyanin powder

The determination of physico-chemical characteristics viz., powder yield, moisture content, water activity, pH, titratable acidity, colour, density, particle size and morphology of microencapsulated Roselle anthocyanin powder are explained below.

3.16.1 Powder yield

The powder yield was determined by measuring the total solid content of the resulting powder and the total solid content in the feed mixture. The relationship between them measures the powder yield of a material (Tee *et al.*, 2012).

$$\text{Powder yield (\%)} = \frac{\text{Total solid content in powder}}{\text{Total solid content in feed mixture}} \times 100 \quad \dots (3.33)$$

3.16.2 Moisture content

Moisture content of microencapsulated Roselle anthocyanin powder was determined using the method AOAC (2000) and the detailed procedure is explained in Section 3.6.1.

3.16.3 Water activity

The water activity of microencapsulated Roselle anthocyanin powder was measured by Rotronic Hygrolab water activity analyzer (Manjula and Ramachandra, 2014) and the detailed procedure is explained in Section 3.6.5.

3.16.4 pH

The pH of microencapsulated Roselle anthocyanin powder was measured by using digital pH meter (Systronics; 361) and the detailed procedure is given in Section 3.6.4.

3.16.5 Titratable acidity

The titratable acidity of microencapsulated Roselle anthocyanin powder was measured by using the method AOAC (2000) and the detailed procedure is given in Section 3.6.6.

3.16.6 Colour

Colour values namely L^* , a^* and b^* of microencapsulated Roselle anthocyanin powder were measured by using Hunters lab colourimeter (Manjula and Ramachandra, 2014) and the detailed procedure followed is explained in Section 3.6.1.

3.16.7 Bulk density

The bulk density of microencapsulated Roselle anthocyanin powder obtained from different treatments were measured according to the procedure described by Caparino *et al.* (2012) and Lebrun *et al.* (2012) and the detailed procedure is given in Section 3.6.2.

3.16.8 Flowability and cohesiveness of microencapsulated Roselle anthocyanin powder

The flowability and cohesiveness of microencapsulated Roselle anthocyanin powder obtained from different treatments were measured in terms of Carr's index and Hausner ratio according to formula given by Olayemi *et al.* (2008) and the detailed procedure is given in Section 3.6.3.

3.16.9 Particle size analysis

Particle size of microencapsulated Roselle anthocyanin powder was measured according to the method employed by Tee *et al.* (2012) and the detailed procedure is given in Section 3.6.9.

3.16.10 Scanning electron microscopy (SEM)

The SEM (Scanning Electron Microscope) is an instrument that produces a largely magnified image by using electrons instead of light to form an image. A beam of electrons is produced at the top of the microscope by an electron gun. The electron beam follows a vertical path through the microscope, which is held within a vacuum. The beam travels through electromagnetic fields and lenses, which focus the beam down toward the sample. Once the beam hits the sample, electrons and X-rays are ejected from the sample. Detectors identify these X-rays, backscattered electrons and secondary electrons will convert them into a signal that is sent to a screen, this produces the final image

(Linna *et al.*, 2014). SEM analysis of encapsulated Roselle powder was conducted at the centre for Nano technology, CAE, UAS, Raichur.

The size and morphology of the microcapsules produced with the different encapsulating agents at different temperature were evaluated by scanning electron microscopy (SEM) according to the modified methodology described by Silva *et al.* (2013). To obtain SEM images, 10 mg of powder were taken from well mixed powder samples. For examining their outer structure, microcapsules were attached to SEM stubs using a two sided adhesive tape (Ted Pella, Redding, CA) and for analyzing the inner structure; microcapsules were fractured by moving a doctor blade perpendicularly through a layer of capsules attached to the specimen holder by a two-sided adhesive tape. Gold: palladium coating of 60:40 ratio was carried out in a sputter coater under a low vacuum in the presence of inert gas. Sputter coater is shown in Plate 14. Scanning electron microscope (Carl Zeiss) was used for the observation. SEM was operated under low vacuum mode with working distance (WD) of 9 mm using SE (secondary electron) detector with 0-30 kV (EHT-Extra High Tension) and scan speed of 7 under the LAB 6 aperture with HV (high vacuum) mode. Samples were systematically observed at 1000×, 3000×, 5000×, 7000× and 10,000× magnification (Silva *et al.*, 2013). Scanning electron microscope is shown in Plate 15. Technical specifications of SEM are given in Appendix A12.



Plate 14. Sputter coater of gold-palladium for microencapsulated Roselle anthocyanin powder



Plate 15. Scanning electron microscope for morphology of microencapsulated Roselle anthocyanin powder

3.17 Reconstitution properties of microencapsulated Roselle anthocyanin powder

The reconstitution properties of microencapsulated Roselle anthocyanin powder are very important criteria for its acceptability. Reconstitution properties such as solubility, wettability and dispersibility of spray dried Roselle anthocyanin powder were determined using standard procedures as explained below.

3.17.1 Solubility

The microencapsulated Roselle anthocyanin powder has to be soluble in water. However, not all components in the powder are soluble when reconstituted in water. The solubility of the product was measured according to the method reported (Shittu and Lawal, 2007; Zhang *et al.*, 2013). One gram of (m_1) spray dried Roselle anthocyanin powder was put into a 50 ml beaker and then 10 ml distilled water (30 ± 2 °C) was added. The suspension was stirred intermittently for 30 min before it was finally centrifuged at 9000 rpm for 10 min and then the supernatant was completely drained into an evaporating dish and dried to constant weight in a hot air oven at 105 ± 2 °C for 4 h. The samples were removed from the oven, cooled in a desiccator and weighed as m_2 . The drying and weighing processes were repeated until constant weight was obtained. Determination of solubility was carried out thrice for spray dried Roselle anthocyanin powder and the average value was considered as solubility of spray dried Roselle anthocyanin powder. The solubility was calculated using the following expression:

$$\text{Solubility (\%)} = \frac{m_2}{m_1} \times 100 \quad \dots (3.34)$$

where,

m_1 = Initial weight of sample, g

m_2 = Final weight of sample, g

3.17.2 Wettability

The wettability is characterized as the rehydration ability of a powder in water. The capacity of the microcapsules to mix with water is one of the most important reconstitution properties (Fernandes *et al.*, 2013)

Wettability of the microencapsulated Roselle anthocyanin powder was determined using the method of Fuchs *et al.* (2006) with some modifications. The powder samples (0.1 g) were sprinkled over the surface of 100 ml of distilled water at 20 °C (water bath) in a 200 ml beaker without agitation. The time it took until the last powder particles submerged was recorded and used for a relative comparison of the extent of wettability between the samples.

3.17.3 Dispersibility

Dispersibility is the ability to disperse in water by gentle stirring. This means the microencapsulated Roselle anthocyanin powder should integrate into single primary particles. The dispersibility of microencapsulated Roselle anthocyanin powder was measured according to the method reported by (Jaya and Das 2004; Fonseca *et al.*, 2011). One gram of microencapsulated Roselle anthocyanin powder was stirred with 10 ml of water at 30±2 °C in 50 ml beaker. Stirring was carried out to make 25 complete back and forth movements across the whole diameter of the beaker for 15 s. The reconstituted microencapsulated Roselle anthocyanin powder was then poured through the sieve of 150 μ size (Taylor series 100 mesh). The dry matter of the filtered spray dried Roselle anthocyanin powder was estimated by measuring its moisture content using an oven at 105 °C for 7 h. Determination of dispersibility was carried out thrice for spray dried Roselle anthocyanin powder and the average value was considered dispersibility of spray dried Roselle anthocyanin powder. The dispersibility (DS) was calculated using the following expression;

$$\text{Dispersibility (\%)} = \frac{(w + a)S_p}{aS_j} \quad \dots (3.35)$$

where,

a = Amount of powder used, g

w = Weight of water taken for reconstitution, g

S_p = Total solid present in encapsulated Roselle anthocyanin powder, %

S_j = Per cent dry matter present in encapsulated Roselle anthocyanin powder after it has been passed through the sieve

3.18 Functional properties of microencapsulated Roselle anthocyanin powder

The functional properties of microencapsulated Roselle anthocyanin powder include total phenolic content, antioxidant activity, total flavonoids and total anthocyanin content are explained below.

3.18.1 Total phenolic content

The total phenolic content of microencapsulated Roselle anthocyanin powder was determined with Folin Ciocalteu reagent by the method described by Sirag *et al.* (2014) and the detailed procedure is given in Section 3.7.1.

3.18.2 Antioxidant activity

Antioxidant activity of microencapsulated Roselle anthocyanin powder measured included radical-scavenging activity. The free radical scavenging capacity of the extracts was determined using the 1,1-diphenyl-1-picrylhydrazyl (DPPH) assay by the method described by Pacome *et al.* (2014) and the detailed procedure is given in Section 3.9.2.

3.18.3 Total flavonoids

Total flavonoids content (TFC) of microencapsulated Roselle anthocyanin powder was determined using the method described by Wang *et al.* (2007) and the detailed procedure is explained in Section 3.7.3.

3.18.4 Total anthocyanin content

Total anthocyanin content of microencapsulated Roselle anthocyanin powder was determined using the method described by Pacome *et al.* (2014) and the detailed procedure is given in Section 3.7.4.

3.19 Experimental design for microencapsulation of Roselle anthocyanin

All the experiments in the study were conducted in triplicate and mean values were reported. Factorial completely randomised design (FCRD) was used to analyse the data. After proper analysis, data were accommodated in the tables as per the needs of objectives for interpretation of results. Statistical significance of the terms in the quadratic equation was examined by analysis of variance (ANOVA) for each response. The p-values were used as a tool to check the significance of each of the coefficients, which, in turn were necessary to understand the pattern of the mutual interactions between the test variables. The smaller the magnitude of the P, the more significant is the

corresponding coefficient. Values of P less than 0.05 indicate the model terms to be significant. The adequacy of regression model was checked by R^2 and Fisher's F-test (Montgomery, 2001).

The significance of all terms in the polynomial were judged statistically by computing the F-values at a probability (P) of 0.01 or 0.05. A complete second order quadratic model was employed to fit the data and adequacy of the model was tested considering R^2 (the coefficient of multiple determination, a measure of the amount of variation around the mean explained by the model). Coefficient of determination, R^2 is defined as the ratio of the explained variation to the total variation and is a measure of the degree of fit (Haber and Runyon, 1977).

It is also the proportion of the variability in the response variables, which is accounted for the quadratic analysis (Mc-Laren *et al.*, 1977). For any term in the models, a large F-value and a small P-value would indicate a more significant effect on respective response variables (Yuan *et al.*, 2008). The quadratic coefficients were then used to make statistical calculation to generate three-dimensional plots from the quadratic model. The models provide several comparative measures for model selection. R^2 statistics, which give a correlation between the experimental response and the predicted response and it, should be high for a particular model to be significant. Adjusted R^2 , which gives a similar correlation after ignoring the insignificant model terms, should have good agreement with predicted R^2 for the model to be fit (Gonnissen *et al.*, 2008).

The experimental design was done with the aid of the Design Expert software version 7.7.0 (Statease Inc., Minneapolis, USA) to identify optimum levels of two independent variables viz., coating materials and inlet air temperature. Twenty responses were selected as dependent variables such as powder yield, moisture content, water activity, pH, titratable acidity, L^* , a^* , b^* , loose bulk density, tapped bulk density, Carr's index, Hausner ratio, particle size, solubility, wettability, dispersibility, total phenolic content, antioxidant activity, total flavonoids and total anthocyanin content and were examined for spray dried anthocyanin of calyces of Roselle.

The experiments were designed according to the general factorial design. The order of experiments was fully randomised. The test of statistical significance was performed on the total error criteria, with a confidence level of 95%. The significant terms in the model were found by analysis of variance (ANOVA) for each response. The

adequacy of the model was checked by calculating R^2 and adjusted R^2 values. The treatment combinations for the production of microencapsulated Roselle anthocyanin powder are given in Table 5.

3.20 Optimization of process parameters for microencapsulation of Roselle anthocyanin

The numerical optimization technique was used for the simultaneous optimization of the multiple responses using Design Expert software version 7.7.0. The desired goals for each variable and response were chosen. The values of all the responses at operating conditions were converted to a desirability function. The desirability values of the minimum and maximum were configured as 0 and 1, respectively. All of the independent variables were kept within range, while the responses were either maximized or minimized. Numerical optimization was applied for encapsulation of anthocyanin of calyces of Roselle using different coating materials and with inlet air temperature on the basis of physico-chemical properties and functional properties of encapsulated anthocyanin of calyces of Roselle.

The quadratic response surface analysis was based on multiple linear regressions taking into account linear, quadratic and interaction effects according to the equation below:

$$Y = b_0 + \sum a_i x_i + \sum a_{ij} x_i x_j + \sum a_{ii} x_i^2 \quad \dots (3.36)$$

Where Y is the response value predicted by the model; b_0 is offset value, a_i , a_{ij} and a_{ii} are main (linear), interaction and quadratic coefficients, respectively. The adequacy of the models was determined using model analysis; lack-of-fit test and coefficient of determination (R^2) analysis. For model to be suited, R^2 should be at least 0.80 for a good fitness of a response model (Mirhosseini *et al.*, 2009).

Table 5. Treatment combinations for production of microencapsulated Roselle anthocyanin powder

Sl. No.	Treatment	Coating materials	Temperature
1	M1	Maltodextrin	150 °C
2	M2	Maltodextrin	160 °C
3	M3	Maltodextrin	170 °C
4	M4	Gum Arabica	150 °C
5	M5	Gum Arabica	160 °C
6	M6	Gum Arabica	170 °C
7	M7	Whey Protein	150 °C
8	M8	Whey Protein	160 °C
9	M9	Whey Protein	170 °C

3.20.1 Verification of predicted and actual responses

To verify the predicted and actual responses of encapsulated Roselle anthocyanin powder, the mean relative per cent deviation modulus was calculated by using the following relation:

$$\text{Per cent relative deviation} = \frac{100}{N} \sum_{i=1}^N \frac{|e_i - p_i|}{p_i} \quad \dots (3.37)$$

where,

N = Total number of observations

e_i = Experimental value

p_i = Predicted value

3.21 Stability of anthocyanin

Anthocyanins are an important group of water soluble plant pigments commonly found in various fruits and vegetables. Several factors influences the pigment stability such as species, environmental and agronomic conditions; extraction and processing parameters such as pH, storage temperature, concentration, chemical structure, light, oxygen, proteins, ascorbic acid, sugars, sulfites, enzymes and metallic ions. Among these factors, pH and temperature are major factors significantly influenced the pigment colour variations and stability (Aishah *et al.*, 2013). Therefore different pH and temperature were studied for stability of Roselle anthocyanin and the detailed procedure is explained here under.

3.21.1 Degradation of anthocyanin

The influence of key factors, pH and temperature on the stability of anthocyanins was studied. In the pH study, buffers at five pH values (2.0, 3.0, 4.0, 5.0, 6.0 and 7.0) were measured by a pH meter and prepared as follows: pH=2.0 (67.0 mL of 0.2 mol/L of hydrochloric acid and 25.0 mL of 0.2 mol/L of KCl), pH=3.0 (20.55 mL of 0.2 mol/L of Na_2HPO_3 and 79.45 mL of 0.1 mol/L of citric acid), pH=5.0 (51.50 mL of 0.2 mol/L of Na_2HPO_3 and 48.50 mL of 0.1 mol/L of citric acid), pH=6.0 (82.35 mL of 0.2 mol/L of Na_2HPO_3 and 17.65 mL of 0.1 mol/L of citric acid), and pH=7.0 (100 mL of 0.04 mol/L of barbituric acid and 1.65 mL of 0.2 mol/L of hydrochloric acid). The extract of anthocyanins was dissolved in different pH buffers (5 mg per 5 mL) in separate

volumetric flasks and placed for 10 days at room temperature under the same conditions. The absorbance of each extract was detected every day at the same time using a UV/VIS spectrophotometer.

In the study of stability of anthocyanin at different temperature, anthocyanin solutions were prepared as follows: a mass of 20 mg of anthocyanin was dissolved in 10 ml of citric acid/Na₂HPO₃ buffer (pH=2.0). The samples were diluted to appropriate concentrations and the initial absorbance was taken. In order to study the effect of temperature on the stability of anthocyanins, diluted samples were placed at room temperature, and at temperatures of 5, 20, 35, 50, 65 and 80 °C for 2 h. Then the absorbance was determined at 30 minute intervals at 535 nm (Reshmi *et al.*, 2012).

The amount of the remaining anthocyanins was calculated using the following formula:

$$\text{Pigment retention (\%)} = \left(\frac{\text{Anthocyanin content after treatment}}{\text{Anthocyanin content before treatment}} \right) \times 100$$

... (3.38)

3.22 Economics for drying, milling, extraction and microencapsulation of Roselle anthocyanin

The cost of production of drying (dehumidified drying, solar tunnel drying and shade drying), milling (normal pulverizer, water cooled pulverizer and LN₂ cooled pulverizer), solvent extraction (ethanol + citric acid) and microencapsulation by spray drying (maltodextrin) was estimated by considering the fixed, variable costs and other related costs. The costs of operation were determined by estimating the fixed cost and variable cost. The following assumptions and relationships were used for calculating the cost of various drying methods, milling methods, solvent extraction and microencapsulation techniques for Roselle anthocyanin. The fixed cost was calculated by using the following relationship.

Fixed cost of the equipment/year = Fixed cost × Capital recovery factor

where,

Capital Recovery Factor (CRF) was derived by using the equation

$$CRF = \frac{R_i \times (1+R_i)^n}{(1+R_i)^n - 1} \quad \dots (3.39)$$

where,

R_i = Existing rate of interest for long term (11.5%) bank loans, per cent

n = Life span of the equipment, years

The variable costs, which were incurred on wages, electricity charges, repairs and maintenance, raw materials, *etc.*, were calculated by collecting data during the operation of equipment and assuming certain data reasonably wherever necessary.

*Experimental Results*__

IV. EXPERIMENTAL RESULTS

The results of the present investigation entitled “Studies on extraction and encapsulation of anthocyanin from calyces of Roselle (*Hibiscus sabdariffa* L.)” conducted at the Department of Processing and Food Engineering, College of Agricultural Engineering, University of Agricultural Sciences, Raichur, Karnataka during the year 2015-17 are presented in this Chapter.

The present study was undertaken to standardize the drying, milling, extraction and encapsulation methods on quality of calyces of Roselle anthocyanin powder. The results of investigations on physico-chemical properties, proximate composition and functional properties of fresh Roselle and dried calyces of Roselle powder and physico-chemical and functional properties of extracted Roselle anthocyanin and microencapsulated Roselle anthocyanin powder and stability of microencapsulated Roselle anthocyanin are discussed in this section.

4.1 Proximate composition of fresh calyces of Roselle

The proximate composition of fresh calyces of Roselle viz., moisture content, crude protein, crude fat, carbohydrates, crude fibre and total ash were estimated by standard procedure as explained in Section 3.7 of Chapter 3. The data obtained are presented in Table 6.

4.1.1 Moisture content

The results obtained for moisture content of the fresh calyces of Roselle are presented in Table 6. The data shows that the average moisture content of calyces of Roselle was recorded as $86.08 \pm 0.65\%$ (w.b.).

4.1.2 Crude protein

The results on the crude protein content of fresh calyces of Roselle are presented in Table 6. From the data, it was observed that the average protein content of fresh calyces of Roselle was $2.95 \pm 0.14\%$.

Table 6. Proximate composition of fresh calyces of Roselle

Sl. No.	Parameter	Average value
1	Moisture content(%) (w.b.)	86.08±0.65
2	Crude protein (%)	2.95±0.14
3	Crude fat (%)	0.90±0.02
4	Carbohydrates (%)	6.21±0.21
5	Crude fibre (%)	2.59±0.27
6	Total ash (%)	1.09±0.02

4.1.3 Crude fat

The average fat content of fresh calyces of Roselle was found to be $0.90 \pm 0.02\%$. The data on fat content of fresh calyces of Roselle are presented in Table 6.

4.1.4 Carbohydrates

The results on the carbohydrate content of fresh calyces of Roselle are presented in Table 6. From the data, it was observed that the average carbohydrate content of fresh calyces of Roselle was $6.21 \pm 0.21\%$.

4.1.5 Crude fibre

The average crude fibre content of fresh calyces of Roselle was found to be $2.59 \pm 0.27\%$. The data on crude fibre content of fresh calyces of Roselle are presented in Table 6.

4.1.6 Total ash

The results on the total ash content of fresh calyces of Roselle are presented in Table 6. From the data, it was observed that the average total ash content of fresh calyces of Roselle was $1.09 \pm 0.02\%$.

4.2 Physico-chemical properties of fresh calyces of Roselle

The physico-chemical properties of fresh calyces of Roselle viz., pH, water activity, L^* value, a^* value, b^* value, ascorbic acid and titratable acidity were estimated by standard procedure as explained in Section 3.8 of Chapter 3. The data obtained are presented in Table 7.

4.2.1 pH

The average pH value of fresh calyces of Roselle was found to be 2.16 ± 0.02 . The data on pH value of fresh calyces of Roselle are presented in Table 7.

4.2.2 Water activity

The results on the water activity of fresh calyces of Roselle are presented in Table 7. From the data, it was observed that the average water activity of fresh calyces of Roselle was 0.75 ± 0.03 .

Table 7. Physico-chemical properties of fresh calyces of Roselle

Sl. No.	Parameter	Average value
1	pH	2.16±0.02
2	Water activity (a_w)	0.75±0.03
3	L^*	32.44±0.26
4	a^*	26.83±0.05
5	b^*	9.38±0.18
6	Ascorbic acid (mg.100g ⁻¹)	46.36±0.49
7	Titrateable acidity (%)	20.12±0.80

4.2.3 L^* value

The average L^* value of fresh calyces of Roselle was found to be 32.44 ± 0.26 . The data on L^* value of fresh calyces of Roselle are presented in Table 7.

4.2.4 a^* value

The results on a^* value of fresh calyces of Roselle are presented in Table 7. From the data, it was observed that the average a^* value of fresh calyces of Roselle was 26.83 ± 0.05 .

4.2.5 b^* value

The average b^* value of fresh calyces of Roselle was found to be 9.38 ± 0.18 . The data on b^* value of fresh calyces of Roselle are presented in Table 7.

4.2.6 Ascorbic acid

The results on the ascorbic acid content of fresh calyces of Roselle are presented in Table 7. From the data, it was observed that the average ascorbic content of fresh calyces of Roselle was $46.36 \pm 0.49 \text{ mg} \cdot 100\text{g}^{-1}$.

4.2.7 Titratable acidity

The results on the titratable acidity of fresh calyces of Roselle are presented in Table 7. From the data, it was observed that the average titratable acidity of fresh calyces of Roselle was $20.12 \pm 0.80\%$.

4.3 Functional properties of fresh calyces of Roselle

The functional properties of fresh calyces of Roselle viz., total phenolic content, antioxidant activity, total flavonoids and total anthocyanin content were estimated by standard procedure as explained in Section 3.9 of Chapter 3. The data obtained are presented in Table 8.

4.3.1 Total phenolic content

The results on the total phenolic content of fresh calyces of Roselle are presented in Table 8. From the data, it was observed that the average total phenolic content of fresh calyces of Roselle was $20.85 \pm 0.08 \text{ mg GAE} \cdot \text{g}^{-1}$.

Table 8. Functional properties of fresh calyces of Roselle

Sl. No.	Parameter	Average value
1	Total phenolic content (mg GAE.g ⁻¹)	20.85±0.08
2	Antioxidant activity (%)	92.23±0.77
3	Total flavonoids (mg RE.g ⁻¹)	38.34±0.11
4	Total anthocyanin content (mg CyaG.100g ⁻¹)	718.72±1.19

4.3.2 Antioxidant activity

The average antioxidant activity of fresh calyces of Roselle was found to be $92.23 \pm 0.77\%$. The data on antioxidant activity of fresh calyces of Roselle are presented in Table 8.

4.3.3 Total flavonoids

The results on the total flavonoid content of fresh calyces of Roselle are presented in Table 8. From the data, it was observed that the average total flavonoid content of fresh calyces of Roselle was $38.34 \pm 0.11 \text{ mg RE.g}^{-1}$.

4.3.4 Total anthocyanin content

The average total anthocyanin content of fresh calyces of Roselle was found to be $718.72 \pm 1.19 \text{ mg CyaG.100g}^{-1}$. The data on total anthocyanin content of fresh calyces of Roselle are presented in Table 8.

4.4 Drying behaviour of calyces of Roselle

Irrespective of drying method, an increasing trend was observed in the reduction of moisture content in the beginning of the drying process. As the drying process proceeded, the loss of moisture in calyces of Roselle decreased with drying time due to the evaporation of water with respect to time. The dried calyces of Roselle obtained by different drying methods are given in Plate 16.

The results of drying characteristics namely, reduction in moisture content, drying rate and moisture ratio of fresh calyces of Roselle dried under three different drying methods are presented here under.

4.4.1 Effect of drying methods on moisture content of calyces of Roselle

In order to produce the Roselle calyces powder by different drying methods, calyces of Roselle was dried in solar tunnel dryer, shade drying and dehumidified air dryer at $40 \pm 1 \text{ }^{\circ}\text{C}$ and $15 \pm 1\% \text{ RH}$.



Solar tunnel dried calyces



Shade dried calyces



Dehumidified air dried calyces

Plate 16. Dried calyces of Roselle obtained from different drying methods

4.4.1.1 Solar tunnel drying

In solar tunnel drying, the moisture content of calyces of Roselle was decreased from 618.44% (d.b.) to 10.21% (d.b.) in 19 hours. The average temperature and humidity in solar tunnel dryer was found to be 35.07 °C and 31.26%, respectively. The reduction in moisture content in solar tunnel drying with respect to drying time is shown in Table 9.

4.4.1.2 Shade drying

In shade drying, the moisture content of calyces of Roselle was decreased from 618.44% (d.b.) to 10.11% (d.b.) in 26 hours. The average temperature and humidity in shade drying was found to be 28.83 °C and 49.70%, respectively. The reduction in moisture content in shade drying with respect to drying time is shown in Table 10.

4.4.1.3 Dehumidified air drying

In dehumidified air drying, the moisture content of calyces of Roselle was decreased from 618.44% (d.b.) to 10.03% (d.b.) in 14 hours at 40±1 °C and 15±1% RH. The reduction in moisture content at 40±1 °C and 15±1% RH with respect to drying time is shown in Table 11.

4.5 Effect of drying methods on drying rate of calyces of Roselle

The drying rate of calyces of Roselle dried under solar tunnel drying, shade drying and dehumidified air drying at 40±1 °C and 15±1% RH are presented in Tables 9, 10 and 11, respectively. The drying rate of fresh calyces of Roselle dried in solar tunnel drying ranged from 0.0173 g.g⁻¹ dm. min⁻¹ in the initial stage of drying to 0.0001 g.g⁻¹ dm. min⁻¹ at the final stage of drying. In case of shade drying, drying rate ranged from 0.0110 g.g⁻¹ dm. min⁻¹ in initial stage of drying to 0.0001 g.g⁻¹ dm. min⁻¹ at the final stage of drying. In both the drying method, the drying rate was mainly dependent on drying temperature. Here the temperature varied according to the climatic condition. The drying rate of fresh calyces of Roselle dried under dehumidified air dryer at 40±1 °C and 15±1% RH ranged from 0.0287 g.g⁻¹ dm. min⁻¹ at initial stage of drying to 0.0008 g.g⁻¹ dm. min⁻¹ at final stage of drying.

Table 9. Moisture content, drying rate and moisture ratio of calyces of Roselle in solar tunnel drying

Time (h)	Drying time	Temp. (°C)	Relative humidity (%)	Moisture content (% d.b.)	Drying rate (g/g of dm/min)	Moisture ratio
0.00	9.00	27.30	47.12	618.44	0.0000	1.0000
0.50	9.30	31.20	44.10	566.66	0.0173	0.9163
1.00	10.00	34.60	38.24	530.28	0.0121	0.8574
1.50	10.30	36.80	26.31	493.20	0.0124	0.7975
2.00	11.00	41.30	23.43	464.78	0.0095	0.7515
2.50	11.30	38.70	21.68	437.32	0.0092	0.7071
3.00	12.00	37.10	33.14	396.11	0.0137	0.6405
3.50	12.30	35.30	34.12	356.78	0.0131	0.5769
4.00	13.00	36.80	38.14	305.56	0.0171	0.4941
4.50	13.30	37.20	31.10	262.45	0.0144	0.4244
5.00	14.00	37.00	30.52	229.78	0.0109	0.3715
5.50	14.30	35.40	32.04	202.34	0.0091	0.3272
6.00	15.00	33.80	31.00	182.00	0.0068	0.2943
6.50	15.30	33.10	33.40	169.45	0.0042	0.2740
7.00	16.00	32.80	42.12	145.73	0.0079	0.2356
7.50	16.30	31.20	43.05	138.76	0.0023	0.2244
8.00	17.00	31.00	44.25	129.67	0.0030	0.2097
8.50	9.30	27.60	49.25	122.45	0.0024	0.1980
9.00	10.00	32.80	30.56	114.78	0.0026	0.1856
9.50	10.30	34.60	21.54	108.86	0.0020	0.1760
10.00	11.00	36.30	27.28	103.45	0.0018	0.1673
10.50	11.30	38.20	21.45	93.34	0.0034	0.1509
11.00	12.00	39.60	20.32	82.45	0.0036	0.1333
11.50	12.30	38.80	21.05	69.34	0.0044	0.1121
12.00	13.00	42.30	23.13	55.16	0.0047	0.0892
12.50	13.30	37.40	23.45	41.12	0.0047	0.0665
13.00	14.00	37.50	20.89	32.12	0.0030	0.0519

13.50	14.30	37.00	22.57	26.45	0.0019	0.0428
14.00	15.00	36.30	24.56	23.23	0.0011	0.0376
14.50	15.30	33.20	32.47	20.17	0.0010	0.0326
15.00	16.00	32.40	36.16	18.79	0.0005	0.0304
15.50	16.30	30.20	40.29	16.45	0.0008	0.0266
16.00	17.00	29.20	48.34	15.15	0.0004	0.0245
16.50	9.30	30.10	38.15	14.58	0.0002	0.0236
17.00	10.00	34.50	33.40	13.78	0.0003	0.0223
17.50	10.30	35.90	21.59	12.26	0.0005	0.0198
18.00	11.00	36.70	22.54	11.97	0.0001	0.0194
18.50	11.30	36.90	22.15	11.06	0.0003	0.0179
19.00	12.00	39.80	24.19	10.21	0.0003	0.0165
Avg.		35.07	31.26			

Table 10. Moisture content, drying rate and moisture ratio of calyces of Roselle in shade drying

Time (h)	Drying time	Temp. (°C)	Relative humidity (%)	Moisture content (% d.b.)	Drying rate (g/g of dm/min)	Moisture ratio
0.00	9.00	27.80	51.42	618.44	0.0000	1.0000
0.50	9.30	27.90	51.21	585.57	0.0110	0.9468
1.00	10.00	27.90	50.24	568.79	0.0056	0.9197
1.50	10.30	28.20	48.52	543.23	0.0085	0.8784
2.00	11.00	29.00	48.27	528.67	0.0049	0.8548
2.50	11.30	29.80	47.19	502.35	0.0088	0.8123
3.00	12.00	30.50	46.28	469.56	0.0109	0.7593
3.50	12.30	30.60	46.05	435.79	0.0113	0.7047
4.00	13.00	30.50	45.68	392.46	0.0144	0.6346
4.50	13.30	30.60	45.26	358.77	0.0112	0.5801
5.00	14.00	30.10	45.04	324.59	0.0114	0.5249
5.50	14.30	29.80	44.34	307.67	0.0056	0.4975
6.00	15.00	29.60	45.32	285.45	0.0074	0.4616
6.50	15.30	28.90	45.65	279.63	0.0019	0.4522
7.00	16.00	28.80	45.98	256.67	0.0077	0.4150
7.50	16.30	28.60	48.56	242.45	0.0047	0.3920
8.00	17.00	28.50	50.20	235.67	0.0023	0.3811
8.50	9.30	27.80	49.27	213.67	0.0073	0.3455
9.00	10.00	28.00	48.52	191.23	0.0075	0.3092
9.50	10.30	28.70	48.26	172.45	0.0063	0.2788
10.00	11.00	28.50	48.16	162.42	0.0033	0.2626
10.50	11.30	29.00	48.02	145.67	0.0056	0.2355
11.00	12.00	29.40	47.15	134.23	0.0038	0.2170
11.50	12.30	29.80	46.19	118.56	0.0052	0.1917
12.00	13.00	30.30	45.23	108.39	0.0034	0.1753
12.50	13.30	30.50	44.15	89.45	0.0063	0.1446
13.00	14.00	30.60	45.28	75.34	0.0047	0.1218

13.50	14.30	30.60	45.98	68.34	0.0023	0.1105
14.00	15.00	30.50	47.85	62.45	0.0020	0.1010
14.50	15.30	29.80	47.98	58.67	0.0013	0.0949
15.00	16.00	29.40	48.27	53.56	0.0017	0.0866
15.50	16.30	29.10	49.56	49.78	0.0013	0.0805
16.00	17.00	28.80	49.25	47.23	0.0009	0.0764
16.50	9.30	27.10	56.02	41.56	0.0019	0.0672
17.00	10.00	27.70	51.85	38.78	0.0009	0.0627
17.50	10.30	28.00	51.56	35.76	0.0010	0.0578
18.00	11.00	28.10	51.28	32.45	0.0011	0.0525
18.50	11.30	28.00	51.12	29.65	0.0009	0.0479
19.00	12.00	29.00	50.02	25.83	0.0013	0.0418
19.50	12.30	29.20	49.50	22.38	0.0012	0.0362
20.00	13.00	28.90	50.12	18.76	0.0012	0.0303
20.50	13.30	28.70	51.22	15.21	0.0012	0.0246
21.00	14.00	28.80	51.36	13.46	0.0006	0.0218
21.50	14.30	28.30	51.48	12.86	0.0002	0.0208
22.00	15.00	28.20	51.66	12.45	0.0001	0.0201
22.50	15.30	28.20	52.39	11.99	0.0002	0.0194
23.00	16.00	27.80	53.33	11.76	0.0001	0.0190
23.50	16.30	27.70	53.87	11.52	0.0001	0.0186
24.00	17.00	27.50	53.99	11.36	0.0001	0.0184
24.50	9.30	26.60	63.24	10.95	0.0001	0.0177
25.00	10.00	26.70	62.04	10.64	0.0001	0.0172
25.50	10.30	27.70	57.89	10.26	0.0001	0.0166
26.00	11.00	28.10	55.64	10.11	0.0001	0.0163
Avg.		28.83	49.70			

Table 11. Moisture content, drying rate and moisture ratio of calyces of Roselle in dehumidified air drying

Time (h)	Temp. (°C)	Relative humidity (%)	Moisture content (% d.b.)	Drying rate (g/g of dm/min)	Moisture ratio
0.00	40.00	15.00	618.44	0.0000	1.0000
0.50	40.00	15.00	532.22	0.0287	0.8606
1.00	40.00	15.00	455.33	0.0256	0.7363
1.50	40.00	15.00	398.67	0.0189	0.6446
2.00	40.00	15.00	355.43	0.0144	0.5747
2.50	40.00	15.00	301.23	0.0181	0.4871
3.00	40.00	15.00	245.63	0.0185	0.3972
3.50	40.00	15.00	201.35	0.0148	0.3256
4.00	40.00	15.00	175.43	0.0086	0.2837
4.50	40.00	15.00	143.25	0.0107	0.2316
5.00	40.00	15.00	126.78	0.0055	0.2050
5.50	40.00	15.00	101.23	0.0085	0.1637
6.00	40.00	15.00	89.76	0.0038	0.1451
6.50	40.00	15.00	75.46	0.0048	0.1220
7.00	40.00	15.00	67.54	0.0026	0.1092
7.50	40.00	15.00	58.39	0.0031	0.0944
8.00	40.00	15.00	49.67	0.0029	0.0803
8.50	40.00	15.00	46.45	0.0011	0.0751
9.00	40.00	15.00	42.12	0.0014	0.0681
9.50	40.00	15.00	37.43	0.0016	0.0605
10.00	40.00	15.00	33.76	0.0012	0.0546
10.50	40.00	15.00	28.87	0.0016	0.0467
11.00	40.00	15.00	26.45	0.0008	0.0428
11.50	40.00	15.00	23.32	0.0010	0.0377
12.00	40.00	15.00	21.37	0.0006	0.0346
12.50	40.00	15.00	18.76	0.0009	0.0303
13.00	40.00	15.00	15.99	0.0009	0.0259
13.50	40.00	15.00	12.46	0.0012	0.0201
14.00	40.00	15.00	10.03	0.0008	0.0162

4.6 Effect of drying methods on moisture ratio of calyces of Roselle

The results of moisture ratio for calyces of Roselle dried under solar tunnel drying, shade drying and dehumidified air drying at 40 ± 1 °C and $15\pm 1\%$ RH are presented in Tables 9, 10 and 11, respectively. From the tables, it is noticed that the moisture ratio of calyces of Roselle dried in solar tunnel drying ranged from 0.9163 in the initial stage of drying to 0.0165 at the final stage of drying. In case of shade drying, moisture ratio ranged from 0.9468 in initial stage of drying to 0.0163 at the final stage of drying. In both the drying method, the moisture ratio was mainly dependent on drying temperature. Here the temperature varied according to the climatic condition. The moisture ratio of calyces of Roselle dried under dehumidified air dryer at 40 ± 1 °C and $15\pm 1\%$ RH ranged from 0.8606 at initial stage of drying to 0.0162 at final stage of drying.

4.7 Mathematical modelling of drying of calyces of Roselle in different drying methods

The different drying models namely, Newton, Page, Modified Page, Henderson and Pabis, Modified Henderson and Pabis and Logarithmic models were selected and fitted to the drying data based on their ability to model the drying phenomenon of fresh calyces of Roselle. The drying constants and the statistical validity of models was evaluated in MATLAB 2007 Software and compared by means of the coefficient of determination (R^2). In order to select the best model, this factor was used as the main selection criteria, but also the goodness of the fit was determined using additionally others statistical parameters such as reduced chi-square (χ^2), sum square error (SSE), adjusted R^2 and root mean square error (RMSE) values. For quality fit, R^2 value should be higher and, χ^2 , SSE and RMSE values should be lower. The constants of various drying models for different drying methods are shown in Table 12. Table 13 presents, the estimated values of statistical parameters of various models for different drying methods.

Table 12. Effect of different drying methods on constants of drying models of calyces of Roselle

Sl. No.	Model	Constants	Drying method		
			STD	SD	DD
1	Newton	k	0.1884	0.1353	0.3081
2	Page	k	0.1391	0.0734	0.2960
		n	1.1640	1.2800	1.0290
3	Modified Page	k	1.1780	0.1741	0.6677
		n	0.1598	0.7771	0.4614
4	Henderson- Pabis	a	1.0550	1.0920	1.0110
		k	0.1983	0.1469	0.3114
5	Modifeid Henderson- Pabis	a	-107.70	0.5972	-3.0940
		b	108.20	0.4935	4.1860
		c	0.4915	-0.0003	-0.0933
		g	0.6823	0.1666	0.3586
		h	0.1310	0.2635	0.7683
		k	0.6869	0.1341	0.3709
6	Logarithmic	a	1.0720	1.1200	1.0080
		c	-0.0347	-0.0562	0.0064
		k	0.1797	0.1264	0.3180

Table 13. Effect of different drying methods on estimated values of statistical parameters of different drying models of calyces of Roselle

Sl. No.	Parameter	Drying method	Model					
			Newton	Page	Modified Page	Henderson- Pabis	Modified Henderson- Pabis	Logarithmic
1	R^2	STD	0.9886	0.9952	0.9886	0.9915	0.9862	0.9934
		SD	0.9815	0.9980	0.9815	0.9891	0.9884	0.9937
		DD	0.9981	0.9983	0.9981	0.9982	0.9983	0.9983
2	SSE	STD	0.0371	0.0156	0.0371	0.0276	0.0449	0.0213
		SD	0.0869	0.0094	0.0869	0.0514	0.0544	0.0295
		DD	0.0042	0.0037	0.0042	0.0039	0.0037	0.0037
3	Adjusted- R^2	STD	0.9886	0.9951	0.9883	0.9913	0.9841	0.9931
		SD	0.9815	0.9980	0.9812	0.9889	0.9872	0.9935
		DD	0.9981	0.9982	0.9980	0.9981	0.9979	0.9982
4	RMSE	STD	0.0313	0.0205	0.0317	0.0273	0.0369	0.0244
		SD	0.0409	0.0136	0.0413	0.0317	0.0340	0.0243
		DD	0.0122	0.0117	0.0124	0.0120	0.0127	0.0119
5	χ^2	STD	0.000977	0.000422	0.001004	0.000746	0.001427	0.000593
		SD	0.001672	0.000184	0.001704	0.001007	0.001158	0.000589
		DD	0.000149	0.000136	0.000154	0.000145	0.000162	0.000140

4.8 Effect of different drying and milling methods on proximate composition of calyces of Roselle powder

The proximate composition of calyces of Roselle powder viz., moisture content, crude protein, crude fat, carbohydrates, crude fibre and total ash content of calyces of Roselle powder in different drying and milling methods were analyzed. Solar tunnel drying, shade drying and dehumidified air drying at 40 ± 1 °C and $15\pm 1\%$ RH were taken as different drying methods. The various milling methods considered were normal pulverizer, water cooled pulverizer and LN_2 cooled pulverizer. The data obtained are presented in Table 14. The optimization of process methods were carried out in Design Expert software by general factorial method based on goal of response. The detailed results are presented hereunder.

4.8.1 Moisture content

The moisture content of calyces of Roselle powder obtained by different drying and milling methods were determined and analyzed statistically and presented in Table 14. From the data, it was observed that in solar tunnel drying, the moisture content of calyces of Roselle powder ranged from 9.22% (d.b.) to 9.38% (d.b.) among the different milling methods. Similarly in shade drying, the moisture content of calyces of Roselle powder ranged from 9.06% (d.b.) to 9.16% (d.b.) and in dehumidified air drying, the moisture content of calyces of Roselle powder ranged from 8.98% (d.b.) to 9.03% (d.b.) among the different milling methods.

In case of milling methods, calyces of Roselle powder obtained from LN_2 cooled pulverizer showed significantly lower moisture content of 8.98% (d.b.) and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had moisture content of 9.02% (d.b.). Milling with normal pulverizer showed significantly higher moisture content value of 9.38% (d.b.).

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by dehumidified air drying and milling with LN_2 cooled pulverizer had lower moisture content value (8.98% d.b.) compared to all other treatments. Calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer gave the highest moisture content of 9.38% (d.b.).

Table 14. Effect of different drying and milling methods on proximate composition of calyces of Roselle powder

Sl. No.	Drying method	STD			SD			DD		
	Milling method	NP	WCP	LN ₂ CP	NP	WCP	LN ₂ CP	NP	WCP	LN ₂ CP
1	Moisture content (% d.b.)	9.38	9.28	9.22	9.16	9.14	9.06	9.03	9.02	8.98
2	Crude protein (%)	7.89	8.10	8.35	8.48	8.77	9.22	8.84	8.97	9.46
3	Crude fat (%)	2.19	2.24	2.28	2.30	2.31	2.32	2.74	2.83	2.98
4	Carbohydrates (%)	62.01	61.59	60.89	60.00	58.64	57.90	58.27	57.68	56.52
5	Crude fibre (%)	9.78	9.89	10.10	10.24	10.58	11.03	10.37	10.55	11.24
6	Total ash (%)	7.92	8.04	8.28	9.64	9.80	10.04	10.02	10.12	10.57

Table 15 shows the analysis of variance (ANOVA) and estimated coefficient values for moisture content of calyces of Roselle powder. The Model (2FI) F-value of 97.92 implies that the model was significant ($p < 0.0001$). Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B, AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R^2 value of 0.98 whereas the optimized predictive model had an R^2 value of 0.95 was in reasonable agreement with the Adjusted R^2 of 0.97. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 28.92 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for moisture content obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\text{Moisture content (\% d.b.)} = 9.139 + 0.154*A - 0.023*A^2 + 0.046*B + 6.155E-003*B^2 + 0.038*A*B - 5.329E-003*A^2*B - 0.016*A*B^2 + 0.014*A^2*B^2 \dots (4.1)$$

4.8.2 Crude protein

The crude protein content of calyces of Roselle powder obtained by different drying and milling methods were determined and analyzed statistically and presented in Table 14. From the data, it was observed that in solar tunnel drying, the crude protein content of calyces of Roselle powder ranged from 7.89% to 8.35% among the different milling methods. Similarly in shade drying, the crude protein content of calyces of Roselle powder ranged from 8.48% to 9.22% and in dehumidified air drying, the crude protein content of calyces of Roselle powder ranged from 8.84% to 9.46% among the different milling methods.

Table 15. Analysis of variance (ANOVA) of moisture content in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	0.431	8	0.054	97.92	< 0.0001	Significant	Intercept	9.139	1	0.005
A-Drying method	0.373	2	0.187	339.52	< 0.0001		A[1]	0.154	1	0.006
B-Milling method	0.044	2	0.022	40.15	< 0.0001		A[2]	-0.023	1	0.006
AB	0.013	4	0.003	6.00	0.0030		B[1]	0.046	1	0.006
Pure Error	0.010	18	0.001				B[2]	0.006	1	0.006
Cor Total	0.441	26					A[1]B[1]	0.038	1	0.009
							A[2]B[1]	-0.005	1	0.009
Std. Dev.	0.023		R-Squared	0.98			A[1]B[2]	-0.016	1	0.009
Mean	9.139		Adj R-Squared	0.97			A[2]B[2]	0.014	1	0.009
C.V. %	0.257		Pred R-Squared	0.95						
PRESS	0.022		Adeq Precision	28.92						

Considering the different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly higher crude protein content of 9.46% and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had crude protein content of 8.97%. Milling with normal pulverizer showed significantly lower crude protein content of 7.89%.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had lower crude protein content (7.89%) compared to all other treatments. Calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the highest crude protein content of 9.46%.

Table 16 shows the analysis of variance (ANOVA) and estimated coefficient values for crude protein content of calyces of Roselle powder. The Model (2FI) F-value of 24.29 implies that the model was significant ($p < 0.0001$). Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R² value of 0.92 whereas the optimized predictive model had an R² value of 0.81 was in reasonable agreement with the Adjusted R² of 0.88. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 15.03 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for crude protein content obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\begin{aligned} \text{Crude protein (\%)} = & 8.677 - 0.561*A + 0.146*A^2 - 0.271*B - 0.064*B^2 + 0.047*A*B - 0.068*A^2 \\ & *B + 0.049*A*B^2 + 8.063E-003*A^2B^2 \end{aligned} \quad \dots (4.2)$$

Table 16. Analysis of variance (ANOVA) of crude protein in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	6.359	8	0.795	24.29	< 0.0001	Significant	Intercept	8.677	1	0.035
A-Drying method	4.571	2	2.286	69.83	< 0.0001		A[1]	-0.561	1	0.049
B-Milling method	1.706	2	0.853	26.06	< 0.0001		A[2]	0.146	1	0.049
AB	0.082	4	0.021	0.63	0.6490		B[1]	-0.271	1	0.049
Pure Error	0.589	18	0.033				B[2]	-0.064	1	0.049
Cor Total	6.948	26					A[1]B[1]	0.047	1	0.070
							A[2]B[1]	-0.068	1	0.070
Std. Dev.	0.181		R-Squared	0.92			A[1]B[2]	0.049	1	0.070
Mean	8.677		Adj R-Squared	0.88			A[2]B[2]	0.008	1	0.070
C.V. %	2.085		Pred R-Squared	0.81						
PRESS	1.326		Adeq Precision	15.03						

4.8.3 Crude fat

The crude fat content of calyces of Roselle powder obtained by different drying and milling methods were determined and analyzed statistically and presented in Table 14. From the data, it was observed that in solar tunnel drying, the crude fat content of calyces of Roselle powder ranged from 2.19% to 2.28% among the different milling methods. Similarly in shade drying, the crude fat content of calyces of Roselle powder ranged from 2.30% to 2.32% and in dehumidified air drying, the crude protein content of calyces of Roselle powder ranged from 2.74% to 2.98% among the different milling methods.

Considering the different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly higher crude fat content of 2.98% and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had crude protein content of 2.83%. Milling with normal pulverizer showed significantly lower crude fat content of 2.19%.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had lower crude fat content (2.19%) compared to all other treatments. Calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the higher crude fat content of 2.98%.

Table 17 shows the analysis of variance (ANOVA) and estimated coefficient values for crude fat content of calyces of Roselle powder. The Model (2FI) F-value of 63.91 implies that the model was significant ($p < 0.0001$). Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B, AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R^2 value of 0.97 whereas the optimized predictive model had an R^2 value of 0.92 was in reasonable agreement with the Adjusted R^2 of 0.95. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 21.44 indicates an adequate signal. Hence, this model could be used to navigate the design space.

Table 17. Analysis of variance (ANOVA) of crude fat in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	Df	Standard Error
Model	2.113	8	0.264	63.91	< 0.0001	Significant	Intercept	2.467	1	0.012
A-Drying method	1.985	2	0.992	240.13	< 0.0001		A[1]	-0.229	1	0.017
B-Milling method	0.099	2	0.049	7.95	0.0034		A[2]	-0.154	1	0.017
AB	0.029	4	0.007	2.56	0.0741		B[1]	-0.058	1	0.017
Pure Error	0.074	18	0.004				B[2]	-0.005	1	0.017
Cor Total	2.187	26					A[1]B[1]	0.009	1	0.025
							A[2]B[1]	0.047	1	0.025
Std. Dev.	0.064		R-Squared	0.97			A[1]B[2]	0.010	1	0.025
Mean	2.467		Adj R-Squared	0.95			A[2]B[2]	0.006	1	0.025
C.V. %	2.606		Pred R-Squared	0.92						
PRESS	0.167		Adeq Precision	21.44						

The optimized predictive model (in terms of actual factors) for crude fat content obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\text{Crude fat (\%)} = 2.467 - 0.229*A - 0.154*A^2 - 0.058*B - 5.100E-003*B^2 + 8.861*A*B + 0.047*A^2*B + 9.966E-003*A*B^2 + 6.251*A^2*B^2 \quad \dots (4.3)$$

4.8.4 Carbohydrates

The carbohydrate content of calyces of Roselle powder obtained by different drying and milling methods were determined and analyzed statistically and presented in Table 14. From the data, it was observed that in solar tunnel drying, the carbohydrate content of calyces of Roselle powder ranged from 60.89% to 62.01% among the different milling methods. Similarly in shade drying, the carbohydrate content of calyces of Roselle powder ranged from 57.90% to 60.00% and in dehumidified air drying, the carbohydrate content of calyces of Roselle powder ranged from 56.52% to 58.27% among the different milling methods.

In case of different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly lower carbohydrate content of 56.52% and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had carbohydrate content of 58.64%. Milling with normal pulverizer showed significantly higher carbohydrate content of 62.01%.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had higher carbohydrate content (62.01%) compared to all other treatments. Calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the lower carbohydrate content of 56.52%.

Table 18 shows the analysis of variance (ANOVA) and estimated coefficient values for carbohydrate content of calyces of Roselle powder. The Model (2FI) F-value of 168.09 implies that the model was significant ($p < 0.0001$). Values of "Prob > F" less than 0.05 indicate model terms were significant. In this case A, B, AB were significant model terms.

Table 18. Analysis of variance (ANOVA) of carbohydrate in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	88.153	8	11.019	168.09	< 0.0001	Significant	Intercept	59.279	1	0.049
A-Drying method	74.665	2	37.332	569.50	< 0.0001		A[1]	2.218	1	0.070
B-Milling method	12.376	2	6.188	94.40	< 0.0001		A[2]	-0.433	1	0.070
AB	1.112	4	0.278	4.24	0.0136		B[1]	0.816	1	0.070
Pure Error	1.180	18	0.066				B[2]	0.026	1	0.070
Cor Total	89.333	26					A[1]B[1]	-0.299	1	0.099
							A[2]B[1]	0.336	1	0.099
Std. Dev.	0.256		R-Squared	0.99			A[1]B[2]	0.063	1	0.099
Mean	59.279		Adj R-Squared	0.98			A[2]B[2]	-0.228	1	0.099
C.V. %	0.432		Pred R-Squared	0.97						
PRESS	2.655		Adeq Precision	37.14						

Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R^2 value of 0.99 whereas the optimized predictive model had an R^2 value of 0.97 was in reasonable agreement with the Adjusted R^2 of 0.98. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 37.14 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for carbohydrate content obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\text{Carbohydrates (\%)} = 59.279 + 2.218*A - 0.433*A^2 + 0.816*B + 0.026*B^2 - 0.299*A*B + 0.336*A^2*B + 0.063*A*B^2 - 0.228*A^2*B^2 \quad \dots (4.4)$$

4.8.5 Crude fibre

The crude fibre content of calyces of Roselle powder obtained by different drying and milling methods were determined and analyzed statistically and presented in Table 14. From the data, it was observed that in solar tunnel drying, the crude fibre content of calyces of Roselle powder ranged from 9.78% to 10.10% among the different milling methods. Similarly in shade drying, the crude fibre content of calyces of Roselle powder ranged from 10.24% to 11.03% and in dehumidified air drying, the crude fibre content of calyces of Roselle powder ranged from 10.37% to 11.24% among the different milling methods.

Considering the different milling methods, calyces of Roselle powder obtained from LN_2 cooled pulverizer showed significantly higher crude fibre content of 11.24% and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had crude fibre content of 10.58%. Milling with normal pulverizer showed significantly lower crude fibre content of 9.78%.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had lower crude fibre content (9.78%) compared to all other treatments. Calyces of Roselle powder obtained by dehumidified air

drying and milling with LN₂ cooled pulverizer gave the higher crude fibre content of 11.24%.

Table 19 shows the analysis of variance (ANOVA) and estimated coefficient values for crude fibre content of calyces of Roselle powder. The Model (2FI) F-value of 35.06 implies that the model was significant ($p < 0.0001$). Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B, AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R² value of 0.94 whereas the optimized predictive model had an R² value of 0.86 was in reasonable agreement with the Adjusted R² of 0.91. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 17.61 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for crude fibre content obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\begin{aligned} \text{Crude fibre (\%)} = & 10.422 - 0.498*A + 0.197*A^2 - 0.289*B - 0.080*B^2 + 0.150*A*B - 0.090*A^2 \\ & *B + 0.046*A*B^2 + 0.043*A^2*B^2 \end{aligned} \quad \dots (4.5)$$

4.8.6 Total ash

The total ash content of calyces of Roselle powder obtained by different drying and milling methods were determined and analyzed statistically and presented in Table 14. From the data, it was observed that in solar tunnel drying, the total ash content of calyces of Roselle powder ranged from 7.92% to 8.28% among the different milling methods. Similarly in shade drying, the total ash content of calyces of Roselle powder ranged from 9.64% to 10.04% and in dehumidified air drying, the total ash content of calyces of Roselle powder ranged from 10.02% to 10.57% among the different milling methods.

Table 19. Analysis of variance (ANOVA) of crude fibre in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	5.762	8	0.720	35.06	< 0.0001	Significant	Intercept	10.422	1	0.028
A-Drying method	3.401	2	1.701	82.79	< 0.0001		A[1]	-0.498	1	0.039
B-Milling method	2.034	2	1.017	49.51	< 0.0001		A[2]	0.197	1	0.039
AB	0.326	4	0.082	3.97	0.0176		B[1]	-0.289	1	0.039
Pure Error	0.370	18	0.021				B[2]	-0.080	1	0.039
Cor Total	6.132	26					A[1]B[1]	0.150	1	0.055
							A[2]B[1]	-0.090	1	0.055
Std. Dev.	0.143		R-Squared	0.94			A[1]B[2]	0.046	1	0.055
Mean	10.422		Adj R-Squared	0.91			A[2]B[2]	0.043	1	0.055
C.V. %	1.375		Pred R-Squared	0.86						
PRESS	0.832		Adeq Precision	17.61						

Considering the different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly higher total ash content of 10.57% and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had total ash content of 10.12%. Milling with normal pulverizer showed significantly lower total ash content of 7.92%.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had lower total ash content (7.92%) compared to all other treatments. Calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the higher total ash content of 10.57%.

Table 20 shows the analysis of variance (ANOVA) and estimated coefficient values for total ash content of calyces of Roselle powder. The Model (2FI) F-value of 121.68 implies that the model was significant ($p < 0.0001$). Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R² value of 0.98 whereas the optimized predictive model had an R² value of 0.96 was in reasonable agreement with the Adjusted R² of 0.97. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 28.86 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for total ash content obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\text{Total ash (\%)} = 9.381 - 1.304*A + 0.448*A^2 - 0.186*B - 0.063*B^2 + 0.028*A*B + 1.197E-004*A^2*B + 0.021*A*B^2 + 0.035*A^2*B^2 \quad \dots (4.6)$$

Table 20. Analysis of variance (ANOVA) of total ash in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	24.658	8	3.082	121.68	< 0.0001	Significant	Intercept	9.381	1	0.031
A-Drying method	23.699	2	11.849	467.78	< 0.0001		A[1]	-1.304	1	0.043
B-Milling method	0.906	2	0.453	17.89	< 0.0001		A[2]	0.448	1	0.043
AB	0.053	4	0.013	0.52	0.7229		B[1]	-0.186	1	0.043
Pure Error	0.456	18	0.025				B[2]	-0.063	1	0.043
Cor Total	25.114	26					A[1]B[1]	0.028	1	0.061
							A[2]B[1]	0.000	1	0.061
Std. Dev.	0.159		R-Squared	0.98			A[1]B[2]	0.021	1	0.061
Mean	9.381		Adj R-Squared	0.97			A[2]B[2]	0.035	1	0.061
C.V. %	1.697		Pred R-Squared	0.96						
PRESS	1.026		Adeq Precision	28.86						

4.9 Physico-chemical properties of calyces of Roselle powder

Physico-chemical properties viz., L^* , a^* , b^* , loose bulk density, tapped bulk density, Carr's index, Hausner ratio, pH, water activity, titratable acidity, ascorbic acid, solubility index and particle size of calyces of Roselle powder obtained from different drying and milling methods are given in Table 21. The detailed results are presented hereunder.

4.9.1 Effect of different drying and milling methods on colour L^* value of calyces of Roselle powder

The effect of different drying and milling methods on colour L^* value of calyces of Roselle powder were analyzed statistically and presented in Table 21. From the data, it was observed that in solar tunnel drying, the L^* value of calyces of Roselle powder were found to be in the range of 45.48 to 46.50 among the different milling methods. Similarly in shade drying, the L^* value of calyces of Roselle powder were found to be in the range of 40.50 to 42.27 and in dehumidified air drying, the L^* value of calyces of Roselle powder were found to be in the range of 38.04 to 39.67 among the different milling methods.

Considering the different milling methods, calyces of Roselle powder obtained from LN_2 cooled pulverizer showed significantly lower L^* value of 38.04 and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had L^* value of 38.26. In case of milling with normal pulverizer, calyces of Roselle powder showed significantly higher L^* value of 46.50.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had higher L^* value (46.50) compared to all other treatments. Whereas, in case of calyces of Roselle powder obtained by dehumidified air drying and milling with LN_2 cooled pulverizer gave the lower L^* value of 38.04.

Table 22 shows the analysis of variance (ANOVA) and estimated coefficient values for L^* value of calyces of Roselle powder. The Model (2FI) F-value of 175.73 implies that the model was significant ($p < 0.0001$).

Table 21. Effect of different drying and milling methods on physico-chemical properties of calyces of Roselle powder

Sl. No.	Drying method	STD			SD			DD		
	Milling method	NP	WCP	LN ₂ CP	NP	WCP	LN ₂ CP	NP	WCP	LN ₂ CP
1	<i>L</i> [*]	46.50	45.97	45.48	42.27	40.94	40.50	39.67	38.26	38.04
2	<i>a</i> [*]	17.79	18.82	19.26	20.79	20.97	21.29	21.16	22.43	23.77
3	<i>b</i> [*]	11.21	11.12	10.42	10.04	9.97	9.72	9.57	9.19	9.18
4	Loose bulk density (g.cc ⁻¹)	0.60	0.57	0.54	0.53	0.52	0.51	0.50	0.48	0.47
5	Tapped bulk density (g.cc ⁻¹)	0.73	0.68	0.64	0.63	0.61	0.60	0.59	0.57	0.55
6	Carr's index	18.03	17.01	16.09	15.79	15.54	15.27	15.00	14.53	14.08
7	Hausner ratio	1.22	1.20	1.19	1.19	1.18	1.18	1.18	1.17	1.16
8	pH	3.27	3.19	3.17	3.16	3.07	2.95	3.06	3.03	2.77
9	Water activity	0.43	0.41	0.40	0.39	0.37	0.36	0.37	0.36	0.35
10	Titrateable acidity (%)	16.73	17.32	18.05	18.01	19.46	20.82	18.30	20.76	21.40
11	Ascorbic acid (mg.100g ⁻¹)	16.68	18.94	22.61	26.29	32.51	37.88	28.55	35.05	40.42
12	Solubility index (g.g ⁻¹)	0.26	0.27	0.28	0.29	0.30	0.32	0.30	0.33	0.33
13	Particle size (d.nm)	3801.33	3570.67	3419.00	3485.67	3078.00	2638.33	2972.00	2619.33	2519.33

Table 22. Analysis of variance (ANOVA) of L^* value in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	Df	Standard Error
Model	259.883	8	32.485	175.73	< 0.0001	Significant	Intercept	41.959	1	0.083
A-Drying method	248.602	2	124.301	672.42	< 0.0001		A[1]	4.024	1	0.117
B-Milling method	10.458	2	5.229	28.29	< 0.0001		A[2]	-0.722	1	0.117
AB	0.823	4	0.206	1.11	0.3810		B[1]	0.852	1	0.117
Pure Error	3.327	18	0.185				B[2]	-0.236	1	0.117
Cor Total	263.211	26					A[1]B[1]	-0.336	1	0.165
							A[2]B[1]	0.178	1	0.165
Std. Dev.	0.430		R-Squared	0.99			A[1]B[2]	0.219	1	0.165
Mean	41.959		Adj R-Squared	0.98			A[2]B[2]	-0.058	1	0.165
C.V. %	1.025		Pred R-Squared	0.97						
PRESS	7.487		Adeq Precision	34.07						

Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R^2 value of 0.99 whereas the optimized predictive model had an R^2 value of 0.97 was in reasonable agreement with the Adjusted R^2 of 0.98. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 34.07 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for L^* value obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$L^* \text{ value} = 41.959 + 4.024*A - 0.722*A^2 + 0.852*B - 0.236*B^2 - 0.336*A*B + 0.178*A^2*B + 0.219*A*B^2 - 0.058*A^2*B^2 \quad \dots (4.7)$$

4.9.2 Effect of different drying and milling methods on colour a^* value of calyces of Roselle powder

The effect of different drying and milling methods on colour a^* value of calyces of Roselle powder were analyzed statistically and presented in Table 21. From the data, it was observed that in solar tunnel drying, the a^* value of calyces of Roselle powder were found to be in the range of 17.79 to 19.26 among the different milling methods. Similarly in shade drying, the a^* value of calyces of Roselle powder were found to be in the range of 20.79 to 21.29 and in dehumidified air drying, the a^* value of calyces of Roselle powder were found to be in the range of 21.16 to 23.77 among the different milling methods.

Considering the different milling methods, calyces of Roselle powder obtained from LN_2 cooled pulverizer showed significantly higher a^* value of 23.77 and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had a^* value of 22.43. In case of milling with normal pulverizer, calyces of Roselle powder showed significantly lower a^* value of 17.79.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had lower a^* value (17.79) compared to

all other treatments. Whereas, in case of calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the higher a^* value of 23.77.

Table 23 shows the analysis of variance (ANOVA) and estimated coefficient values for a^* value of calyces of Roselle powder. The Model (2FI) F-value of 146.07 implies that the model was significant ($p < 0.0001$). Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B, AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R^2 value of 0.98 whereas the optimized predictive model had an R^2 value of 0.97 was in reasonable agreement with the Adjusted R^2 of 0.98. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 39.27 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for a^* value obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$a^* \text{ value} = 20.689 - 2.072*A + 0.319*A^2 - 0.787*B + 0.042*B^2 - 0.049*A*B + 0.557*A^2B + 0.156*A*B^2 - 0.089*A^2*B^2 \quad \dots (4.8)$$

4.9.3 Effect of different drying and milling methods on colour b^* value of calyces of Roselle powder

The effect of different drying and milling methods on colour b^* value of calyces of Roselle powder were analyzed statistically and presented in Table 21. From the data, it was observed that in solar tunnel drying, the b^* value of calyces of Roselle powder were found to be in the range of 10.42 to 11.21 among the different milling methods. Similarly in shade drying, the b^* value of calyces of Roselle powder were found to be in the range of 9.72 to 10.04 and in dehumidified air drying, the b^* value of calyces of Roselle powder were found to be in the range of 9.18 to 9.57 among the different milling methods.

Table 23. Analysis of variance (ANOVA) of a^* value in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	81.304	8	10.163	146.07	< 0.0001	Significant	Intercept	20.698	1	0.051
A-Drying method	67.230	2	33.615	483.13	< 0.0001		A[1]	-2.072	1	0.072
B-Milling method	10.573	2	5.287	75.98	< 0.0001		A[2]	0.319	1	0.072
AB	3.500	4	0.875	12.58	< 0.0001		B[1]	-0.787	1	0.072
Pure Error	1.252	18	0.070				B[2]	0.042	1	0.072
Cor Total	82.556	26					A[1]B[1]	-0.049	1	0.102
							A[2]B[1]	0.557	1	0.102
Std. Dev.	0.264		R-Squared	0.98			A[1]B[2]	0.156	1	0.102
Mean	20.698		Adj R-Squared	0.98			A[2]B[2]	-0.089	1	0.102
C.V. %	1.274		Pred R-Squared	0.97						
PRESS	2.818		Adeq Precision	39.27						

Considering the different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly lower b^* value of 9.18 and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had b^* value of 9.19. In case of milling with normal pulverizer, calyces of Roselle powder showed significantly higher b^* value of 11.21.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had higher b^* value (11.21) compared to all other treatments. Whereas, in case of calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the lower b^* value of 9.18.

Table 24 shows the analysis of variance (ANOVA) and estimated coefficient values for b^* value of calyces of Roselle powder. The Model (2FI) F-value of 46.61 implies that the model was significant ($p < 0.0001$). Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B, AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R^2 value of 0.95 whereas the optimized predictive model had an R^2 value of 0.90 was in reasonable agreement with the Adjusted R^2 of 0.93. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 18.56 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for b^* value obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$b^* \text{ value} = 10.047 + 0.870*A - 0.137*A^2 + 0.229*B + 0.044*B^2 + 0.068*A*B - 0.099*A^2B + 0.159*A*B^2 + 0.012*A^2*B^2 \quad \dots (4.9)$$

Table 24. Analysis of variance (ANOVA) of b^* value in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	Df	Standard Error
Model	13.427	8	1.678	46.61	< 0.0001	Significant	Intercept	10.047	1	0.037
A-Drying method	11.820	2	5.910	164.12	< 0.0001		A[1]	0.870	1	0.052
B-Milling method	1.162	2	0.581	16.13	< 0.0001		A[2]	-0.137	1	0.052
AB	0.446	4	0.111	3.09	0.0421		B[1]	0.229	1	0.052
Pure Error	0.648	18	0.036				B[2]	0.044	1	0.052
Cor Total	14.076	26					A[1]B[1]	0.068	1	0.073
							A[2]B[1]	-0.099	1	0.073
Std. Dev.	0.190		R-Squared	0.95			A[1]B[2]	0.159	1	0.073
Mean	10.047		Adj R-Squared	0.93			A[2]B[2]	0.012	1	0.073
C.V. %	1.889		Pred R-Squared	0.90						
PRESS	1.458		Adeq Precision	18.56						

4.9.4 Effect of different drying and milling methods on loose bulk density of calyces of Roselle powder

The effect of different drying and milling methods on loose bulk density of calyces of Roselle powder were analyzed statistically and presented in Table 21. From the data, it was observed that in solar tunnel drying, the loose bulk density of calyces of Roselle powder were found to be in the range of 0.54 to 0.60 g.cc⁻¹ among the different milling methods. Similarly in shade drying, the loose bulk density of calyces of Roselle powder were found to be in the range of 0.51 to 0.53 g.cc⁻¹ and in dehumidified air drying, the loose bulk density of calyces of Roselle powder were found to be in the range of 0.47 to 0.50 g.cc⁻¹ among the different milling methods.

Considering the different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly lower loose bulk density of 0.47 g.cc⁻¹ and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had loose bulk density of 0.48 g.cc⁻¹. In case of milling with normal pulverizer, calyces of Roselle powder showed significantly higher loose bulk density of 0.60 g.cc⁻¹.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had higher loose bulk density (0.60 g.cc⁻¹) compared to all other treatments. Whereas, in case of calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the lower loose bulk density of 0.47 g.cc⁻¹.

Table 25 shows the analysis of variance (ANOVA) and estimated coefficient values for loose bulk density of calyces of Roselle powder. The Model (2FI) F-value of 23.27 implies that the model was significant ($p < 0.0001$). Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R² value of 0.91 whereas the optimized predictive model had an R² value of 0.80 was in reasonable agreement with the Adjusted R² of 0.87. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 15.58 indicates an adequate signal. Hence, this model could be used to navigate the design space.

Table 25. Analysis of variance (ANOVA) of loose bulk density in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	0.080	8	0.010	23.27	< 0.0001	Significant	Intercept	0.621	1	0.004
A-Drying method	0.064	2	0.032	73.60	< 0.0001		A[1]	0.063	1	0.006
B-Milling method	0.013	2	0.006	14.97	0.0001		A[2]	-0.009	1	0.006
AB	0.004	4	0.001	2.25	0.1041		B[1]	0.027	1	0.006
Pure Error	0.008	18	0.000				B[2]	-0.001	1	0.006
Cor Total	0.088	26					A[1]B[1]	0.021	1	0.008
							A[2]B[1]	-0.015	1	0.008
Std. Dev.	0.021		R-Squared	0.91			A[1]B[2]	-0.001	1	0.008
Mean	0.621		Adj R-Squared	0.87			A[2]B[2]	0.001	1	0.008
C.V. %	3.343		Pred R-Squared	0.80						
PRESS	0.017		Adeq Precision	15.58						

The optimized predictive model (in terms of actual factors) for loose bulk density obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\begin{aligned} \text{Loose bulk density (g.cc}^{-1}\text{)} = & 0.621 + 0.063*A - 8.672E-003*A^2 + 0.027*B - 8.397E-004*B^2 \\ & + 0.021*A*B - 0.015*A^2*B - 1.297E-003*A*B^2 + 8.397E- \\ & 004*A^2*B^2 \end{aligned} \quad \dots (4.10)$$

4.9.5 Effect of different drying and milling methods on tapped bulk density of calyces of Roselle powder

The effect of different drying and milling methods on tapped bulk density of calyces of Roselle powder were analyzed statistically and presented in Table 21. From the data, it was observed that in solar tunnel drying, the tapped bulk density of calyces of Roselle powder were found to be in the range of 0.64 to 0.73 g.cc⁻¹ among the different milling methods. Similarly in shade drying, the tapped bulk density of calyces of Roselle powder were found to be in the range of 0.60 to 0.63 g.cc⁻¹ and in dehumidified air drying, the tapped bulk density of calyces of Roselle powder were found to be in the range of 0.55 to 0.59 g.cc⁻¹ among the different milling methods.

Considering the different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly lower tapped bulk density of 0.55 g.cc⁻¹ and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had tapped bulk density of 0.57 g.cc⁻¹. In case of milling with normal pulverizer, calyces of Roselle powder showed significantly higher tapped bulk density of 0.73 g.cc⁻¹.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had higher tapped bulk density (0.73 g.cc⁻¹) compared to all other treatments. Whereas, in case of calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the lower tapped bulk density of 0.55 g.cc⁻¹.

Table 26 shows the analysis of variance (ANOVA) and estimated coefficient values for tapped bulk density of calyces of Roselle powder. The Model (2FI) F-value of 23.58 implies that the model was significant (p<0.0001).

Table 26. Analysis of variance (ANOVA) of tapped bulk density in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	0.040	8	0.005	23.58	< 0.0001	Significant	Intercept	0.523	1	0.003
A-Drying method	0.032	2	0.016	75.04	< 0.0001		A[1]	0.044	1	0.004
B-Milling method	0.006	2	0.003	15.14	0.0001		A[2]	-0.006	1	0.004
AB	0.002	4	0.000	2.08	0.1264		B[1]	0.019	1	0.004
Pure Error	0.004	18	0.000				B[2]	0.000	1	0.004
Cor Total	0.044	26					A[1]B[1]	0.014	1	0.006
							A[2]B[1]	-0.010	1	0.006
Std. Dev.	0.015		R-Squared	0.91			A[1]B[2]	-0.001	1	0.006
Mean	0.523		Adj R-Squared	0.87			A[2]B[2]	0.000	1	0.006
C.V. %	2.776		Pred R-Squared	0.80						
PRESS	0.009		Adeq Precision	15.68						

Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R^2 value of 0.91 whereas the optimized predictive model had an R^2 value of 0.80 was in reasonable agreement with the Adjusted R^2 of 0.87. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 15.68 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for tapped bulk density obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\begin{aligned} \text{Tapped bulk density (g.cc}^{-1}\text{)} = & 0.523 + 0.044*A - 5.649E-003*A^2 + 0.019*B - 4.863E-004*B^2 \\ & + 0.014*A*B - 0.010*A^2*B - 7.320E-004*A*B^2 + 4.863E- \\ & 004*A^2*B^2 \end{aligned} \quad \dots (4.11)$$

4.9.6 Effect of different drying and milling methods on Carr's index of calyces of Roselle powder

The effect of different drying and milling methods on Carr's index of calyces of Roselle powder were analyzed statistically and presented in Table 21. From the data, it was observed that in solar tunnel drying, the Carr's index of calyces of Roselle powder were found to be in the range of 16.09 to 18.03 among the different milling methods. Similarly in shade drying, the Carr's index of calyces of Roselle powder were found to be in the range of 15.27 to 15.79 and in dehumidified air drying, the Carr's index of calyces of Roselle powder were found to be in the range of 14.08 to 15.00 among the different milling methods.

Considering the different milling methods, calyces of Roselle powder obtained from LN_2 cooled pulverizer showed significantly lower Carr's index of 14.08 and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had Carr's index of 14.53. In case of milling with normal pulverizer, calyces of Roselle powder showed significantly higher Carr's index of 18.03.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar

tunnel drying and milling with normal pulverizer had higher Carr's index (18.03) compared to all other treatments. Whereas, in case of calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the lower Carr's index of 14.08.

Table 27 shows the analysis of variance (ANOVA) and estimated coefficient values for Carr's index of calyces of Roselle powder. The Model (2FI) F-value of 23.58 implies that the model was significant ($p < 0.0001$). Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R² value of 0.91 whereas the optimized predictive model had an R² value of 0.80 was in reasonable agreement with the Adjusted R² of 0.87. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 15.68 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for Carr's index obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\text{Carr's index} = 15.769 + 1.334*A - 0.169*A^2 + 0.572*B - 0.015*B^2 + 0.412*A*B - 0.309*A^2*B - 0.022*A*B^2 + 0.015*A^2*B^2 \quad \dots (4.12)$$

4.9.7 Effect of different drying and milling methods on Hausner ratio of calyces of Roselle powder

The effect of different drying and milling methods on Hausner ratio of calyces of Roselle powder were analyzed statistically and presented in Table 21. From the data, it was observed that in solar tunnel drying, the Hausner ratio of calyces of Roselle powder were found to be in the range of 1.19 to 1.22 among the different milling methods. Similarly in shade drying, the Hausner ratio of calyces of Roselle powder were found to be in the range of 1.18 to 1.19 and in dehumidified air drying, the Hausner ratio of calyces of Roselle powder were found to be in the range of 1.18 to 1.16 among the different milling methods.

Table 27. Analysis of variance (ANOVA) of Carr's index in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	35.815	8	4.477	23.58	< 0.0001	Significant	Intercept	15.696	1	0.084
A-Drying method	28.490	2	14.245	75.04	< 0.0001		A[1]	1.334	1	0.119
B-Milling method	5.749	2	2.874	15.14	0.0001		A[2]	-0.169	1	0.119
AB	1.576	4	0.394	2.08	0.1264		B[1]	0.572	1	0.119
Pure Error	3.417	18	0.190				B[2]	-0.015	1	0.119
Cor Total	39.232	26					A[1]B[1]	0.412	1	0.168
							A[2]B[1]	-0.309	1	0.168
Std. Dev.	0.436		R-Squared	0.91			A[1]B[2]	-0.022	1	0.168
Mean	15.696		Adj R-Squared	0.87			A[2]B[2]	0.015	1	0.168
C.V. %	2.776		Pred R-Squared	0.80						
PRESS	7.688		Adeq Precision	15.68						

Considering the different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly lower Hausner ratio of 1.16 and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had Hausner ratio of 1.17. In case of milling with normal pulverizer, calyces of Roselle powder showed significantly higher Hausner ratio of 1.22.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had higher Hausner ratio (1.22) compared to all other treatments. Whereas, in case of calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the lower Hausner ratio of 1.16.

Table 28 shows the analysis of variance (ANOVA) and estimated coefficient values for Hausner ratio of calyces of Roselle powder. The Model (2FI) F-value of 23.27 implies that the model was significant ($p < 0.0001$). Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R² value of 0.91 whereas the optimized predictive model had an R² value of 0.80 was in reasonable agreement with the Adjusted R² of 0.87. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 15.58 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for Hausner ratio obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\begin{aligned} \text{Hausner ratio} = & 1.186 + 0.019 \cdot A - 2.602 \times 10^{-3} \cdot A^2 + 8.158 \times 10^{-3} \cdot B - 2.519 \times 10^{-4} \cdot B^2 + 6.219 \times 10^{-4} \cdot A \cdot B \\ & - 4.482 \times 10^{-3} \cdot A^2 \cdot B - 3.891 \times 10^{-4} \cdot A \cdot B^2 + 2.519 \times 10^{-4} \cdot A^2 \cdot B^2 \end{aligned}$$

... (4.13)

Table 28. Analysis of variance (ANOVA) of Hausner ratio in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	0.007	8	0.001	23.27	< 0.0001	Significant	Intercept	1.186	1	0.001
A-Drying method	0.006	2	0.003	73.60	< 0.0001		A[1]	0.019	1	0.002
B-Milling method	0.001	2	0.001	14.96	0.0001		A[2]	-0.003	1	0.002
AB	0.000	4	0.000	2.25	0.1041		B[1]	0.008	1	0.002
Pure Error	0.001	18	0.000				B[2]	0.000	1	0.002
Cor Total	0.008	26					A[1]B[1]	0.006	1	0.002
							A[2]B[1]	-0.004	1	0.002
Std. Dev.	0.006		R-Squared	0.91			A[1]B[2]	0.000	1	0.002
Mean	1.186		Adj R-Squared	0.87			A[2]B[2]	0.000	1	0.002
C.V. %	0.525		Pred R-Squared	0.80						
PRESS	0.002		Adeq Precision	15.58						

4.9.8 Effect of different drying and milling methods on pH of calyces of Roselle powder

The effect of different drying and milling methods on pH of calyces of Roselle powder were analyzed statistically and presented in Table 21. From the data, it was observed that in solar tunnel drying, the pH of calyces of Roselle powder were found to be in the range of 3.17 to 3.27 among the different milling methods. Similarly in shade drying, the pH of calyces of Roselle powder were found to be in the range of 2.95 to 3.16 and in dehumidified air drying, the pH of calyces of Roselle powder were found to be in the range of 2.77 to 3.06 among the different milling methods.

In case of different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly lower pH of 2.77 and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had pH of 3.03. In case of milling with normal pulverizer, calyces of Roselle powder showed significantly higher pH of 3.27.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had higher pH (3.27) compared to all other treatments. Whereas, in case of calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the lower pH of 2.77.

Table 29 shows the analysis of variance (ANOVA) and estimated coefficient values for pH of calyces of Roselle powder. The Model (2FI) F-value of 23.95 implies that the model was significant ($p < 0.0001$). Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B, AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R^2 value of 0.91 whereas the optimized predictive model had an R^2 value of 0.81 was in reasonable agreement with the Adjusted R^2 of 0.88. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 16.33 indicates an adequate signal. Hence, this model could be used to navigate the design space.

Table 29. Analysis of variance (ANOVA) of pH in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	0.532	8	0.066	23.95	< 0.0001	Significant	Intercept	3.075	1	0.010
A-Drying method	0.299	2	0.149	53.89	< 0.0001		A[1]	0.135	1	0.014
B-Milling method	0.182	2	0.091	32.77	< 0.0001		A[2]	-0.014	1	0.014
AB	0.051	4	0.013	4.57	0.0101		B[1]	0.089	1	0.014
Pure Error	0.050	18	0.003				B[2]	0.021	1	0.014
Cor Total	0.581	26					A[1]B[1]	-0.032	1	0.020
							A[2]B[1]	0.010	1	0.020
Std. Dev.	0.053		R-Squared	0.91			A[1]B[2]	-0.041	1	0.020
Mean	3.075		Adj R-Squared	0.88			A[2]B[2]	-0.012	1	0.020
C.V. %	1.713		Pred R-Squared	0.81						
PRESS	0.112		Adeq Precision	16.33						

The optimized predictive model (in terms of actual factors) for pH obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\text{pH} = 3.075 + 0.135*A - 0.014*A^2 + 0.089*B + 0.021*B^2 - 0.032*A*B + 0.010*A^2*B - 0.041*A*B^2 - 0.012*A^2*B^2 \quad \dots (4.14)$$

4.9.9 Effect of different drying and milling methods on water activity of calyces of Roselle powder

The effect of different drying and milling methods on water activity of calyces of Roselle powder were analyzed statistically and presented in Table 21. From the data, it was observed that in solar tunnel drying, the water activity of calyces of Roselle powder were found to be in the range of 0.40 to 0.43 among the different milling methods. Similarly in shade drying, the water activity of calyces of Roselle powder were found to be in the range of 0.36 to 0.39 and in dehumidified air drying, the water activity of calyces of Roselle powder were found to be in the range of 0.35 to 0.37 among the different milling methods.

Considering the different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly lower water activity of 0.35 and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had water activity of 0.36. In case of milling with normal pulverizer, calyces of Roselle powder showed significantly higher water activity of 0.43.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had higher water activity (0.43) compared to all other treatments. Whereas, in case of calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the lower water activity of 0.35.

Table 30 shows the analysis of variance (ANOVA) and estimated coefficient values for water activity of calyces of Roselle powder. The Model (2FI) F-value of 34.55 implies that the model was significant (p<0.0001). Values of "Prob> F" less than 0.05 indicate model terms were significant.

Table 30. Analysis of variance (ANOVA) of water activity in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	0.019	8	0.002	34.55	< 0.0001	Significant	Intercept	0.383	1	0.002
A-Drying method	0.016	2	0.008	115.32	< 0.0001		A[1]	0.034	1	0.002
B-Milling method	0.003	2	0.002	21.53	< 0.0001		A[2]	-0.011	1	0.002
AB	0.000	4	0.000	0.68	0.6120		B[1]	0.014	1	0.002
Pure Error	0.001	18	0.000				B[2]	-0.003	1	0.002
Cor Total	0.021	26					A[1]B[1]	0.003	1	0.003
							A[2]B[1]	0.001	1	0.003
Std. Dev.	0.008		R-Squared	0.94			A[1]B[2]	-0.001	1	0.003
Mean	0.383		Adj R-Squared	0.91			A[2]B[2]	0.001	1	0.003
C.V. %	2.193		Pred R-Squared	0.86						
PRESS	0.003		Adeq Precision	16.52						

In this case A, B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R^2 value of 0.94 whereas the optimized predictive model had an R^2 value of 0.86 was in reasonable agreement with the Adjusted R^2 of 0.91. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 16.52 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for water activity obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\text{Water activity} = 0.383 + 0.034*A - 0.011*A^2 + 0.014*B - 2.593E-003*B^2 + 2.593E-003*A*B + 1.481E-003*A^2*B - 7.407E-004*A*B^2 + 1.481E-003*A^2*B^2 \quad \dots (4.15)$$

4.9.10 Effect of different drying and milling methods on titratable acidity of calyces of Roselle powder

The effect of different drying and milling methods on titratable acidity of calyces of Roselle powder were analyzed statistically and presented in Table 21. From the data, it was observed that in solar tunnel drying, the titratable acidity of calyces of Roselle powder were found to be in the range of 16.73% to 18.05% among the different milling methods. Similarly in shade drying, the titratable acidity of calyces of Roselle powder were found to be in the range of 18.01% to 20.82% and in dehumidified air drying, the titratable acidity of calyces of Roselle powder were found to be in the range of 18.30% to 21.40% among the different milling methods.

In case of different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly higher titratable acidity of 21.40% and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had titratable acidity of 20.76%. In case of milling with normal pulverizer, calyces of Roselle powder showed significantly lower titratable acidity of 16.73%.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had lower titratable acidity (16.73%)

compared to all other treatments. Whereas, in case of calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the higher titratable acidity of 21.40%.

Table 31 shows the analysis of variance (ANOVA) and estimated coefficient values for titratable acidity of calyces of Roselle powder. The Model (2FI) F-value of 51.30 implies that the model was significant ($p < 0.0001$). Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B, AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R² value of 0.96 whereas the optimized predictive model had an R² value of 0.91 was in reasonable agreement with the Adjusted R² of 0.94. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 19.85 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for titratable acidity obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\begin{aligned} \text{Titratable acidity (\%)} = & 18.982 - 1.617*A + 0.446*A^2 - 1.304*B + 0.197*B^2 + 0.664*A*B - \\ & 0.119*A^2*B - 0.239*A*B^2 - 0.168*A^2*B^2 \end{aligned} \quad \dots (4.16)$$

4.9.11 Effect of different drying and milling methods on ascorbic acid of calyces of Roselle powder

The effect of different drying and milling methods on ascorbic acid of calyces of Roselle powder were analyzed statistically and presented in Table 21. From the data, it was observed that in solar tunnel drying, the ascorbic acid of calyces of Roselle powder were found to be in the range of 16.68 to 22.61 mg.100g⁻¹ among the different milling methods. Similarly in shade drying, the ascorbic acid of calyces of Roselle powder were found to be in the range of 26.29 to 37.88 mg.100g⁻¹ and in dehumidified air drying, the ascorbic acid of calyces of Roselle powder were found to be in the range of 28.55 to 40.42 mg.100g⁻¹ among the different milling methods.

Table 31. Analysis of variance (ANOVA) of titratable acidity in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	68.176	8	8.522	51.30	< 0.0001	Significant	Intercept	18.982	1	0.078
A-Drying method	37.648	2	18.824	113.32	< 0.0001		A[1]	-1.617	1	0.111
B-Milling method	26.673	2	13.337	80.27	< 0.0001		A[2]	0.446	1	0.111
AB	3.854	4	0.964	5.80	0.0035		B[1]	-1.304	1	0.111
Pure Error	2.990	18	0.166				B[2]	0.197	1	0.111
Cor Total	71.166	26					A[1]B[1]	0.664	1	0.157
							A[2]B[1]	-0.119	1	0.157
Std. Dev.	0.408		R-Squared	0.96			A[1]B[2]	-0.239	1	0.157
Mean	18.982		Adj R-Squared	0.94			A[2]B[2]	-0.168	1	0.157
C.V. %	2.147		Pred R-Squared	0.91						
PRESS	6.728		Adeq Precision	19.85						

Considering the different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly higher ascorbic acid of 40.42 mg.100g⁻¹ and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had ascorbic acid of 35.05 mg.100g⁻¹. In case of milling with normal pulverizer, calyces of Roselle powder showed significantly lower ascorbic acid of 16.68 mg.100g⁻¹.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had lower ascorbic acid (16.68 mg.100g⁻¹) compared to all other treatments. Whereas, in case of calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the higher ascorbic acid of 40.42 mg.100g⁻¹.

Table 32 shows the analysis of variance (ANOVA) and estimated coefficient values for ascorbic acid of calyces of Roselle powder. The Model (2FI) F-value of 715.66 implies that the model was significant (p<0.0001). Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B, AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R² value of 0.99 whereas the optimized predictive model had an R² value of 0.99 was in reasonable agreement with the Adjusted R² of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 75.98 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for ascorbic acid obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\text{Ascorbic acid (mg.100 g}^{-1}\text{)} = 28.769 - 9.359*A + 3.455*A^2 - 4.931*B + 0.063*B^2 + 2.199A \\ *B - 1.005 *A^2*B - 0.534*A*B^2 + 0.220*A^2*B^2 \quad \dots (4.17)$$

Table 32. Analysis of variance (ANOVA) of ascorbic acid in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	1677.323	8	209.665	715.66	< 0.0001	Significant	Intercept	28.769	1	0.104
A-Drying method	1209.586	2	604.793	2064.36	< 0.0001		A[1]	-9.359	1	0.147
B-Milling method	432.155	2	216.077	737.55	< 0.0001		A[2]	3.455	1	0.147
AB	35.582	4	8.896	30.36	< 0.0001		B[1]	-4.931	1	0.147
Pure Error	5.273	18	0.293				B[2]	0.063	1	0.147
Cor Total	1682.597	26					A[1]B[1]	2.199	1	0.208
							A[2]B[1]	-1.005	1	0.208
Std. Dev.	0.541		R-Squared	0.99			A[1]B[2]	-0.534	1	0.208
Mean	28.769		Adj R-Squared	0.99			A[2]B[2]	0.220	1	0.208
C.V. %	1.881		Pred R-Squared	0.99						
PRESS	11.865		Adeq Precision	75.98						

4.9.12 Effect of different drying and milling methods on solubility index of calyces of Roselle powder

The effect of different drying and milling methods on solubility index of calyces of Roselle powder were analyzed statistically and presented in Table 21. From the data, it was observed that in solar tunnel drying, the solubility index of calyces of Roselle powder were found to be in the range of 0.26 to 0.28 g.g⁻¹ among the different milling methods. Similarly in shade drying, the solubility index of calyces of Roselle powder were found to be in the range of 0.29 to 0.32 g.g⁻¹ and in dehumidified air drying, the solubility index of calyces of Roselle powder were found to be in the range of 0.30 to 0.33 g.g⁻¹ among the different milling methods.

In case of different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly higher solubility index of 0.33 g.g⁻¹ and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had solubility index of 0.33 g.g⁻¹. In case of milling with normal pulverizer, calyces of Roselle powder showed significantly lower solubility index of 0.26 g.g⁻¹.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had lower solubility index (0.26 g.g⁻¹) compared to all other treatments. Whereas, in case of calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the higher solubility index of 0.33 g.g⁻¹.

Table 33 shows the analysis of variance (ANOVA) and estimated coefficient values for solubility index of calyces of Roselle powder. The Model (2FI) F-value of 18.60 implies that the model was significant ($p < 0.0001$). Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R² value of 0.89 whereas the optimized predictive model had an R² value of 0.76 was in reasonable agreement with the Adjusted R² of 0.84. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 12.47 indicates an adequate signal. Hence, this model could be used to navigate the design space.

Table 33. Analysis of variance (ANOVA) of solubility index in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	0.015	8	0.002	18.60	< 0.0001	Significant	Intercept	0.298	1	0.002
A-Drying method	0.011	2	0.005	53.78	< 0.0001		A[1]	-0.027	1	0.003
B-Milling method	0.004	2	0.002	18.96	< 0.0001		A[2]	0.005	1	0.003
AB	0.000	4	0.000	0.82	0.5300		B[1]	-0.016	1	0.003
Pure Error	0.002	18	0.000				B[2]	0.004	1	0.003
Cor Total	0.017	26					A[1]B[1]	0.003	1	0.004
							A[2]B[1]	0.001	1	0.004
Std. Dev.	0.010		R-Squared	0.89			A[1]B[2]	0.000	1	0.004
Mean	0.298		Adj R-Squared	0.84			A[2]B[2]	-0.005	1	0.004
C.V. %	3.380		Pred R-Squared	0.76						
PRESS	0.004		Adeq Precision	12.47						

The optimized predictive model (in terms of actual factors) for solubility index obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\begin{aligned} \text{Solubility index (g.g}^{-1}\text{)} = & 0.298 - 0.027*A + 5.436E-003*A^2 - 0.016*B + 3.964E-003*B^2 \\ & + 3.320E-003*A*B + 6.976E-004*A^2*B + 1.461E-004*A*B^2 \\ & - 5.102E-003*A^2*B^2 \end{aligned} \quad \dots (4.18)$$

4.9.13 Effect of different drying and milling methods on particle size of calyces of Roselle powder

The effect of different drying and milling methods on particle size of calyces of Roselle powder were analyzed statistically and presented in Table 21. From the data, it was observed that in solar tunnel drying, the particle size of calyces of Roselle powder were found to be in the range of 3419.00 to 3801.33 d.nm among the different milling methods. Similarly in shade drying, the particle size of calyces of Roselle powder were found to be in the range of 2638.33 to 3485.67 d.nm and in dehumidified air drying, the particle size of calyces of Roselle powder were found to be in the range of 2519.33 to 2972.00 d.nm among the different milling methods.

Considering the different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly lower particle size of 2519.33 d.nm and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had particle size of 2619.33 d.nm. In case of milling with normal pulverizer, calyces of Roselle powder showed significantly higher particle size of 3801.33 d.nm.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had higher particle size (3801.33 d.nm) compared to all other treatments. Whereas, in case of calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the lower particle size of 2519.33 d.nm.

Table 34 shows the analysis of variance (ANOVA) and estimated coefficient values for particle size of calyces of Roselle powder. The Model (2FI) F-value of 33.92 implies that the model was significant ($p < 0.0001$).

Table 34. Analysis of variance (ANOVA) of particle size in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	Df	Standard Error
Model	5272514.296	8	659064.287	33.92	< 0.0001	Significant	Intercept	3122.630	1	26.828
A-Drying method	3633372.074	2	1816686.037	93.49	< 0.0001		A[1]	474.370	1	37.940
B-Milling method	1430089.407	2	715044.704	36.80	< 0.0001		A[2]	-55.296	1	37.940
AB	209052.815	4	52263.204	2.69	0.0644		B[1]	297.037	1	37.940
Pure Error	349788.000	18	19432.667				B[2]	-33.296	1	37.940
Cor Total	5622302.296	26					A[1]B[1]	-92.704	1	53.656
							A[2]B[1]	121.296	1	53.656
Std. Dev.	139.401		R-Squared	0.94			A[1]B[2]	6.963	1	53.656
Mean	3122.630		Adj R-Squared	0.91			A[2]B[2]	43.963	1	53.656
C.V. %	4.464		Pred R-Squared	0.86						
PRESS	787023.000		Adeq Precision	15.93						

Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B, AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R² value of 0.94 whereas the optimized predictive model had an R² value of 0.86 was in reasonable agreement with the Adjusted R² of 0.91. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 15.93 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for particle size obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\text{Particle size (d.nm)} = 3122.630 + 474.370 * A - 55.296 * A^2 + 297.037 * B - 33.296 * B^2 - 92.704 * A * B + 121.296 * A^2 * B + 6.963 * A * B^2 + 43.963 * A^2 * B^2 \quad \dots (4.19)$$

4.10 Functional properties of calyces of Roselle powder

Functional properties viz., total phenolic content, antioxidant activity, total flavonoids and total anthocyanin content of calyces of Roselle powder obtained from different drying and milling methods are given in Table 35. The detailed results are presented hereunder.

4.10.1 Effect of different drying and milling methods on total phenolic content of calyces of Roselle powder

The effect of different drying and milling methods on total phenolic content of calyces of Roselle powder were analyzed statistically and presented in Table 35. From the data, it was observed that in solar tunnel drying, the total phenolic content of calyces of Roselle powder were found to be in the range of 14.66 to 16.12 mg GAE.g⁻¹ among the different milling methods. Similarly in shade drying, the total phenolic content of calyces of Roselle powder were found to be in the range of 16.47 to 18.65 mg GAE.g⁻¹ and in dehumidified air drying, the total phenolic content of calyces of Roselle powder were found to be in the range of 17.32 to 20.66 mg GAE.g⁻¹ among the different milling methods.

Table 35. Effect of different drying and milling methods on functional properties of calyces of Roselle powder

Sl. No.	Drying method	STD			SD			DD		
	Milling method	NP	WCP	LN ₂ CP	NP	WCP	LN ₂ CP	NP	WCP	LN ₂ CP
1	Total phenolic content (mg GAE.g ⁻¹)	14.66	15.25	16.12	16.47	18.31	18.65	17.32	19.47	20.66
2	Antioxidant activity (%)	23.67	25.27	32.46	33.84	40.96	47.49	36.82	42.85	48.58
3	Total flavonoids (mg RE.g ⁻¹)	15.85	17.54	21.79	21.76	24.22	27.39	22.29	24.33	27.85
4	Total anthocyanin content (mg CyaG.100g ⁻¹)	383.12	400.30	440.85	518.07	543.53	561.06	539.42	557.07	593.86

Considering the different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly higher total phenolic content of 20.66 mg GAE.g⁻¹ and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had total phenolic content of 19.47 mg GAE.g⁻¹. In case of milling with normal pulverizer, calyces of Roselle powder showed significantly lower total phenolic content of 14.66 mg GAE.g⁻¹.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had lower total phenolic content (14.66 mg GAE.g⁻¹) compared to all other treatments. Whereas, in case of calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the higher total phenolic content of 20.66 mg GAE.g⁻¹.

Table 36 shows the analysis of variance (ANOVA) and estimated coefficient values for total phenolic content of calyces of Roselle powder. The Model (2FI) F-value of 493.07 implies that the model was significant (p<0.0001). Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B, AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R² value of 0.99 whereas the optimized predictive model had an R² value of 0.98 was in reasonable agreement with the Adjusted R² of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 66.68 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for total phenolic content obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\begin{aligned} \text{Total phenolic content (mg GAE.g}^{-1}\text{)} = & 17.434 - 2.091*A + 0.376*A^2 - 1.286*B + 0.243*B^2 \\ & + 0.602*A*B - 0.058*A^2*B - 0.333*A*B^2 + 0.257 \\ & *A^2*B^2 \quad \dots (4.20) \end{aligned}$$

Table 36. Analysis of variance (ANOVA) of total phenolic content in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	95.823	8	11.978	493.07	< 0.0001	Significant	Intercept	17.434	1	0.030
A-Drying method	67.112	2	33.556	1381.33	< 0.0001		A[1]	-2.091	1	0.042
B-Milling method	25.183	2	12.591	518.32	< 0.0001		A[2]	0.376	1	0.042
AB	3.528	4	0.882	36.31	< 0.0001		B[1]	-1.286	1	0.042
Pure Error	0.437	18	0.024				B[2]	0.243	1	0.042
Cor Total	96.260	26					A[1]B[1]	0.602	1	0.060
							A[2]B[1]	-0.058	1	0.060
Std. Dev.	0.156		R-Squared	0.99			A[1]B[2]	-0.333	1	0.060
Mean	17.434		Adj R-Squared	0.99			A[2]B[2]	0.257	1	0.060
C.V. %	0.894		Pred R-Squared	0.98						
PRESS	0.984		Adeq Precision	66.68						

4.10.2 Effect of different drying and milling methods on antioxidant activity of calyces of Roselle powder

The effect of different drying and milling methods on antioxidant activity of calyces of Roselle powder were analyzed statistically and presented in Table 35. From the data, it was observed that in solar tunnel drying, the antioxidant activity of calyces of Roselle powder were found to be in the range of 23.67 to 32.46% among the different milling methods. Similarly in shade drying, the antioxidant activity of calyces of Roselle powder were found to be in the range of 33.84 to 47.49% and in dehumidified air drying, the antioxidant activity of calyces of Roselle powder were found to be in the range of 36.82 to 48.58% among the different milling methods.

In case of different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly higher antioxidant activity of 48.58% and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had antioxidant activity of 42.85%. In case of milling with normal pulverizer, calyces of Roselle powder showed significantly lower antioxidant activity of 23.67%.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had lower antioxidant activity (23.67%) compared to all other treatments. Whereas, in case of calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the lower antioxidant activity of 48.58%.

Table 37 shows the analysis of variance (ANOVA) and estimated coefficient values for antioxidant activity of calyces of Roselle powder. The Model (2FI) F-value of 5686.26 implies that the model was significant ($p < 0.0001$). Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B, AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R² value of 0.99 whereas the optimized predictive model had an R² value of 0.99 was in reasonable agreement with the Adjusted R² of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 210.04 indicates an adequate signal. Hence, this model could be used to navigate the design space.

Table 37. Analysis of variance (ANOVA) of antioxidant activity in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	1919.282	8	239.910	5686.26	< 0.0001	Significant	Intercept	36.884	1	0.040
A-Drying method	1300.400	2	650.200	15410.79	< 0.0001		A[1]	-9.747	1	0.056
B-Milling method	588.698	2	294.349	6976.54	< 0.0001		A[2]	3.881	1	0.056
AB	30.184	4	7.546	178.85	< 0.0001		B[1]	-5.439	1	0.056
Pure Error	0.759	18	0.042				B[2]	-0.524	1	0.056
Cor Total	1920.042	26					A[1]B[1]	1.977	1	0.079
							A[2]B[1]	-1.485	1	0.079
Std. Dev.	0.205		R-Squared	0.99			A[1]B[2]	-1.339	1	0.079
Mean	36.884		Adj R-Squared	0.99			A[2]B[2]	0.718	1	0.079
C.V. %	0.557		Pred R-Squared	0.99						
PRESS	1.709		Adeq Precision	210.04						

The optimized predictive model (in terms of actual factors) for antioxidant activity obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\text{Antioxidant activity (\%)} = 36.884 - 9.747*A + 3.881*A^2 - 5.439*B - 0.524*B^2 + 1.977*A*B - 1.485*A^2*B - 1.339*A*B^2 + 0.718*A^2*B^2 \quad \dots (4.21)$$

4.10.3 Effect of different drying and milling methods on total flavonoids of calyces of Roselle powder

The effect of different drying and milling methods on total flavonoids of calyces of Roselle powder were analyzed statistically and presented in Table 35. From the data, it was observed that in solar tunnel drying, the total flavonoids of calyces of Roselle powder were found to be in the range of 15.85 to 21.79 mg RE.g⁻¹ among the different milling methods. Similarly in shade drying, the total flavonoids of calyces of Roselle powder were found to be in the range of 21.76 to 27.39 mg RE.g⁻¹ and in dehumidified air drying, the total flavonoids of calyces of Roselle powder were found to be in the range of 22.29 to 27.85 mg RE.g⁻¹ among the different milling methods.

Considering the different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly higher total flavonoids of 27.85 mg RE.g⁻¹ and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had total flavonoids of 24.33 mg RE.g⁻¹. In case of milling with normal pulverizer, calyces of Roselle powder showed significantly lower total flavonoids of 15.85 mg RE.g⁻¹.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had lower total flavonoids (15.85 mg RE.g⁻¹) compared to all other treatments. Whereas, in case of calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the higher total flavonoids of 27.85 mg RE.g⁻¹.

Table 38 shows the analysis of variance (ANOVA) and estimated coefficient values for total flavonoids of calyces of Roselle powder. The Model (2FI) F-value of 1929.73 implies that the model was significant (p<0.0001).

Table 38. Analysis of variance (ANOVA) of total flavonoids in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	386.460	8	48.307	1929.73	< 0.0001	Significant	Intercept	22.559	1	0.030
A-Drying method	234.897	2	117.448	4691.68	< 0.0001		A[1]	-4.166	1	0.043
B-Milling method	150.583	2	75.291	3007.64	< 0.0001		A[2]	1.899	1	0.043
AB	0.980	4	0.245	9.79	0.0002		B[1]	-2.592	1	0.043
Pure Error	0.451	18	0.025				B[2]	-0.527	1	0.043
Cor Total	386.910	26					A[1]B[1]	0.046	1	0.061
							A[2]B[1]	-0.106	1	0.061
Std. Dev.	0.158		R-Squared	0.99			A[1]B[2]	-0.323	1	0.061
Mean	22.559		Adj R-Squared	0.99			A[2]B[2]	0.291	1	0.061
C.V. %	0.701		Pred R-Squared	0.99						
PRESS	1.014		Adeq Precision	131.44						

Values of "Prob> F" less than 0.05 indicate model terms were significant. In this case A, B, AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R² value of 0.99 whereas the optimized predictive model had an R² value of 0.99 was in reasonable agreement with the Adjusted R² of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 131.44 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for total flavonoids obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\text{Total flavonoids (mg RE.g}^{-1}\text{)} = 22.559 - 4.166*A + 1.899*A^2 - 2.592*B - 0.527*B^2 + 0.046*A*B - 0.106*A^2*B - 0.323*A*B^2 + 0.291*A^2*B^2 \quad \dots (4.22)$$

4.10.4 Effect of different drying and milling methods on total anthocyanin content of calyces of Roselle powder

The effect of different drying and milling methods on total anthocyanin content of calyces of Roselle powder were analyzed statistically and presented in Table 35. From the data, it was observed that in solar tunnel drying, the total anthocyanin content of calyces of Roselle powder were found to be in the range of 383.12 to 440.85 mg CyaG.100g⁻¹ among the different milling methods. Similarly in shade drying, the total anthocyanin content of calyces of Roselle powder were found to be in the range of 518.07 to 561.06 mg CyaG.100g⁻¹ and in dehumidified air drying, the total anthocyanin content of calyces of Roselle powder were found to be in the range of 539.42 to 593.86 mg CyaG.100g⁻¹ among the different milling methods.

In case of different milling methods, calyces of Roselle powder obtained from LN₂ cooled pulverizer showed significantly higher total anthocyanin content of 593.83 mg CyaG.100g⁻¹ and was followed by calyces of Roselle powder obtained by water cooled pulverizer which had total anthocyanin content of 557.07 mg CyaG.100g⁻¹. In case of milling with normal pulverizer, calyces of Roselle powder showed significantly lower total anthocyanin content of 383.12 mg CyaG.100g⁻¹.

The interaction between drying and milling conditions were analysed statistically and it was revealed from the results that, calyces of Roselle powder obtained by solar tunnel drying and milling with normal pulverizer had lower total anthocyanin content (383.12 mg CyaG.100g⁻¹) compared to all other treatments. Whereas, in case of calyces of Roselle powder obtained by dehumidified air drying and milling with LN₂ cooled pulverizer gave the higher total anthocyanin content of 593.86 mg CyaG.100g⁻¹.

Table 39 shows the analysis of variance (ANOVA) and estimated coefficient values for total anthocyanin content of calyces of Roselle powder. The Model (2FI) F-value of 1521.88 implies that the model was significant (p<0.0001). Values of "Prob>F" less than 0.05 indicate model terms were significant. In this case A, B, AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that proposed models fitted the experimental data with R² value of 0.99 whereas the optimized predictive model had an R² value of 0.98 was in reasonable agreement with the Adjusted R² of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 107.81 indicates an adequate signal. Hence, this model could be used to navigate the design space.

The optimized predictive model (in terms of actual factors) for total anthocyanin content obtained from different drying and milling methods of calyces of Roselle is presented in the following equation.

$$\begin{aligned} \text{Total anthocyanin content (mg CyaG.100g}^{-1}\text{)} = & 504.141 - 96.051*A + 36.746*A^2 - 23.938 \\ & *B - 3.843*B^2 - 1.027*A*B + 1.120*A^2*B - \\ & 3.949*A*B^2 + 6.486*A^2*B^2 \quad \dots (4.23) \end{aligned}$$

Table 39. Analysis of variance (ANOVA) of total anthocyanin content in calyces of Roselle powder for different drying and milling methods

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	139543.164	8	17442.895	1521.88	< 0.0001	Significant	Intercept	504.141	1	0.652
A-Drying method	126838.932	2	63419.466	5533.29	< 0.0001		A[1]	-96.051	1	0.921
B-Milling method	12236.366	2	6118.183	533.81	< 0.0001		A[2]	36.746	1	0.921
AB	467.866	4	116.967	10.21	0.0002		B[1]	-23.938	1	0.921
Pure Error	206.306	18	11.461				B[2]	-3.843	1	0.921
Cor Total	139749.470	26					A[1]B[1]	-1.027	1	1.303
							A[2]B[1]	1.120	1	1.303
Std. Dev.	3.385		R-Squared	0.99			A[1]B[2]	-3.949	1	1.303
Mean	504.141		Adj R-Squared	0.99			A[2]B[2]	6.486	1	1.303
C.V. %	0.672		Pred R-Squared	0.98						
PRESS	464.188		Adeq Precision	107.81						

4.11 Optimization of process parameters for drying and milling of calyces of Roselle

Optimization of two variables namely, drying method and milling method was performed using the general factorial design in Design Expert Software 7.7.0. Numerical optimization was performed using statistical models to find the optimal drying and milling method. In the present investigation, the independent variables were kept within the range and dependent variables were chosen as maximum and minimum. Table 40 shows the process parameters and responses of calyces of Roselle powder achieved from General Factorial in Design Expert Software 7.7.0. The calyces of Roselle powder obtained from optimized drying and milling methods is depicted in Plate 17.

4.11.1 Verification of the predicted variables

The optimum response values were tested using the recommended optimum conditions of the variables and was also used to validate the experimental and predicted values of the responses. The experimental results had the optimum process conditions of dehumidified air drying method and milling with LN₂ cooled pulverizer. This optimized parameter was carried out thrice and the average values of each response are presented. The verification of the predicted and actual values of the responses and the percentage variation at the optimized condition of experiments are presented in Table 41.

4.12 Cost of production of calyces of Roselle powder under different drying and milling methods

The cost of production of dried and milled calyces of Roselle obtained using different drying and milling methods was carried out and detailed calculation is presented in Appendix II. Estimated cost of production per 100 kg dried Roselle calyces was computed as ₹ 16,200, 16,500 and 48,300 for STD, SD and DD, respectively and Gross return was computed as ₹ 28,000, 25,000 and 70,000 for STD, SD and DD, respectively. Similarly, Net return obtained was ₹ 11,800, 8,500 and 21,200 with benefit cost ratio of 1.51, 1.50 and 1.45 for STD, SD and DD drying methods, respectively.

Estimated cost of production per 100 kg milled calyces of Roselle was computed as ₹ 49,900, 52,600 and 59,400 for NP, WCP and LN₂CP, respectively and Gross return was computed as ₹ 75,000, 80,000 and 1,00,000 for NP, WCP and LN₂CP, respectively. Similarly, Net return obtained was ₹ 25,100, 27,400 and 40,600 with benefit cost ratio of 1.50, 1.52 and 1.68 for NP, WCP and LN₂CP milling methods, respectively.

Table 40. Effect of different drying and milling methods on optimization of calyces of Roselle powder

Sl. No.	Parameter	Goal	Lower limit	Upper limit
1	Drying method	is in range	STD	DD
2	Milling method	is in range	NP	LN ₂ CP
3	Moisture content (% d.b.)	Minimize	8.96	9.41
4	Crude protein (%)	Maximize	7.78	9.60
5	Crude fat (%)	Maximize	2.15	3.05
6	Carbohydrates (%)	Minimize	56.00	62.21
7	Crude fibre (%)	Maximize	9.62	11.47
8	Total ash (%)	Maximize	7.71	10.68
9	L^*	Minimize	37.58	46.89
10	a^*	Maximize	17.71	23.97
11	b^*	Minimize	9.03	11.34
12	Loose bulk density (g.cc ⁻¹)	Minimize	0.53	0.77
13	Tapped bulk density(g.cc ⁻¹)	Minimize	0.45	0.63
14	Carr's index	Minimize	13.64	18.75
15	Hausner ratio	Minimize	1.16	1.23
16	pH	Minimize	2.71	3.37
17	Water activity	Minimize	0.35	0.44
18	Titrateable acidity (%)	Maximize	16.51	21.89
19	Ascorbic acid (mg.100g ⁻¹)	Maximize	16.11	40.70
20	Solubility index (g.g ⁻¹)	Maximize	0.25	0.36
21	Particle size (d.nm)	Minimize	2316.00	3828.00
22	Total phenolic content (mg GAE.g ⁻¹)	Maximize	14.55	20.93
23	Antioxidant activity (%)	Maximize	23.53	48.80
24	Total flavonoids (mg RE.g ⁻¹)	Maximize	15.65	28.25
25	Total anthocyanin content (mg CyaG.100g ⁻¹)	Maximize	380.86	596.60



Plate 17. Calyces of Roselle powder obtained from optimized drying and milling methods

Table 41. Predicted and actual values of responses for optimized drying and milling methods

Sl. No.	Responses	Predicted value	Actual value	Variation %
1	Moisture content (% d.b.)	8.99	9.23	0.099
2	Crude protein (%)	9.46	9.53	0.027
3	Crude fat (%)	2.98	2.94	-0.068
4	Carbohydrates (%)	56.52	55.63	-0.057
5	Crude fibre (%)	11.24	11.11	-0.043
6	Total ash (%)	10.57	10.99	0.147
7	L^*	38.04	36.56	-0.144
8	a^*	23.77	24.69	0.143
9	b^*	9.18	9.5	0.129
10	Loose bulk density (g.cc ⁻¹)	0.55	0.58	0.202
11	Tapped bulk density(g.cc ⁻¹)	0.47	0.45	-0.158
12	Carr's index	14.07	14.05	-0.005
13	Hausner ratio	1.16	1.15	-0.032
14	pH	2.77	2.75	-0.027
15	Water activity	0.35	0.33	-0.212
16	Titrateable acidity (%)	21.40	21.88	0.083
17	Ascorbic acid (mg.100g ⁻¹)	40.42	40.45	0.003
18	Solubility index (g.g ⁻¹)	0.33	0.35	0.224
19	Particle size (d.nm)	2519.33	2524.92	0.008
20	Total phenolic content (mg GAE.g ⁻¹)	20.66	20.21	-0.081
21	Antioxidant activity (%)	48.58	47.98	-0.046
22	Total flavonoids (mg RE.g ⁻¹)	27.85	27.59	-0.035
23	Total anthocyanin content (mg CyaG.100g ⁻¹)	593.86	591.39	-0.015
24	Desirability	0.93		

4.13 Extraction of anthocyanin from calyces of Roselle powder

The optimized calyces of Roselle powder was subjected to solvent extraction of anthocyanin using three different solid to solvent ratio and concentration of citric acid (%) and were studied for physico-chemical and functional properties. The detailed results are presented hereunder.

4.14 Physico-chemical properties of solvent extracted anthocyanin from calyces of Roselle

The physico-chemical properties of solvent extracted anthocyanin from calyces of Roselle viz., extraction yield, pH, TSS, colour (L^* , a^* and b^*) and titratable acidity were studied and presented hereunder.

4.14.1 Effect of solid to solvent ratios and concentration of citric acid (%) on extraction yield of solvent extracted anthocyanin from calyces of Roselle

The extraction yield of solvent extracted anthocyanin from calyces of Roselle obtained from different solid to solvent ratios and concentration of citric acid (%) using ethanol as a solvent are presented in Table 42. From the table, it is noticed that the extraction yield of Roselle anthocyanin extract was found to be in the range of 54.67 to 68.00% using 1:5 solid to solvent ratio. Similarly, using 1:10 solid to solvent ratio the extraction yield was found to be in the range of 93.33 to 97.33% whereas, in case of Roselle anthocyanin extract obtained using 1:15 solid to solvent ratio the extraction yield was found to be in the range of 87.33 to 91.33%.

In case of different solid to solvent ratios and concentration of citric acid (%) interaction, it is revealed that the Roselle anthocyanin extract obtained from 1:10 solid to solvent ratio with 2% citric acid showed highest extraction yield of 97.33 whereas, minimum of 54.67% was recorded in case of 1:5 solid to solvent ratio with 1% citric acid. The extraction yield of Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio and 70% ethanol (control) was found to be 48.67%.

Table 43 shows the analysis of variance (ANOVA) and estimated coefficient values for extraction yield of solvent extracted anthocyanin from calyces of Roselle. The Model (2FI) F-value of 160.51 implies that the model was significant ($p < 0.0001$). Values of “Prob>F” less than 0.05 indicate model terms were significant.

Table 42. Effect of different solid to solvent ratios and concentration of citric acid (%) on physico-chemical properties of solvent extracted anthocyanin from calyces of Roselle

Treatment	Solid: solvent	% citric acid	Extraction yield (%)	pH	TSS (°Brix)	<i>L</i>[*]	<i>a</i>[*]	<i>b</i>[*]	Titrateable acidity (%)
E1	1:5	1%	54.67	1.48	32.43	3.24	0.29	0.18	17.15
E2	1:5	2%	68.00	1.39	34.50	3.14	0.38	0.13	17.66
E3	1:5	3%	58.67	1.30	33.90	3.09	0.34	0.14	18.07
E4	1:10	1%	93.33	1.29	38.23	2.10	0.62	0.11	21.18
E5	1:10	2%	97.33	1.27	45.60	1.74	0.75	0.06	22.46
E6	1:10	3%	95.33	1.19	39.67	1.56	0.66	0.09	23.94
E7	1:15	1%	87.33	1.39	35.73	1.45	0.57	0.22	19.43
E8	1:15	2%	91.33	1.33	43.57	1.38	0.65	0.16	20.69
E9	1:15	3%	90.67	1.31	38.73	1.26	0.60	0.19	20.82
Control	1:5	0	48.67	1.53	30.50	4.14	0.25	0.19	15.68

Table 43. Analysis of variance (ANOVA) of extraction yield in solvent extracted anthocyanin from calyces of Roselle for different solid to solvent ratios and concentration of citric acid (%)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	6658.074	8	832.259	160.51	< 0.0001	Significant	Intercept	81.852	1	0.438
A-Solid:solvent	6325.630	2	3162.815	609.97	< 0.0001		A[1]	-21.407	1	0.620
B-Citric acid	228.741	2	114.370	22.06	< 0.0001		A[2]	13.481	1	0.620
AB	103.704	4	25.926	5.00	0.0069		B[1]	-3.407	1	0.620
Pure Error	93.333	18	5.185				B[2]	3.704	1	0.620
Cor Total	6751.407	26					A[1]B[1]	-2.370	1	0.876
							A[2]B[1]	1.407	1	0.876
Std. Dev.	2.277		R-Squared	0.99			A[1]B[2]	3.852	1	0.876
Mean	81.852		Adj R-Squared	0.98			A[2]B[2]	-1.704	1	0.876
C.V. %	2.782		Pred R-Squared	0.97						
PRESS	210.000		Adeq Precision	32.45						

In this case, A, B and AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.97 and was in reasonable agreement with the "Adj R-Squared" of 0.98. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 32.45 indicates an adequate signal. Hence, this model could be used to navigate the design space. The optimized predictive model (in terms of actual factors) for extraction yield of solvent extracted anthocyanin from calyces of Roselle is presented in the following equation.

$$\begin{aligned} \text{Extraction yield (\%)} = & 81.852 - 21.407*A + 13.481*A^2 - 3.407*B + 3.704*B^2 - 2.370*A*B \\ & + 1.407*A^2*B + 3.852*A*B^2 - 704*A^2*B^2 \quad \dots (4.24) \end{aligned}$$

4.14.2 Effect of solid to solvent ratios and concentration of citric acid (%) on pH of solvent extracted anthocyanin from calyces of Roselle

The pH of solvent extracted anthocyanin from calyces of Roselle obtained from different solid to solvent ratios and concentration of citric acid (%) using ethanol as a solvent are presented in Table 42. From the table, it is noticed that the pH of Roselle anthocyanin extract was found to be in the range of 1.30 to 1.48 using 1:5 solid to solvent ratio. Similarly, using 1:10 solid to solvent ratio the pH was found to be in the range of 1.19 to 1.29 whereas, in case of Roselle anthocyanin extract obtained using 1:15 solid to solvent ratio the pH was found to be in the range of 1.31 to 1.39.

In case of different solid to solvent ratios and concentration of citric acid (%) interaction, it is revealed that the Roselle anthocyanin extract obtained from 1:10 solid to solvent ratio with 3% citric acid showed lowest pH of 1.19 whereas, maximum of 1.48 pH was recorded in case of 1:5 solid to solvent ratio with 1% citric acid. The pH of Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio and 70% ethanol (control) was found to be 1.53.

Table 44 shows the analysis of variance (ANOVA) and estimated coefficient values for pH of solvent extracted anthocyanin from calyces of Roselle. The Model (2FI) F-value of 174.95 implies that the model was significant ($p < 0.0001$). Values of "Prob>F" less than 0.05 indicate model terms were significant.

Table 44. Analysis of variance (ANOVA) of pH in solvent extracted anthocyanin from calyces of Roselle for different solid to solvent ratios and concentration of citric acid (%)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	0.166	8	0.021	174.95	< 0.0001	Significant	Intercept	1.328	1	0.002
A-Solid:solvent	0.092	2	0.046	387.22	< 0.0001		A[1]	0.062	1	0.003
B-Citric acid	0.065	2	0.032	273.88	< 0.0001		A[2]	-0.078	1	0.003
AB	0.009	4	0.002	19.34	< 0.0001		B[1]	0.059	1	0.003
Pure Error	0.002	18	0.000				B[2]	0.003	1	0.003
Cor Total	0.168	26					A[1]B[1]	0.028	1	0.004
							A[2]B[1]	-0.015	1	0.004
Std. Dev.	0.011		R-Squared	0.99			A[1]B[2]	-0.003	1	0.004
Mean	1.328		Adj R-Squared	0.98			A[2]B[2]	0.017	1	0.004
C.V. %	0.820		Pred R-Squared	0.97						
PRESS	0.005		Adeq Precision	46.14						

In this case, A, B and AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.97 and was in reasonable agreement with the "Adj R-Squared" of 0.98. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 46.14 indicates an adequate signal. Hence, this model could be used to navigate the design space. The optimized predictive model (in terms of actual factors) for pH of solvent extracted anthocyanin from calyces of Roselle is presented in the following equation.

$$\text{pH} = 1.328 + 0.062*A - 0.078*A^2 + 0.059*B + 2.963E-003*B^2 + 0.028*A*B - 0.015*A^2*B - 2.963E-003*A*B^2 + 0.017*A^2*B^2 \quad \dots (4.25)$$

4.14.3 Effect of solid to solvent ratios and concentration of citric acid (%) on TSS of solvent extracted anthocyanin from calyces of Roselle

The TSS of solvent extracted anthocyanin from calyces of Roselle obtained from different solid to solvent ratios and concentration of citric acid (%) using ethanol as a solvent are presented in Table 42. From the table, it is noticed that the TSS of extract was found to be in the range of 32.43 to 34.50 °Brix using 1:5 solid to solvent ratio. Similarly, using 1:10 solid to solvent ratio the TSS was found to be in the range of 38.23 to 45.60 °Brix whereas, in case of Roselle anthocyanin extract obtained using 1:15 solid to solvent ratio the TSS was found to be in the range of 35.73 to 43.57 °Brix.

Considering the different solid to solvent ratios and concentration of citric acid (%) interaction, it is revealed that the Roselle anthocyanin extract obtained from 1:10 solid to solvent ratio with 2% citric acid showed highest TSS of 45.60 °Brix whereas, minimum of 32.43 °Brix was recorded in case of 1:5 solid to solvent ratio with 1% citric acid. The TSS of Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio and 70% ethanol (control) was found to be 30.50 °Brix.

Table 45 shows the analysis of variance (ANOVA) and estimated coefficient values for TSS of solvent extracted anthocyanin from calyces of Roselle. The Model (2FI) F-value of 1769.49 implies that the model was significant ($p < 0.0001$). Values of "Prob>F" less than 0.05 indicate model terms were significant.

Table 45. Analysis of variance (ANOVA) of TSS in solvent extracted anthocyanin from calyces of Roselle for different solid to solvent ratios and concentration of citric acid (%)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	471.865	8	58.983	1769.49	< 0.0001	Significant	Intercept	38.041	1	0.035
A-Solid:solvent	279.834	2	139.917	4197.51	< 0.0001		A[1]	-4.430	1	0.050
B-Citric acid	154.050	2	77.025	2310.74	< 0.0001		A[2]	3.126	1	0.050
AB	37.981	4	9.495	284.86	< 0.0001		B[1]	-2.574	1	0.050
Pure Error	0.600	18	0.033				B[2]	3.181	1	0.050
Cor Total	472.465	26					A[1]B[1]	1.396	1	0.070
							A[2]B[1]	-0.359	1	0.070
Std. Dev.	0.183		R-Squared	0.99			A[1]B[2]	-2.293	1	0.070
Mean	38.041		Adj R-Squared	0.99			A[2]B[2]	1.252	1	0.070
C.V. %	0.480		Pred R-Squared	0.99						
PRESS	1.350		Adeq Precision	124.91						

In this case, A, B and AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.99 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 124.91 indicates an adequate signal. Hence, this model could be used to navigate the design space. The optimized predictive model (in terms of actual factors) for TSS of solvent extracted anthocyanin from calyces of Roselle is presented in the following equation.

$$\text{TSS (°Brix)} = 38.041 - 4.430*A + 3.126*A^2 - 2.574*B + 3.181*B^2 + 1.396*A*B - 0.359*A^2*B - 2.293*A*B^2 + 1.252*A^2*B^2 \quad \dots (4.26)$$

4.14.4 Effect of solid to solvent ratios and concentration of citric acid (%) on L^* value of solvent extracted anthocyanin from calyces of Roselle

The L^* value of solvent extracted anthocyanin from calyces of Roselle obtained from different solid to solvent ratios and concentration of citric acid (%) using ethanol as a solvent are presented in Table 42. From the table, it is noticed that the L^* value of Roselle anthocyanin extract was found to be in the range of 3.09 to 3.24 using 1:5 solid to solvent ratio. Similarly, using 1:10 solid to solvent ratio the L^* value was found to be in the range of 1.56 to 2.10 whereas, in case of Roselle anthocyanin extract obtained using 1:15 solid to solvent ratio the L^* value was found to be in the range of 1.26 to 1.45.

In case of the different solid to solvent ratios and concentration of citric acid (%) interaction, it is revealed that the Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio with 1% citric acid showed highest L^* value of 3.24 whereas, minimum of 1.26 was recorded in case of 1:15 solid to solvent ratio with 3% citric acid. The L^* value of Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio and 70% ethanol (control) was found to be 4.14.

Table 46 shows the analysis of variance (ANOVA) and estimated coefficient values for L^* value of solvent extracted anthocyanin from calyces of Roselle. The Model (2FI) F-value of 4510.08 implies that the model was significant ($p < 0.0001$). Values of "Prob>F" less than 0.05 indicate model terms were significant.

Table 46. Analysis of variance (ANOVA) of L^* value in solvent extracted anthocyanin from calyces of Roselle for different solid to solvent ratios and concentration of citric acid (%)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	16.303	8	2.038	4510.08	< 0.0001	Significant	Intercept	2.106	1	0.004
A-Solid:solvent	15.748	2	7.874	17426.00	< 0.0001		A[1]	1.050	1	0.006
B-Citric acid	0.397	2	0.198	438.84	< 0.0001		A[2]	-0.307	1	0.006
AB	0.159	4	0.040	87.73	< 0.0001		B[1]	0.158	1	0.006
Pure Error	0.008	18	0.000				B[2]	-0.022	1	0.006
Cor Total	16.311	26					A[1]B[1]	-0.075	1	0.008
							A[2]B[1]	0.146	1	0.008
Std. Dev.	0.021		R-Squared	0.99			A[1]B[2]	0.002	1	0.008
Mean	2.106		Adj R-Squared	0.99			A[2]B[2]	-0.040	1	0.008
C.V. %	1.009		Pred R-Squared	0.99						
PRESS	0.018		Adeq Precision	161.33						

In this case, A, B and AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.99 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 161.33 indicates an adequate signal. Hence, this model could be used to navigate the design space. The optimized predictive model (in terms of actual factors) for L^* value of solvent extracted anthocyanin from calyces of Roselle is presented in the following equation.

$$L^* \text{ value} = 2.106 + 1.050 * A - 0.307 * A^2 + 0.158 * B - 0.022 * B^2 - 0.075 * A * B + 0.146 * A^2 * B + 1.852E-003 * A * B^2 - 0.040 * A^2 \quad \dots (4.27)$$

4.14.5 Effect of solid to solid to solvent ratios and concentration of citric acid (%) on a^* value of solvent extracted anthocyanin from calyces of Roselle

The a^* value of solvent extracted anthocyanin from calyces of Roselle obtained from different solid to solvent ratios and concentration of citric acid (%) using ethanol as a solvent are presented in Table 42. From the table, it is noticed that the a^* value of Roselle anthocyanin extract was found to be in the range of 0.29 to 0.38 using 1:5 solid to solvent ratio. Similarly, using 1:10 solid to solvent ratio the a^* value was found to be in the range of 0.62 to 0.75 whereas, in case of Roselle anthocyanin extract obtained using 1:15 solid to solvent ratio the a^* value was found to be in the range of 0.57 to 0.65.

Considering the different solid to solvent ratios and concentration of citric acid (%) interaction, it is revealed that the Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio with 1% citric acid showed lowest a^* value of 0.29 whereas, maximum of 0.75 was recorded in case of 1:10 solid to solvent ratio with 2% citric acid. The a^* value of Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio and 70% ethanol (control) was found to be 0.25.

Table 47 shows the analysis of variance (ANOVA) and estimated coefficient values for a^* value of solvent extracted anthocyanin from calyces of Roselle. The Model (2FI) F-value of 687.12 implies that the model was significant ($p < 0.0001$). Values of "Prob>F" less than 0.05 indicate model terms were significant.

Table 47. Analysis of variance (ANOVA) of a^* value in solvent extracted anthocyanin from calyces of Roselle for different solid to solvent ratios and concentration of citric acid (%)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	0.631	8	0.079	687.12	< 0.0001	Significant	Intercept	0.540	1	0.002
A-Solid:solvent	0.583	2	0.292	2539.26	< 0.0001		A[1]	-0.203	1	0.003
B-Citric acid	0.046	2	0.023	198.58	< 0.0001		A[2]	0.139	1	0.003
AB	0.002	4	0.001	5.32	0.0052		B[1]	-0.047	1	0.003
Pure Error	0.002	18	0.000				B[2]	0.053	1	0.003
Cor Total	0.633	26					A[1]B[1]	0.000	1	0.004
							A[2]B[1]	-0.009	1	0.004
Std. Dev.	0.011		R-Squared	0.99			A[1]B[2]	-0.010	1	0.004
Mean	0.540		Adj R-Squared	0.99			A[2]B[2]	0.018	1	0.004
C.V. %	1.984		Pred R-Squared	0.98						
PRESS	0.005		Adeq Precision	74.36						

In this case, A, B and AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.98 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 74.36 indicates an adequate signal. Hence, this model could be used to navigate the design space. The optimized predictive model (in terms of actual factors) for a^* value of solvent extracted anthocyanin from calyces of Roselle is presented in the following equation.

$$a^* \text{ value} = 0.540 - 0.203*A + 0.139*A^2 - 0.047*B + 0.053*B^2 + 0.000*A*B - 8.889E-003*A^2 * B - 0.010*A*B^2 + 0.018*A^2*B^2 \quad \dots (4.28)$$

4.14.6 Effect of solid to solvent ratios and concentration of citric acid (%) on b^* value of solvent extracted anthocyanin from calyces of Roselle

The b^* value of solvent extracted anthocyanin from calyces of Roselle obtained from different solid to solvent ratios and concentration of citric acid (%) using ethanol as a solvent are presented in Table 42. From the table, it is noticed that the b^* value of Roselle anthocyanin extract was found to be in the range of 0.13 to 0.18 using 1:5 solid to solvent ratio. Similarly, using 1:10 solid to solvent ratio the b^* value was found to be in the range of 0.06 to 0.11 whereas, in case of Roselle anthocyanin extract obtained using 1:15 solid to solvent ratio the b^* value was found to be in the range of 0.16 to 0.22.

In case of the different solid to solvent ratios and concentration of citric acid (%) interaction, it is revealed that the Roselle anthocyanin extract obtained from 1:10 solid to solvent ratio with 2% citric acid showed lowest b^* value of 0.06 whereas, maximum of 0.22 was recorded in case of 1:15 solid to solvent ratio with 1% citric acid. The b^* value of Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio and 70% ethanol (control) was found to be 0.19.

Table 48 shows the analysis of variance (ANOVA) and estimated coefficient values for b^* value of solvent extracted anthocyanin from calyces of Roselle. The Model (2FI) F-value of 157.15 implies that the model was significant ($p < 0.0001$). Values of "Prob>F" less than 0.05 indicate model terms were significant.

Table 48. Analysis of variance (ANOVA) of b^* value in solvent extracted anthocyanin from calyces of Roselle for different solid to solvent ratios and concentration of citric acid (%)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	0.061	8	0.008	157.15	< 0.0001	Significant	Intercept	0.143	1	0.001
A-Solid:solvent	0.048	2	0.024	503.31	< 0.0001		A[1]	0.006	1	0.002
B-Citric acid	0.012	2	0.006	122.08	< 0.0001		A[2]	-0.054	1	0.002
AB	0.000	4	0.000	1.62	0.2136		B[1]	0.026	1	0.002
Pure Error	0.001	18	0.000				B[2]	-0.026	1	0.002
Cor Total	0.061	26					A[1]B[1]	0.002	1	0.003
							A[2]B[1]	-0.004	1	0.003
Std. Dev.	0.007		R-Squared	0.99			A[1]B[2]	0.003	1	0.003
Mean	0.143		Adj R-Squared	0.98			A[2]B[2]	0.000	1	0.003
C.V. %	4.841		Pred R-Squared	0.97						
PRESS	0.002		Adeq Precision	39.11						

In this case, A and B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.97 and was in reasonable agreement with the "Adj R-Squared" of 0.98. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 39.11 indicates an adequate signal. Hence, this model could be used to navigate the design space. The optimized predictive model (in terms of actual factors) for b^* value of solvent extracted anthocyanin from calyces of Roselle is presented in the following equation.

$$b^* \text{ value} = 0.143 + 5.556\text{E-}003 * A - 0.054 * A^2 + 0.026 * B - 0.026 * B^2 + 2.222\text{E-}003 * A * B - 4.444\text{E-}003 * A^2 * B + 3.333\text{E-}003 * A * B^2 + 0.000 * A^2 * B^2 \quad \dots (4.29)$$

4.14.7 Effect of solid to solvent ratios and concentration of citric acid (%) on titratable acidity of solvent extracted anthocyanin from calyces of Roselle

The titratable acidity of solvent extracted anthocyanin from calyces of Roselle obtained from different solid to solvent ratios and concentration of citric acid (%) using ethanol as a solvent are presented in Table 42. From the table, it is noticed that the titratable acidity of Roselle anthocyanin extract was found to be in the range of 17.15 to 18.07% using 1:5 solid to solvent ratio. Similarly, using 1:10 solid to solvent ratio the titratable acidity was found to be in the range of 21.18 to 23.94% whereas, in case of Roselle anthocyanin extract obtained using 1:15 solid to solvent ratio the titratable acidity was found to be in the range of 19.43 to 20.82%.

Considering the different solid to solvent ratios and concentration of citric acid (%) interaction, it is revealed that the Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio with 1% citric acid showed lowest titratable acidity of 17.15% whereas, maximum of 23.94% was recorded in case of 1:10 solid to solvent ratio with 2% citric acid. The titratable acidity of Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio and 70% ethanol (control) was found to be 15.68%.

Table 49 shows the analysis of variance (ANOVA) and estimated coefficient values for titratable acidity of solvent extracted anthocyanin from calyces of Roselle. The Model (2FI) F-value of 1298.92 implies that the model was significant ($p < 0.0001$).

Table 49. Analysis of variance (ANOVA) of titratable acidity in solvent extracted anthocyanin from calyces of Roselle for different solid to solvent ratios and concentration of citric acid (%)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	124.536	8	15.567	1298.92	< 0.0001	Significant	Intercept	20.158	1	0.021
A-Solid:solvent	108.366	2	54.183	4521.05	< 0.0001		A[1]	-2.529	1	0.030
B-Citric acid	12.964	2	6.482	540.85	< 0.0001		A[2]	2.370	1	0.030
AB	3.206	4	0.802	66.89	< 0.0001		B[1]	-0.901	1	0.030
Pure Error	0.216	18	0.012				B[2]	0.116	1	0.030
Cor Total	124.752	26					A[1]B[1]	0.424	1	0.042
							A[2]B[1]	-0.443	1	0.042
Std. Dev.	0.109		R-Squared	0.99			A[1]B[2]	-0.081	1	0.042
Mean	20.158		Adj R-Squared	0.99			A[2]B[2]	-0.180	1	0.042
C.V. %	0.543		Pred R-Squared	0.98						
PRESS	0.485		Adeq Precision	107.33						

Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A and B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.98 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 107.33 indicates an adequate signal. Hence, this model could be used to navigate the design space. The optimized predictive model (in terms of actual factors) for titratable acidity of solvent extracted anthocyanin from calyces of Roselle is presented in the following equation.

$$\text{Titratable acidity (\%)} = 20.158 - 2.529*A + 2.370*A^2 - 0.901*B + 0.116*B^2 + 0.424*A*B - 0.443*A^2*B - 0.081*A*B^2 - 0.180*A^2*B^2 \quad \dots (4.30)$$

4.15 Functional properties of solvent extracted anthocyanin from calyces of Roselle

The functional properties of solvent extracted anthocyanin from calyces of Roselle viz., total phenolic content, antioxidant activity, total flavonoids and total anthocyanin content were studied and presented hereunder.

4.15.1 Effect of solid to solvent ratios and concentration of citric acid (%) on total phenolic content of solvent extracted anthocyanin from calyces of Roselle

The total phenolic content of solvent extracted anthocyanin from calyces of Roselle obtained from different solid to solvent ratios and concentration of citric acid (%) using ethanol as a solvent are presented in Table 50. From the data, it was observed that in 1:5 solid to solvent ratio the total phenolic content of Roselle anthocyanin extract was found to be in the range of 26.28 to 30.72 mg GAE.g⁻¹. Similarly, using 1:10 solid to solvent ratio the total phenolic content was found to be in the range of 37.49 to 43.19 mg GAE.g⁻¹ whereas, in case of Roselle anthocyanin extract obtained using 1:15 solid to solvent ratio the total phenolic content was found to be in the range of 34.27 to 41.25 mg GAE.g⁻¹.

In case of the different solid to solvent ratios and concentration of citric acid (%) interaction, it is revealed that the Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio with 3% citric acid showed lowest total phenolic content of 26.28 mg GAE.g⁻¹ whereas, maximum of 43.19 mg GAE.g⁻¹ was recorded in case of 1:10 solid to

Table 50. Effect of different solid to solvent ratios and concentration of citric acid (%) on functional properties of solvent extracted anthocyanin from calyces of Roselle

Treatment	Solid: solvent	% citric acid	Total phenolic content (mg GAE.g⁻¹)	Antioxidant activity (%)	Total flavonoids (mg RE.g⁻¹)	Total anthocyanin content (mg CyaG.100g⁻¹)
E1	1:5	1%	28.57	76.50	39.17	749.31
E2	1:5	2%	30.72	79.82	42.85	768.10
E3	1:5	3%	26.28	71.99	36.85	723.91
E4	1:10	1%	39.28	84.04	50.56	939.42
E5	1:10	2%	43.19	87.29	54.28	1072.27
E6	1:10	3%	37.49	83.30	46.55	908.47
E7	1:15	1%	36.25	82.34	48.96	889.92
E8	1:15	2%	41.25	85.74	53.11	1028.86
E9	1:15	3%	34.27	80.93	44.27	870.72
Control	1:5	0	26.57	61.56	34.28	513.54

solvent ratio with 2% citric acid. The total phenolic content of Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio and 70% ethanol (control) was found to be 26.57 mg GAE.g⁻¹.

Table 51 shows the analysis of variance (ANOVA) and estimated coefficient values for total phenolic content of solvent extracted anthocyanin from calyces of Roselle. The Model (2FI) F-value of 31197.52 implies that the model was significant ($p < 0.0001$). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A and B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.98 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 516.17 indicates an adequate signal. Hence, this model could be used to navigate the design space. The optimized predictive model (in terms of actual factors) for total phenolic content of solvent extracted anthocyanin from calyces of Roselle is presented in the following equation.

$$\begin{aligned} \text{Total phenolic content (mg GAE.g}^{-1}\text{)} = & 35.254 - 6.731*A + 4.729*A^2 - 0.555*B + 3.131*B^2 \\ & + 0.599*A*B - 0.151*A^2*B - 0.931*A*B^2 + 0.072 \\ & *A^2*B^2 \dots (4.31) \end{aligned}$$

4.15.2 Effect of solid to solvent ratios and concentration of citric acid (%) on antioxidant activity of solvent extracted anthocyanin from calyces of Roselle

The antioxidant activity of solvent extracted anthocyanin from calyces of Roselle obtained from different solid to solvent ratios and concentration of citric acid (%) using ethanol as a solvent are presented in Table 51. From the data, it was observed that in 1:5 solid to solvent ratio the antioxidant activity of Roselle anthocyanin extract was found to be in the range of 71.99 to 79.82%. Similarly, using 1:10 solid to solvent ratio the antioxidant activity was found to be in the range of 83.30 to 87.29% whereas, in case of Roselle anthocyanin extract obtained using 1:15 solid to solvent ratio the antioxidant activity was found to be in the range of 80.93 to 85.74%.

Table 51. Analysis of variance (ANOVA) of total phenolic content in solvent extracted anthocyanin from calyces of Roselle for different solid to solvent ratios and concentration of citric acid (%)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	803.278	8	100.410	31197.52	< 0.0001	Significant	Intercept	35.254	1	0.011
A-Solid:solvent	645.072	2	322.536	100212.60	< 0.0001		A[1]	-6.731	1	0.015
B-Citric acid	150.765	2	75.383	23421.55	< 0.0001		A[2]	4.729	1	0.015
AB	7.441	4	1.860	577.96	< 0.0001		B[1]	-0.555	1	0.015
Pure Error	0.058	18	0.003				B[2]	3.131	1	0.015
Cor Total	803.336	26					A[1]B[1]	0.599	1	0.022
							A[2]B[1]	-0.151	1	0.022
Std. Dev.	0.057		R-Squared	0.99			A[1]B[2]	-0.931	1	0.022
Mean	35.254		Adj R-Squared	0.99			A[2]B[2]	0.072	1	0.022
C.V. %	0.161		Pred R-Squared	0.98						
PRESS	0.130		Adeq Precision	516.17						

Considering the different solid to solvent ratios and concentration of citric acid (%) interaction, it is revealed that the Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio with 3% citric acid showed lowest antioxidant activity of 71.99% whereas, maximum of 87.29% was recorded in case of 1:10 solid to solvent ratio with 2% citric acid. The antioxidant activity of Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio and 70% ethanol (control) was found to be 61.56%.

Table 52 shows the analysis of variance (ANOVA) and estimated coefficient values for antioxidant activity of solvent extracted anthocyanin from calyces of Roselle. The Model (2FI) F-value of 403.29 implies that the model was significant ($p < 0.0001$). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A and B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.98 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 64.74 indicates an adequate signal. Hence, this model could be used to navigate the design space. The optimized predictive model (in terms of actual factors) for antioxidant activity of solvent extracted anthocyanin from calyces of Roselle is presented in the following equation.

$$\text{Antioxidant activity (\%)} = 81.325 - 5.223*A + 3.548*A^2 - 0.370*B + 2.956*B^2 + 0.764*A*B - 0.468*A^2*B + 0.764*A*B^2 - 0.542*A^2*B^2 \quad \dots (4.32)$$

4.15.3 Effect of solid to solvent ratios and concentration of citric acid (%) on total flavonoid content of solvent extracted anthocyanin from calyces of Roselle

The total flavonoid content of solvent extracted anthocyanin from calyces of Roselle obtained from different solid to solvent ratios and concentration of citric acid (%) using ethanol as a solvent are presented in Table 50. From the data, it was observed that in 1:5 solid to solvent ratio the total flavonoid content of Roselle anthocyanin extract was found to be in the range of 36.85 to 42.85 mg RE.g⁻¹. Similarly, using 1:10 solid to solvent ratio the total flavonoid content was found to be in the range of 46.55 to 54.28 mg RE.g⁻¹ whereas, in case of Roselle anthocyanin extract obtained using 1:15 solid to solvent ratio the total flavonoid content was found to be in the range of 44.27 to 53.11 mg RE.g⁻¹.

Table 52. Analysis of variance (ANOVA) of antioxidant activity in solvent extracted anthocyanin from calyces of Roselle for different solid to solvent ratios and concentration of citric acid (%)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	540.476	8	67.559	403.29	< 0.0001	Significant	Intercept	81.325	1	0.079
A-Solid:solvent	384.047	2	192.024	1146.26	< 0.0001		A[1]	-5.223	1	0.111
B-Citric acid	140.117	2	70.059	418.21	< 0.0001		A[2]	3.548	1	0.111
AB	16.312	4	4.078	24.34	< 0.0001		B[1]	-0.370	1	0.111
Pure Error	3.015	18	0.168				B[2]	2.956	1	0.111
Cor Total	543.491	26					A[1]B[1]	0.764	1	0.158
							A[2]B[1]	-0.468	1	0.158
Std. Dev.	0.409		R-Squared	0.99			A[1]B[2]	0.764	1	0.158
Mean	81.325		Adj R-Squared	0.99			A[2]B[2]	-0.542	1	0.158
C.V. %	0.503		Pred R-Squared	0.98						
PRESS	6.785		Adeq Precision	64.74						

Considering the different solid to solvent ratios and concentration of citric acid (%) interaction, it is revealed that the Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio with 3% citric acid showed lowest total flavonoid content of 36.85 mg RE.g⁻¹ whereas, maximum of 54.28 mg RE.g⁻¹ was recorded in case of 1:10 solid to solvent ratio with 2% citric acid. The total flavonoid content of Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio and 70% ethanol (control) was found to be 34.28 mg RE.g⁻¹.

Table 53 shows the analysis of variance (ANOVA) and estimated coefficient values for total flavonoid content of solvent extracted anthocyanin from calyces of Roselle. The Model (2FI) F-value of 427762.28 implies that the model was significant (p<0.0001). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A and B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R² value of 0.99, whereas the optimized predictive model had an R² value of 0.99 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 1888.85 indicates an adequate signal. Hence, this model could be used to navigate the design space. The optimized predictive model (in terms of actual factors) for total flavonoid content of solvent extracted anthocyanin from calyces of Roselle is presented in the following equation.

$$\text{Total flavonoids (mg RE.g}^{-1}\text{)} = 46.288 - 6.666*A + 4.176*A^2 - 0.057*B + 3.790*B^2 - 0.392*A*B + 0.153*A^2*B - 0.565*A*B^2 + 0.026*A^2*B^2 \quad \dots (4.33)$$

4.15.4 Effect of solid to solvent ratios and concentration of citric acid (%) on total anthocyanin content of solvent extracted anthocyanin from calyces of Roselle

The total anthocyanin content of solvent extracted anthocyanin from calyces of Roselle powder obtained from different solid to solvent ratios and concentration of citric acid (%) using ethanol as a solvent are presented in Table 50. From the data, it was observed that in 1:5 solid to solvent ratio the total anthocyanin content of Roselle anthocyanin extract was found to be in the range of 723.91 to 768.10 mg CyaG.100g⁻¹.

Table 53. Analysis of variance (ANOVA) of total flavonoids in solvent extracted anthocyanin from calyces of Roselle for different solid to solvent ratios and concentration of citric acid (%)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	874.536	8	109.317	427762.28	< 0.0001	Significant	Intercept	46.288	1	0.003
A-Solid:solvent	612.668	2	306.334	1198699.19	< 0.0001		A[1]	-6.666	1	0.004
B-Citric acid	254.671	2	127.336	498269.62	< 0.0001		A[2]	4.176	1	0.004
AB	7.197	4	1.799	7040.15	< 0.0001		B[1]	-0.057	1	0.004
Pure Error	0.005	18	0.000				B[2]	3.790	1	0.004
Cor Total	874.541	26					A[1]B[1]	-0.392	1	0.006
							A[2]B[1]	0.153	1	0.006
Std. Dev.	0.016		R-Squared	0.99			A[1]B[2]	-0.565	1	0.006
Mean	46.288		Adj R-Squared	0.99			A[2]B[2]	0.026	1	0.006
C.V. %	0.035		Pred R-Squared	0.99						
PRESS	0.010		Adeq Precision	1888.85						

Similarly, using 1:10 solid to solvent ratio the total anthocyanin content was found to be in the range of 908.47 to 1072.27 mg CyaG.100g⁻¹ whereas, in case of Roselle anthocyanin extract obtained using 1:15 solid to solvent ratio the total anthocyanin content was found to be in the range of 870.70 to 1028.86 mg CyaG.100g⁻¹.

In case of the different solid to solvent ratios and concentration of citric acid (%) interaction, it is revealed that the Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio with 3% citric acid showed lowest total anthocyanin content of 723.91 mg CyaG.100g⁻¹ whereas, maximum of 1072.27 mg CyaG.100g⁻¹ was recorded in case of 1:10 solid to solvent ratio with 2% citric acid. The total anthocyanin content of Roselle anthocyanin extract obtained from 1:5 solid to solvent ratio and 70% ethanol (control) was found to be 513.54 mg CyaG.100g⁻¹.

Table 54 shows the analysis of variance (ANOVA) and estimated coefficient values for total anthocyanin content of solvent extracted anthocyanin from calyces of Roselle. The Model (2FI) F-value of 149915.15 implies that the model was significant (p<0.0001). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A and B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R² value of 0.99, whereas the optimized predictive model had an R² value of 0.99 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 1112.90 indicates an adequate signal. Hence, this model could be used to navigate the design space. The optimized predictive model (in terms of actual factors) for total anthocyanin content of solvent extracted anthocyanin from calyces of Roselle is presented in the following equation.

$$\begin{aligned} \text{Total anthocyanin content (mg CyaG.100g}^{-1}\text{)} = & 883.443 - 136.335*A + 89.942*A^2 - 23.892 \\ & *B + 72.968*B^2 + 26.098*A*B - 10.078*A^2 \\ & *B - 51.978*A*B^2 + 25.919*A^2*B^2 \dots (4.34) \end{aligned}$$

Table 54. Analysis of variance (ANOVA) of total anthocyanin content in solvent extracted anthocyanin from calyces of Roselle for different solid to solvent ratios and concentration of citric acid (%)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	352533.029	8	44066.629	149915.15	< 0.0001	Significant	Intercept	883.443	1	0.104
A-Solid:solvent	259461.329	2	129730.665	441345.13	< 0.0001		A[1]	-136.335	1	0.148
B-Citric acid	74731.676	2	37365.838	127118.99	< 0.0001		A[2]	89.942	1	0.148
AB	18340.024	4	4585.006	15598.24	< 0.0001		B[1]	-23.892	1	0.148
Pure Error	5.291	18	0.294				B[2]	72.968	1	0.148
Cor Total	352538.320	26					A[1]B[1]	26.098	1	0.209
							A[2]B[1]	-10.078	1	0.209
Std. Dev.	0.542		R-Squared	0.99			A[1]B[2]	-51.978	1	0.209
Mean	883.443		Adj R-Squared	0.99			A[2]B[2]	25.919	1	0.209
C.V. %	0.061		Pred R-Squared	0.99						
PRESS	11.905		Adeq Precision	1112.90						

4.16 Optimization of process parameters for solvent extraction of anthocyanin from calyces of Roselle

Optimization of two process variables namely, solid to solvent ratio and concentration of citric acid (%) was performed using the general factorial design in Design Expert Software 7.7.0. Numerical optimization was performed using statistical models to find optimal process parameters of solvent extracted anthocyanin from calyces of Roselle. In the present investigation, the independent variables were kept within the range and dependent variables were chosen as maximum and minimum. Table 55 shows the process parameters and responses of solvent extracted anthocyanin from calyces of Roselle achieved from General Factorial in Design Expert Software 7.7.0.

4.16.1 Verification of predicted variables for solvent extracted anthocyanin from calyces of Roselle

The suitability of the model equation for predicting the optimum response values was verified using the optimal condition. The experimental sample had the optimum process conditions of 1:10 solid to solvent ratio along with 2% citric acid. The extracted anthocyanin from calyces of Roselle obtained from optimized solid to solvent ratio and concentration of citric acid (%) is depicted in Plate 18. This optimized parameter was carried out thrice and the average values of each response are presented and verification of the predicted and actual values of responses for optimized solid to solvent ratio and concentration of citric acid (%) is presented in Table 56.

4.17 Cost of production of solvent extracted anthocyanin from calyces of Roselle

The cost of production of solvent extracted anthocyanin from calyces of Roselle is presented in Appendix II. Estimation of cost of production for 100 kg of solvent extracted anthocyanin from calyces of Roselle was computed as ₹ 1,83,600 with benefit cost ratio of 2.72. The Net returns and Gross returns of solvent extracted anthocyanin from calyces of Roselle was computed as ₹ 3,16,400 and 5,00,000, respectively. Hence, the developed technology could be adopted for commercial production of solvent extracted anthocyanin from calyces of Roselle.

Table 55. Effect of different solid to solvent ratios and concentration of citric acid (%) on optimization of solvent extraction of anthocyanin from calyces of Roselle

Sl. No.	Parameter	Goal	Lower limit	Upper limit
1	Solid: solvent	is in range	1:5	1:15
2	% Citric acid	is in range	1	3
3	Extraction yield (%)	maximize	50.00	98.00
4	pH	minimize	1.18	1.49
5	TSS (°Brix)	maximize	32.30	45.80
6	L^*	minimize	1.24	3.25
7	a^*	maximize	0.28	0.76
8	b^*	minimize	0.06	0.23
9	Titrateable acidity (%)	maximize	17.09	24.00
10	Total phenolic content (mg GAE.g ⁻¹)	maximize	26.25	43.20
11	Antioxidant activity (%)	maximize	71.18	87.36
12	Total flavonoids (mg RE.g ⁻¹)	maximize	36.84	54.30
13	Total anthocyanin content (mg CyaG.100 ⁻¹)	maximize	723.61	1072.99



Plate 18. Extracted anthocyanin from calyces of Roselle obtained from optimized solid to solvent ratio and concentration of citric acid (%)

Table 56. Predicted and actual values of responses for optimized solid to solvent ratio and concentration of citric acid (%)

Sl. No.	Responses	Predicted value	Actual value	Variation %
1	Extraction yield (%)	97.33	96.97	-0.01
2	pH	1.27	1.25	-0.06
3	TSS (°Brix)	45.60	46.00	0.03
4	L^*	1.74	1.65	-0.20
5	a^*	0.75	0.78	0.14
6	b^*	0.06	0.05	-0.74
7	Titrateable acidity (%)	22.46	22.28	-0.03
8	Total phenolic content (mg GAE.g ⁻¹)	43.19	42.88	-0.03
9	Antioxidant activity (%)	87.29	87.96	0.03
10	Total flavonoids (mg RE.g ⁻¹)	54.28	53.98	-0.02
11	Total anthocyanin content (mg CyaG.100 ⁻¹)	1072.27	1075.56	0.01
12	Desirability	0.92		

4.18 Microencapsulation of Roselle anthocyanin

The microencapsulation of Roselle anthocyanin was evaluated in terms of physico-chemical and functional properties by using the procedure explained in Section 3.19 of Chapter III. Spray drying was used for microencapsulation of Roselle anthocyanin and the effect of different process conditions viz., coating material (maltodextrin, gum arabica and whey protein) and inlet air temperature (150, 160 and 170 °C) on physico-chemical and functional properties of microencapsulated Roselle anthocyanin were evaluated and presented hereunder. Microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures is presented in Plate 19.

4.19 Physico-chemical properties of microencapsulated Roselle anthocyanin powder

The physico-chemical properties of microencapsulated Roselle anthocyanin powder viz., powder yield, moisture content, water activity, pH, titratable acidity, loose bulk density, tapped bulk density, Carr's index, Hausner ratio, particle size and colour (L^* , a^* and b^*) were determined and presented here under.

4.19.1 Effect of different coating materials and inlet air temperatures on powder yield of microencapsulated Roselle anthocyanin powder

The powder yield of microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures are presented in Table 57. From the table, it is noticed that the powder yield of microencapsulated Roselle anthocyanin powder varied from 84.07 to 92.04% using maltodextrin (MD) as a coating material and at different inlet air temperatures, whereas, using gum arabica (GA) the powder yield was varied from 78.40 to 83.01% and with whey protein (WP), the powder yield was varied from 63.31 to 78.13% at different inlet air temperatures.

In case of different coating materials and inlet air temperatures interaction, it is revealed that the microencapsulated Roselle anthocyanin powder produced using whey protein as a coating material with 150 °C inlet air temperature showed the lowest powder yield of 63.31% and microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material with 170 °C inlet air temperature showed the highest powder yield of 92.04%.



Plate 19. Microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures

Table 57. Effect of different coating materials and inlet air temperatures on physico-chemical properties of microencapsulated Roselle anthocyanin powder

Treatment	CM	Temp. (°C)	Powder yield (%)	MC (% d.b.)	a _w	pH	TA (%)	LBD (g.cc ⁻¹)	TBD (g.cc ⁻¹)	Carr's index	Hausner ratio	Particle size (d.nm)
M1	MD	150	84.07	2.43	0.24	2.38	15.98	0.26	0.32	18.11	1.22	538.70
M2	MD	160	87.40	2.29	0.22	2.33	16.43	0.25	0.30	17.36	1.21	424.47
M3	MD	170	92.04	2.16	0.21	2.29	17.41	0.23	0.28	16.03	1.19	354.70
M4	GA	150	78.40	3.47	0.27	2.99	14.81	0.37	0.46	21.18	1.27	832.77
M5	GA	160	81.98	3.21	0.26	2.96	15.10	0.35	0.43	19.78	1.25	753.27
M6	GA	170	83.01	3.08	0.25	2.92	15.42	0.33	0.41	19.15	1.24	662.80
M7	WP	150	63.31	3.90	0.31	3.90	13.91	0.40	0.52	23.69	1.31	1170.33
M8	WP	160	76.85	3.67	0.29	3.85	14.19	0.39	0.51	23.08	1.30	1045.17
M9	WP	170	78.13	3.52	0.28	3.82	14.46	0.38	0.49	22.51	1.29	973.43

CM = Coating material; Temp. = Inlet air temperature; MD = Maltodextrin; WP = Whey protein; GA = Gum arabica; MC = Moisture content; a_w = Water activity; TA = Titratable acidity; LBD = Loose bulk density; TBD = Tapped bulk density;

Table 58 shows the analysis of variance (ANOVA) and estimated coefficient values for the powder yield of microencapsulated Roselle anthocyanin powder. The model (2FI) F-value of 392.77 implies that the model was significant ($p < 0.0001$). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A, B and AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.98 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 70.57 indicates an adequate signal. The optimized predictive model (in terms of actual factors) for powder yield of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$\text{Powder yield (\%)} = 80.578 + 7.260*A + 0.553*A^2 - 5.315*B + 1.498*B^2 + 1.552*A*B + 2.584*A^2*B - 1.940*A*B^2 - 0.647*A^2*B^2 \quad \dots (4.35)$$

4.19.2 Effect of different coating materials and inlet air temperatures on moisture content of microencapsulated Roselle anthocyanin powder

The moisture content of microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures are presented in Table 57. From the table, it was observed that the moisture content of microencapsulated Roselle anthocyanin powder using maltodextrin (MD) as a coating material was found to be in the range of 2.16 to 2.43% (d.b.). Similarly, the moisture content of microencapsulated Roselle anthocyanin powder obtained using gum arabica (GA) as a coating material was found to be in the range of 3.08 to 3.47% (d.b) whereas, the moisture content of microencapsulated Roselle anthocyanin powder obtained using whey protein (WP) as a coating material was found to be in the range of 3.52 to 3.90% (d.b.).

Considering the different coating materials and inlet air temperatures interaction, it is revealed that the highest moisture content of 3.90% (d.b.) was recorded using whey protein as a coating material at 150 °C inlet air temperature whereas, minimum of 2.16% (d.b.) moisture content was observed in case of the microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material at inlet air temperature of 170 °C.

Table 58. Analysis of variance (ANOVA) of powder yield in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	1562.293	8	195.287	392.77	< 0.0001	Significant	Intercept	80.578	1	0.136
A-Coating material	1026.601	2	513.301	1032.37	< 0.0001		A[1]	7.260	1	0.192
B-Inlet temperature	405.615	2	202.807	407.90	< 0.0001		A[2]	0.553	1	0.192
AB	130.077	4	32.519	65.40	< 0.0001		B[1]	-5.315	1	0.192
Pure Error	8.950	18	0.497				B[2]	1.498	1	0.192
Cor Total	1571.243	26					A[1]B[1]	1.552	1	0.271
							A[2]B[1]	2.584	1	0.271
Std. Dev.	0.705		R-Squared	0.99			A[1]B[2]	-1.940	1	0.271
Mean	80.578		Adj R-Squared	0.99			A[2]B[2]	-0.647	1	0.271
C.V. %	0.875		Pred R-Squared	0.98						
PRESS	20.137		Adeq Precision	70.57						

Table 59 shows the analysis of variance (ANOVA) and estimated coefficient values for the moisture content of microencapsulated Roselle anthocyanin powder. The Model (2FI) F-value of 1999.03 implies that the model was significant ($p < 0.0001$). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A and B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.99 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 121.36 indicates an adequate signal.

The optimized predictive model (in terms of actual factors) for moisture content of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$\text{Moisture content (\% d.b.)} = 3.081 - 0.786*A + 0.170*A^2 + 0.182*B - 0.022*B^2 - 0.051*A*B + 0.032*A^2*B + 0.022*A*B^2 - 0.018*A^2*B^2 \quad \dots (4.36)$$

4.19.3 Effect of different coating materials and inlet air temperatures on water activity of microencapsulated Roselle anthocyanin powder

The water activity of microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures are presented in Table 57. From the table, it is noticed that the water activity of microencapsulated Roselle anthocyanin powder varied from 0.21 to 0.24 using maltodextrin (MD) as a coating material and at different inlet air temperatures, whereas, using gum arabica (GA) the water activity was varied from 0.25 to 0.27 and with whey protein (WP), the water activity was varied from 0.28 to 0.31 at different inlet air temperatures.

In case of coating materials and inlet air temperatures interaction, it is revealed that the microencapsulated Roselle anthocyanin powder produced using whey protein as a coating material with 150 °C inlet air temperature showed the highest water activity of 0.31 and microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material with 170 °C inlet air temperature showed the lowest water activity of 0.21.

Table 60 shows the analysis of variance (ANOVA) and estimated coefficient values for the water activity of microencapsulated Roselle anthocyanin powder. The model (2FI) F-value of 110.50 implies that the model was significant ($p < 0.0001$).

Table 59. Analysis of variance (ANOVA) of moisture content in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	9.789	8	1.224	1999.03	< 0.0001	Significant	Intercept	3.081	1	0.005
A-Coating material	9.237	2	4.619	7545.31	< 0.0001		A[1]	-0.786	1	0.007
B-Inlet temperature	0.533	2	0.267	435.78	< 0.0001		A[2]	0.170	1	0.007
AB	0.018	4	0.005	7.51	0.0010		B[1]	0.182	1	0.007
Pure Error	0.011	18	0.001				B[2]	-0.022	1	0.007
Cor Total	9.800	26					A[1]B[1]	-0.051	1	0.010
							A[2]B[1]	0.032	1	0.010
Std. Dev.	0.025		R-Squared	0.99			A[1]B[2]	0.022	1	0.010
Mean	3.081		Adj R-Squared	0.99			A[2]B[2]	-0.018	1	0.010
C.V. %	0.803		Pred R-Squared	0.99						
PRESS	0.025		Adeq Precision	121.36						

Table 60. Analysis of variance (ANOVA) of water activity in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	0.023	8	0.003	110.50	< 0.0001	Significant	Intercept	0.261	1	0.001
A-Coating material	0.020	2	0.010	386.29	< 0.0001		A[1]	-0.034	1	0.001
B-Inlet temperature	0.003	2	0.001	54.14	< 0.0001		A[2]	0.001	1	0.001
AB	0.000	4	0.000	0.79	0.5493		B[1]	0.014	1	0.001
Pure Error	0.000	18	0.000				B[2]	-0.003	1	0.001
Cor Total	0.023	26					A[1]B[1]	0.003	1	0.002
							A[2]B[1]	-0.003	1	0.002
Std. Dev.	0.005		R-Squared	0.98			A[1]B[2]	0.000	1	0.002
Mean	0.261		Adj R-Squared	0.97			A[2]B[2]	0.001	1	0.002
C.V. %	1.953		Pred R-Squared	0.96						
PRESS	0.001		Adeq Precision	31.75						

Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A and B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.98, whereas the optimized predictive model had an R^2 value of 0.96 and was in reasonable agreement with the "Adj R-Squared" of 0.97. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 31.75 indicates an adequate signal.

The optimized predictive model (in terms of actual factors) for water activity of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$\begin{aligned} \text{Water activity} = & 0.261 - 0.034*A + 1.481E-003*A^2 + 0.014*B - 2.963E-003*B^2 + 2.963E-003 \\ & *A*B - 2.593E-003*A^2*B - 3.704E-004*A*B^2 + 7.407E-004*A^2*B^2 \\ & \dots (4.37) \end{aligned}$$

4.19.4 Effect of different coating materials and inlet air temperatures on pH of microencapsulated Roselle anthocyanin powder

The pH of microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures are presented in Table 57. From the table, it was observed that the pH of microencapsulated Roselle anthocyanin powder using maltodextrin (MD) as a coating material was found to be in the range of 2.29 to 2.38. Similarly, the pH of microencapsulated Roselle anthocyanin powder obtained using gum arabica (GA) as a coating material was found to be in the range of 2.92 to 2.99 whereas, the pH of microencapsulated Roselle anthocyanin powder obtained using whey protein (WP) as a coating material was found to be in the range of 3.82 to 3.90.

Considering the different coating materials and inlet air temperatures interaction, it is revealed that the highest pH of 3.90 was recorded using whey protein as a coating material at 150 °C inlet air temperature whereas, minimum of 2.29 pH was observed in case of the microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material at inlet air temperature of 170 °C.

Table 61 shows the analysis of variance (ANOVA) and estimated coefficient values for the pH of microencapsulated Roselle anthocyanin powder. The Model (2FI)

F-value of 23852.72 implies that the model was significant ($p < 0.0001$). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A and B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.99 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 374.13 indicates an adequate signal.

The optimized predictive model (in terms of actual factors) for pH of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$\text{pH} = 3.048 - 0.716 * A - 0.093 * A^2 + 0.040 * B - 1.481E-003 * B^2 + 4.815E-003 * A * B - 8.519E-003 * A^2 * B - 7.407E-004 * A * B^2 + 2.593E-003 * A^2 * B^2 \quad \dots (4.38)$$

4.19.5 Effect of different coating materials and inlet air temperatures on titratable acidity of microencapsulated Roselle anthocyanin powder

The titratable acidity of microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures are presented in Table 57. From the table, it is noticed that the titratable acidity of microencapsulated Roselle anthocyanin powder varied from 15.98 to 17.41% using maltodextrin (MD) as a coating material and at different inlet air temperatures, whereas, using gum arabica (GA) the titratable acidity was varied from 14.81 to 15.42% and with whey protein (WP), the titratable acidity was varied from 13.91 to 14.46% at different inlet air temperatures.

In case of coating materials and inlet air temperatures interaction, it is revealed that the microencapsulated Roselle anthocyanin powder produced using whey protein as a coating material with 150 °C inlet air temperature showed the lowest titratable acidity of 13.91% and microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material with 170 °C inlet air temperature showed the highest titratable acidity of 17.41.

Table 62 shows the analysis of variance (ANOVA) and estimated coefficient values for the titratable acidity of microencapsulated Roselle anthocyanin powder.

Table 61. Analysis of variance (ANOVA) of pH in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	10.601	8	1.325	23852.71	< 0.0001	Significant	Intercept	3.048	1	0.001
A-Coating material	10.573	2	5.287	95160.87	< 0.0001		A[1]	-0.716	1	0.002
B-Inlet temperature	0.027	2	0.014	245.27	< 0.0001		A[2]	-0.093	1	0.002
AB	0.001	4	0.000	2.37	0.0915		B[1]	0.040	1	0.002
Pure Error	0.001	18	0.000				B[2]	-0.001	1	0.002
Cor Total	10.602	26					A[1]B[1]	0.005	1	0.003
							A[2]B[1]	-0.009	1	0.003
Std. Dev.	0.007		R-Squared	0.99			A[1]B[2]	-0.001	1	0.003
Mean	3.048		Adj R-Squared	0.99			A[2]B[2]	0.003	1	0.003
C.V. %	0.245		Pred R-Squared	0.99						
PRESS	0.002		Adeq Precision	374.13						

Table 62. Analysis of variance (ANOVA) of titratable acidity in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	31.033	8	3.879	319.63	< 0.0001	Significant	Intercept	15.301	1	0.021
A-Coating material	26.791	2	13.395	1103.75	< 0.0001		A[1]	1.304	1	0.030
B-Inlet temperature	3.438	2	1.719	141.65	< 0.0001		A[2]	-0.190	1	0.030
AB	0.804	4	0.201	16.57	< 0.0001		B[1]	-0.403	1	0.030
Pure Error	0.218	18	0.012				B[2]	-0.062	1	0.030
Cor Total	31.252	26					A[1]B[1]	-0.223	1	0.042
							A[2]B[1]	0.097	1	0.042
Std. Dev.	0.110		R-Squared	0.99			A[1]B[2]	-0.116	1	0.042
Mean	15.301		Adj R-Squared	0.99			A[2]B[2]	0.055	1	0.042
C.V. %	0.720		Pred R-Squared	0.98						
PRESS	0.492		Adeq Precision	55.01						

The model (2FI) F-value of 319.63 implies that the model was significant ($p < 0.0001$). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A, B and AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.98 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 55.01 indicates an adequate signal.

The optimized predictive model (in terms of actual factors) for titratable acidity of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$\begin{aligned} \text{Titratable acidity (\%)} = & 15.301 + 1.304*A - 0.190*A^2 - 0.403*B - 0.062*B^2 - 0.223*A*B \\ & + 0.097*A^2*B - 0.116*A*B^2 + 0.055*A^2*B^2 \end{aligned} \quad \dots (4.39)$$

4.19.6 Effect of different coating materials and inlet air temperatures on loose bulk density of microencapsulated Roselle anthocyanin powder

The loose bulk density of microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures are presented in Table 57. From the table, it was observed that the loose bulk density of microencapsulated Roselle anthocyanin powder using maltodextrin (MD) as a coating material was found to be in the range of 0.23 to 0.26 g.cc⁻¹. Similarly, the loose bulk density of microencapsulated Roselle anthocyanin powder obtained using gum arabica (GA) as a coating material was found to be in the range of 0.33 to 0.37 g.cc⁻¹ whereas, the loose bulk density of microencapsulated Roselle anthocyanin powder obtained using whey protein (WP) as a coating material was found to be in the range of 0.38 to 0.40 g.cc⁻¹.

Considering the different coating materials and inlet air temperatures interaction, it is revealed that the highest loose bulk density of 0.40 g.cc⁻¹ was recorded using whey protein as a coating material at 150 °C inlet air temperature whereas, minimum of 0.23 g.cc⁻¹ loose bulk density was observed in case of the microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material and at inlet air temperature of 170 °C.

Table 63 shows the analysis of variance (ANOVA) and estimated coefficient values for the loose bulk density of microencapsulated Roselle anthocyanin powder. The Model (2FI) F-value of 236.74 implies that the model was significant ($p < 0.0001$). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A and B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.98 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 38.95 indicates an adequate signal.

The optimized predictive model (in terms of actual factors) for loose bulk density of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$\begin{aligned} \text{Loose bulk density (g.cc}^{-1}\text{)} = & 0.328 - 0.080*A + 0.018*A^2 + 0.013*B + 2.391E-004*B^2 - \\ & 5.656E-004*A*B + 6.259E-003*A^2*B + 9.003E-004*A*B^2 \\ & - 1.145E-003*A^2*B^2 \quad \dots (4.40) \end{aligned}$$

4.19.7 Effect of different coating materials and inlet air temperatures on tapped bulk density of microencapsulated Roselle anthocyanin powder

The tapped bulk density of microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures are presented in Table 57. From the table, it is noticed that the tapped bulk density of microencapsulated Roselle anthocyanin powder varied from 0.28 to 0.32 g.cc⁻¹ using maltodextrin (MD) as a coating material and at different inlet air temperatures, whereas, using gum arabica (GA) the tapped bulk density was varied from 0.41 to 0.46 g.cc⁻¹ and with whey protein (WP), the tapped bulk density was varied from 0.49 to 0.52 g.cc⁻¹ at different inlet air temperatures.

In case of coating materials and inlet air temperatures interaction, it is revealed that the microencapsulated Roselle anthocyanin powder produced using whey protein as a coating material with 150 °C inlet air temperature showed the highest tapped bulk density of 0.52 g.cc⁻¹.

Table 63. Analysis of variance (ANOVA) of loose bulk density in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	0.099	8	0.012	236.74	< 0.0001	Significant	Intercept	0.328	1	0.001
A-Coating material	0.095	2	0.048	914.62	< 0.0001		A[1]	-0.080	1	0.002
B-Inlet temperature	0.003	2	0.001	28.57	< 0.0001		A[2]	0.018	1	0.002
AB	0.000	4	0.000	1.87	0.1595		B[1]	0.013	1	0.002
Pure Error	0.001	18	0.000				B[2]	0.000	1	0.002
Cor Total	0.100	26					A[1]B[1]	-0.001	1	0.003
							A[2]B[1]	0.006	1	0.003
Std. Dev.	0.007		R-Squared	0.99			A[1]B[2]	0.001	1	0.003
Mean	0.328		Adj R-Squared	0.99			A[2]B[2]	-0.001	1	0.003
C.V. %	2.200		Pred R-Squared	0.98						
PRESS	0.002		Adeq Precision	38.95						

Whereas, microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material with 170 °C inlet air temperature showed the lowest tapped bulk density of 0.28 g.cc⁻¹.

Table 64 shows the analysis of variance (ANOVA) and estimated coefficient values for the tapped bulk density of microencapsulated Roselle anthocyanin powder. The model (2FI) F-value of 212.17 implies that the model was significant (p<0.0001). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A and B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R² value of 0.99, whereas the optimized predictive model had an R² value of 0.98 and was in reasonable agreement with the "Adj R-Squared" of 0.98. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 37.70 indicates an adequate signal.

The optimized predictive model (in terms of actual factors) for tapped bulk density of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$\begin{aligned} \text{Tapped bulk density (g.cc}^{-1}\text{)} = & 0.414 - 0.114*A + 0.020*A^2 + 0.020*B - 1.372E-004*B^2 - \\ & 2.343E-003*A*B + 9.647E-003*A^2*B + 2.053E-003*A*B^2 - \\ & 2.604E-003*A^2*B^2 \end{aligned} \quad \dots (4.41)$$

4.19.8 Effect of different coating materials and inlet air temperatures on Carr's index of microencapsulated Roselle anthocyanin powder

The Carr's index of microencapsulated Roselle anthocyanin powder obtained from three different coating materials and inlet air temperatures are presented in Table 57. From the table, it was observed that the Carr's index of microencapsulated Roselle anthocyanin powder using maltodextrin (MD) as a coating material was found to be in the range of 16.03 to 18.11. Similarly, the Carr's index of microencapsulated Roselle anthocyanin powder obtained using gum arabica (GA) as a coating material was found to be in the range of 19.15 to 21.18 whereas, the Carr's index of microencapsulated Roselle anthocyanin powder obtained using whey protein (WP) as a coating material was found to be in the range of 22.51 to 23.69.

Table 64. Analysis of variance (ANOVA) of tapped bulk density in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	0.208	8	0.026	212.17	< 0.0001	Significant	Intercept	0.414	1	0.002
A-Coating material	0.200	2	0.100	814.85	< 0.0001		A[1]	-0.114	1	0.003
B-Inlet temperature	0.008	2	0.004	30.65	< 0.0001		A[2]	0.020	1	0.003
AB	0.001	4	0.000	1.58	0.2219		B[1]	0.020	1	0.003
Pure Error	0.002	18	0.000				B[2]	0.000	1	0.003
Cor Total	0.210	26					A[1]B[1]	-0.002	1	0.004
							A[2]B[1]	0.010	1	0.004
Std. Dev.	0.011		R-Squared	0.99			A[1]B[2]	0.002	1	0.004
Mean	0.414		Adj R-Squared	0.98			A[2]B[2]	-0.003	1	0.004
C.V. %	2.676		Pred R-Squared	0.98						
PRESS	0.005		Adeq Precision	37.70						

Considering the different coating materials and inlet air temperatures interaction, it is revealed that the highest Carr's index of 23.69 was recorded using whey protein as a coating material at 150 °C inlet air temperature whereas, minimum of 16.03 Carr's index was observed in case of the microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material at inlet air temperature of 170 °C.

Table 65 shows the analysis of variance (ANOVA) and estimated coefficient values for the Carr's index of microencapsulated Roselle anthocyanin powder. The Model (2FI) F-value of 169.40 implies that the model was significant ($p < 0.0001$). Values of "Prob>F" less than 0.05 indicate model terms were significant. In this case, A and B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.97 and was in reasonable agreement with the "Adj R-Squared" of 0.98. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 37.10 indicates an adequate signal.

The optimized predictive model (in terms of actual factors) for Carr's index of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$\begin{aligned} \text{Carr's index} = & 20.099 - 2.934*A - 0.059*A^2 + 0.894*B - 0.026*B^2 + 0.046*A*B + 0.248*A^2*B \\ & + 0.218*A*B^2 - 0.229*A^2*B^2 \end{aligned} \quad \dots (4.42)$$

4.19.9 Effect of different coating materials and inlet air temperatures on Hausner ratio of microencapsulated Roselle anthocyanin powder

The Hausner ratio of microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures are presented in Table 57. From the table, it is noticed that the Hausner ratio of microencapsulated Roselle anthocyanin powder varied from 1.19 to 1.22 using maltodextrin (MD) as a coating material and at different inlet air temperatures, whereas, using gum arabica (GA) the Hausner ratio was varied from 1.24 to 1.27 and with whey protein (WP), the Hausner ratio was varied from 1.29 to 1.31 at different inlet air temperatures.

Table 65. Analysis of variance (ANOVA) of Carr's index in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	173.306	8	21.663	169.40	< 0.0001	Significant	Intercept	20.099	1	0.069
A-Coating material	158.112	2	79.056	618.20	< 0.0001		A[1]	-2.934	1	0.097
B-Inlet temperature	13.990	2	6.995	54.70	< 0.0001		A[2]	-0.059	1	0.097
AB	1.203	4	0.301	2.35	0.0929		B[1]	0.894	1	0.097
Pure Error	2.302	18	0.128				B[2]	-0.026	1	0.097
Cor Total	175.608	26					A[1]B[1]	0.046	1	0.138
							A[2]B[1]	0.248	1	0.138
Std. Dev.	0.358		R-Squared	0.99			A[1]B[2]	0.218	1	0.138
Mean	20.099		Adj R-Squared	0.98			A[2]B[2]	-0.229	1	0.138
C.V. %	1.779		Pred R-Squared	0.97						
PRESS	5.179		Adeq Precision	37.10						

In case of coating materials and inlet air temperatures interaction, it is revealed that the microencapsulated Roselle anthocyanin powder produced using whey protein as a coating material with 150 °C inlet air temperature showed the highest Hausner ratio of 1.31. Whereas, microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material with 170 °C inlet air temperature showed the lowest Hausner ratio of 1.19.

Table 66 shows the analysis of variance (ANOVA) and estimated coefficient values for the Hausner ratio of microencapsulated Roselle anthocyanin powder. The model (2FI) F-value of 160.37 implies that the model was significant ($p < 0.0001$). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A and B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.97 and was in reasonable agreement with the "Adj R-Squared" of 0.98. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 35.96 indicates an adequate signal.

The optimized predictive model (in terms of actual factors) for Hausner ratio of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$\begin{aligned} \text{Hausner ratio} = & 1.253 - 0.045 * A - 2.040E-003 * A^2 + 0.014 * B - 5.919E-004 * B^2 - 2.325E-004 \\ & * A * B + 4.013E-003 * A^2 * B + 3.272E-003 * A * B^2 - 3.530E-003 * A^2 * B^2 \\ & \dots (4.43) \end{aligned}$$

4.19.10 Effect of different coating materials and inlet air temperatures on particle size of microencapsulated Roselle anthocyanin powder

The particle size of microencapsulated Roselle anthocyanin powder obtained from three different coating materials and inlet air temperatures are presented in Table 57. From the table, it was observed that the particle size of microencapsulated Roselle anthocyanin powder using maltodextrin (MD) as a coating material was found to be in the range of 354.70 to 538.70 d.nm. Similarly, the particle size of microencapsulated Roselle anthocyanin powder obtained using gum arabica (GA) as a coating material was found to be in the range of 662.80 to 832.77 d.nm.

Table 66. Analysis of variance (ANOVA) of Hausner ratio in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	0.043	8	0.005	160.37	< 0.0001	Significant	Intercept	1.253	1	0.001
A-Coating material	0.039	2	0.019	587.22	< 0.0001		A[1]	-0.045	1	0.002
B-Inlet temperature	0.003	2	0.002	50.83	< 0.0001		A[2]	-0.002	1	0.002
AB	0.000	4	0.000	1.71	0.1919		B[1]	0.014	1	0.002
Pure Error	0.001	18	0.000				B[2]	-0.001	1	0.002
Cor Total	0.043	26					A[1]B[1]	0.000	1	0.002
							A[2]B[1]	0.004	1	0.002
Std. Dev.	0.006		R-Squared	0.99			A[1]B[2]	0.003	1	0.002
Mean	1.253		Adj R-Squared	0.98			A[2]B[2]	-0.004	1	0.002
C.V. %	0.460		Pred R-Squared	0.97						
PRESS	0.001		Adeq Precision	35.96						

Whereas, the particle size of microencapsulated Roselle anthocyanin powder obtained using whey protein (WP) as a coating material was found to be in the range of 973.43 to 1170.33 d.nm.

Considering the different coating materials and inlet air temperatures interaction, it is revealed that the highest particle size of 1170.33 d.nm was recorded using whey protein as a coating material at 150 °C inlet air temperature whereas, minimum of 354.70 d.nm particle size was observed in case of the microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material at inlet air temperature of 170 °C.

Table 67 shows the analysis of variance (ANOVA) and estimated coefficient values for the particle size of microencapsulated Roselle anthocyanin powder. The Model (2FI) F-value of 565.09 implies that the model was significant ($p < 0.0001$). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A and B were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.98 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 68.82 indicates an adequate signal.

The optimized predictive model (in terms of actual factors) for particle size of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$\text{Particle size (d.nm)} = 750.626 - 311.337 * A - 1.015 * A^2 + 96.641 * B - 9.659 * B^2 + 2.770 * A * B - 13.485 * A^2 * B - 5.163 * A * B^2 + 13.315 * A^2 * B^2 \quad \dots (4.44)$$

Table 67. Analysis of variance (ANOVA) of particle size in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	1905206.892	8	238150.861	565.09	< 0.0001	Significant	Intercept	750.626	1	3.951
A-Coating material	1750459.139	2	875229.569	2076.80	< 0.0001		A[1]	-311.337	1	5.587
B-Inlet temperature	152986.610	2	76493.305	181.51	< 0.0001		A[2]	-1.015	1	5.587
AB	1761.144	4	440.286	1.05	0.4118		B[1]	96.641	1	5.587
Pure Error	7585.780	18	421.432				B[2]	-9.659	1	5.587
Cor Total	1912792.672	26					A[1]B[1]	2.770	1	7.902
							A[2]B[1]	-13.485	1	7.902
Std. Dev.	20.529		R-Squared	0.99			A[1]B[2]	-5.163	1	7.902
Mean	750.626		Adj R-Squared	0.99			A[2]B[2]	13.315	1	7.902
C.V. %	2.735		Pred R-Squared	0.98						
PRESS	17068.005		Adeq Precision	68.82						

4.19.11 Effect of different coating materials and inlet air temperatures on colour value of microencapsulated Roselle anthocyanin powder

L^* value

The L^* value of microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures are presented in Table 68. From the table, it is noticed that the L^* value of microencapsulated Roselle anthocyanin powder varied from 64.81 to 65.84 using maltodextrin (MD) as a coating material and at different inlet air temperatures, whereas, using gum arabica (GA) the L^* value was varied from 66.14 to 66.36 and with whey protein (WP), the L^* value was varied from 66.63 to 70.43 at different inlet air temperatures.

In case of coating materials and inlet air temperatures interaction, it is revealed that the microencapsulated Roselle anthocyanin powder produced using whey protein as a coating material with 150 °C inlet air temperature showed the highest L^* value of 70.43. Whereas, microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material with 170 °C inlet air temperature showed the lowest L^* value of 64.81.

Table 69 shows the analysis of variance (ANOVA) and estimated coefficient values for the L^* value of microencapsulated Roselle anthocyanin powder. The model (2FI) F-value of 9.23 implies that the model was significant ($p < 0.0001$). Values of "Prob>F" less than 0.05 indicate model terms were significant. In this case, A, B and AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.80, whereas the optimized predictive model had an R^2 value of 0.56 and was in reasonable agreement with the "Adj R-Squared" of 0.72. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 10.91 indicates an adequate signal.

The optimized predictive model (in terms of actual factors) for L^* value of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$L^* = 66.561 - 1.084*A - 0.325*A^2 + 0.981*B - 0.278*B^2 - 0.622*A*B - 0.854*A^2*B + 0.587*A*B^2 + 0.248*A^2*B^2 \quad \dots (4.45)$$

Table 68. Effect of different coating materials and inlet air temperatures on colour value of microencapsulated Roselle anthocyanin powder

Treatment	Coating material	Inlet air temperature (°C)	<i>L</i>[*]	<i>a</i>[*]	<i>b</i>[*]
M1	Maltodextrin	150	65.84	27.52	3.30
M2	Maltodextrin	160	65.79	27.37	3.13
M3	Maltodextrin	170	64.81	28.87	2.93
M4	Gum arabica	150	66.36	19.23	5.41
M5	Gum arabica	160	66.21	19.94	5.34
M6	Gum arabica	170	66.14	20.53	4.93
M7	Whey protein	150	70.43	7.06	8.85
M8	Whey protein	160	66.86	7.24	7.04
M9	Whey protein	170	66.63	8.14	6.29

Table 69. Analysis of variance (ANOVA) of L^* value in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	58.702	8	7.338	9.23	< 0.0001	Significant	Intercept	66.561	1	0.172
A-Coating material	29.375	2	14.687	18.47	< 0.0001		A[1]	-1.084	1	0.243
B-Inlet temperature	13.796	2	6.898	8.68	0.0023		A[2]	-0.325	1	0.243
AB	15.531	4	3.883	4.88	0.0076		B[1]	0.981	1	0.243
Pure Error	14.311	18	0.795				B[2]	-0.278	1	0.243
Cor Total	73.013	26					A[1]B[1]	-0.622	1	0.343
							A[2]B[1]	-0.854	1	0.343
Std. Dev.	0.892		R-Squared	0.80			A[1]B[2]	0.587	1	0.343
Mean	66.561		Adj R-Squared	0.72			A[2]B[2]	0.248	1	0.343
C.V. %	1.340		Pred R-Squared	0.56						
PRESS	32.199		Adeq Precision	10.91						

a^* value

The a^* value of microencapsulated Roselle anthocyanin powder obtained from three different coating materials and inlet air temperatures are presented in Table 68. From the table, it was observed that the a^* value of microencapsulated Roselle anthocyanin powder using maltodextrin (MD) as a coating material was found to be in the range of 27.52 to 28.87. Similarly, the a^* value of microencapsulated Roselle anthocyanin powder obtained using gum arabica (GA) as a coating material was found to be in the range of 19.23 to 20.53 whereas, the a^* value of microencapsulated Roselle anthocyanin powder obtained using whey protein (WP) as a coating material was found to be in the range of 7.06 to 8.14.

Considering the different coating materials and inlet air temperatures interaction, it is revealed that the lowest a^* value of 7.06 was recorded using whey protein as a coating material at 150 °C inlet air temperature whereas, maximum of 28.87 a^* value was observed in case of the microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material at inlet air temperature of 170 °C.

Table 70 shows the analysis of variance (ANOVA) and estimated coefficient values for the a^* value of microencapsulated Roselle anthocyanin powder. The Model (2FI) F-value of 4941.63 implies that the model was significant ($p < 0.0001$). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A, B and AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.98 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 171.51 indicates an adequate signal.

The optimized predictive model (in terms of actual factors) for a^* value of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$a^* = 18.433 + 9.484*A + 1.468*A^2 - 0.498*B - 0.249*B^2 + 0.097*A*B - 0.177*A^2*B - 0.302*A*B^2 + 0.291*A^2*B^2 \quad \dots (4.46)$$

Table 70. Analysis of variance (ANOVA) of a^* value in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	1917.208	8	239.651	4941.63	< 0.0001	Significant	Intercept	18.433	1	0.042
A-Coating material	1908.542	2	954.271	19677.19	< 0.0001		A[1]	9.484	1	0.060
B-Inlet temperature	7.805	2	3.903	80.47	< 0.0001		A[2]	1.468	1	0.060
AB	0.860	4	0.215	4.44	0.0114		B[1]	-0.498	1	0.060
Pure Error	0.873	18	0.048				B[2]	-0.249	1	0.060
Cor Total	1918.081	26					A[1]B[1]	0.097	1	0.085
							A[2]B[1]	-0.177	1	0.085
Std. Dev.	0.220		R-Squared	0.99			A[1]B[2]	-0.302	1	0.085
Mean	18.433		Adj R-Squared	0.99			A[2]B[2]	0.291	1	0.085
C.V. %	1.195		Pred R-Squared	0.98						
PRESS	1.964		Adeq Precision	171.51						

b^* value

The b^* value of microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures are presented in Table 68. From the table, it is noticed that the b^* value of microencapsulated Roselle anthocyanin powder varied from 2.93 to 3.30 using maltodextrin (MD) as a coating material and at different inlet air temperatures, whereas, using gum arabica (GA) the b^* value was varied from 4.93 to 5.41 and with whey protein (WP), the L^* value was varied from 6.29 to 8.85 at different inlet air temperatures.

In case of coating materials and inlet air temperatures interaction, it is revealed that the microencapsulated Roselle anthocyanin powder produced using whey protein as a coating material with 150 °C inlet air temperature showed the highest b^* value of 8.85. Whereas, microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material with 170 °C inlet air temperature showed the lowest b^* value of 2.93.

Table 71 shows the analysis of variance (ANOVA) and estimated coefficient values for the b^* value of microencapsulated Roselle anthocyanin powder. The model (2FI) F-value of 612.83 implies that the model was significant ($p < 0.0001$). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A, B and AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.98 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 74.41 indicates an adequate signal.

The optimized predictive model (in terms of actual factors) for b^* value of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$b^* = 5.246 - 2.127*A - 0.020*A^2 + 0.607*B - 0.076*B^2 - 0.426*A*B - 0.426*A^2*B + 0.087*A*B^2 + 0.190*A^2*B^2 \quad \dots (4.47)$$

Table 71. Analysis of variance (ANOVA) of b^* value in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	93.205	8	11.651	612.834	< 0.0001	Significant	Intercept	5.246	1	0.027
A-Coating material	82.182	2	41.091	2161.410	< 0.0001		A[1]	-2.127	1	0.038
B-Inlet temperature	5.902	2	2.951	155.238	< 0.0001		A[2]	-0.020	1	0.038
AB	5.121	4	1.280	67.345	< 0.0001		B[1]	0.607	1	0.038
Pure Error	0.342	18	0.019				B[2]	-0.076	1	0.038
Cor Total	93.547	26					A[1]B[1]	-0.426	1	0.053
							A[2]B[1]	-0.426	1	0.053
Std. Dev.	0.138		R-Squared	0.99			A[1]B[2]	0.087	1	0.053
Mean	5.246		Adj R-Squared	0.99			A[2]B[2]	0.190	1	0.053
C.V. %	2.629		Pred R-Squared	0.98						
PRESS	0.770		Adeq Precision	74.41						

4.20 Reconstitution properties of microencapsulated Roselle anthocyanin powder

The effect of spray drying processing conditions viz., coating material (MD, GA and WP) and inlet air temperature (150, 160 and 170 °C) on reconstitution properties such as solubility, wettability and dispersibility of microencapsulated Roselle anthocyanin powder were determined and presented hereunder.

4.20.1 Effect of different coating materials and inlet air temperatures on solubility of microencapsulated Roselle anthocyanin powder

The solubility of microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures are presented in Table 72. From the table, it is noticed that the solubility of microencapsulated Roselle anthocyanin powder varied from 91.51 to 96.75% using maltodextrin (MD) as a coating material and at different inlet air temperatures, whereas, using gum arabica (GA) the solubility was varied from 85.61 to 92.79% and with whey protein (WP), the solubility was varied from 35.05 to 49.63% at different inlet air temperatures.

In case of coating materials and inlet air temperatures interaction, it is revealed that the microencapsulated Roselle anthocyanin powder produced using whey protein as a coating material with 150 °C inlet air temperature showed the lowest solubility of 35.05%. Whereas, microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material with 170 °C inlet air temperature showed the highest solubility of 96.75%.

Table 73 shows the analysis of variance (ANOVA) and estimated coefficient values for the solubility of microencapsulated Roselle anthocyanin powder. The model (2FI) F-value of 13979.88 implies that the model was significant ($p < 0.0001$). Values of "Prob>F" less than 0.05 indicate model terms were significant. In this case, A, B and AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.98 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 291.70 indicates an adequate signal.

Table 72. Effect of different coating materials and inlet air temperatures on reconstitution properties of microencapsulated Roselle anthocyanin powder

Treatment	Coating material	Inlet air temperature (°C)	Solubility (%)	Wettability (s)	Dispersibility (%)
M1	Maltodextrin	150	91.51	265.00	80.85
M2	Maltodextrin	160	93.55	249.60	82.39
M3	Maltodextrin	170	96.75	190.80	84.30
M4	Gum Arabic	150	85.61	622.00	69.92
M5	Gum Arabic	160	90.53	573.80	71.17
M6	Gum Arabic	170	92.79	546.20	72.42
M7	Whey protein	150	35.05	500.00	74.47
M8	Whey protein	160	43.30	423.60	76.89
M9	Whey protein	170	49.63	383.00	78.87

Table 73. Analysis of variance (ANOVA) of solubility in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	15007.935	8	1875.992	13979.88	< 0.0001	Significant	Intercept	75.413	1	0.070
A-Coating material	14564.542	2	7282.271	54267.42	< 0.0001		A[1]	18.523	1	0.100
B-Inlet temperature	366.510	2	183.255	1365.61	< 0.0001		A[2]	14.229	1	0.100
AB	76.883	4	19.221	143.23	< 0.0001		B[1]	-4.690	1	0.100
Pure Error	2.415	18	0.134				B[2]	0.380	1	0.100
Cor Total	15010.351	26					A[1]B[1]	2.263	1	0.141
							A[2]B[1]	0.654	1	0.141
Std. Dev.	0.366		R-Squared	0.99			A[1]B[2]	-0.764	1	0.141
Mean	75.413		Adj R-Squared	0.99			A[2]B[2]	0.507	1	0.141
C.V. %	0.486		Pred R-Squared	0.98						
PRESS	5.435		Adeq Precision	291.70						

The optimized predictive model (in terms of actual factors) for solubility of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$\text{Solubility (\%)} = 75.413 + 18.523*A + 14.229*A^2 - 4.690*B + 0.380*B^2 + 2.263*A*B + 0.654*A^2*B - 0.764*A*B^2 + 0.507*A^2*B^2 \quad \dots (4.48)$$

4.20.2 Effect of different coating materials and inlet air temperatures on wettability of microencapsulated Roselle anthocyanin powder

The wettability of microencapsulated Roselle anthocyanin powder obtained from three different coating materials and inlet air temperatures are presented in Table 72. From the table, it was observed that the wettability of microencapsulated Roselle anthocyanin powder using maltodextrin (MD) as a coating material was found to be in the range of 190.80 to 265.00 s. Similarly, the wettability of microencapsulated Roselle anthocyanin powder obtained using gum arabica (GA) as a coating material was found to be in the range of 546.20 to 622.00 s whereas, the wettability of microencapsulated Roselle anthocyanin powder obtained using whey protein (WP) as a coating material was found to be in the range of 383.00 to 500.00 s.

Considering the different coating materials and inlet air temperatures interaction, it is revealed that the highest wettability of 622.00 s was recorded using gum arabica as a coating material at 150 °C inlet air temperature whereas, minimum of 190.80 s wettability was observed in case of the microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material at inlet air temperature of 170 °C.

Table 74 shows the analysis of variance (ANOVA) and estimated coefficient values for the wettability of microencapsulated Roselle anthocyanin powder. The Model (2FI) F-value of 3225.23 implies that the model was significant ($p < 0.0001$). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A, B and AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.98 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 157.38 indicates an adequate signal.

Table 74. Analysis of variance (ANOVA) of wettability in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	581056.587	8	72632.073	3225.23	< 0.0001	Significant	Intercept	417.111	1	0.913
A-Coating material	541851.387	2	270925.693	12030.45	< 0.0001		A[1]	-181.978	1	1.292
B-Inlet temperature	35672.667	2	17836.333	792.02	< 0.0001		A[2]	163.556	1	1.292
AB	3532.533	4	883.133	39.22	< 0.0001		B[1]	45.222	1	1.292
Pure Error	405.360	18	22.520				B[2]	-1.444	1	1.292
Cor Total	581461.947	26					A[1]B[1]	-15.356	1	1.827
							A[2]B[1]	-3.889	1	1.827
Std. Dev.	4.746		R-Squared	0.99			A[1]B[2]	15.911	1	1.827
Mean	417.111		Adj R-Squared	0.99			A[2]B[2]	-5.422	1	1.827
C.V. %	1.138		Pred R-Squared	0.98						
PRESS	912.060		Adeq Precision	157.38						

The optimized predictive model (in terms of actual factors) for wettability of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$\text{Wettability (s)} = 417.111 - 181.978*A + 163.556*A^2 + 45.222*B - 1.444*B^2 - 15.356*A*B - 3.889*A^2*B + 15.911*A*B^2 - 5.422*A^2*B^2 \quad \dots (4.49)$$

4.20.3 Effect of different coating materials and inlet air temperatures on dispersibility of microencapsulated Roselle anthocyanin powder

The dispersibility of microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures are presented in Table 72. From the table, it is noticed that the dispersibility of microencapsulated Roselle anthocyanin powder varied from 80.85 to 84.30% using maltodextrin (MD) as a coating material and at different inlet air temperatures, whereas, using gum arabica (GA) the dispersibility was varied from 69.92 to 72.42% and with whey protein (WP), the dispersibility was varied from 74.47 to 78.87% at different inlet air temperatures.

In case of coating materials and inlet air temperature interaction, it is revealed that the microencapsulated Roselle anthocyanin powder produced using gum arabica as a coating material with 150 °C inlet air temperature showed the lowest dispersibility of 69.92%. Whereas, microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material with 170 °C inlet air temperature showed the highest dispersibility of 84.30%.

Table 75 shows the analysis of variance (ANOVA) and estimated coefficient values for the dispersibility of microencapsulated Roselle anthocyanin powder. The model (2FI) F-value of 2568.19 implies that the model was significant ($p < 0.0001$). Values of "Prob>F" less than 0.05 indicate model terms were significant. In this case, A, B and AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.98 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 141.57 indicates an adequate signal.

Table 75. Analysis of variance (ANOVA) of dispersibility in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	635.313	8	79.414	2568.19	< 0.0001	Significant	Intercept	76.809	1	0.034
A-Coating material	578.964	2	289.482	9361.62	< 0.0001		A[1]	5.704	1	0.048
B-Inlet temperature	53.459	2	26.729	864.41	< 0.0001		A[2]	-5.639	1	0.048
AB	2.890	4	0.722	23.36	< 0.0001		B[1]	-1.727	1	0.048
Pure Error	0.557	18	0.031				B[2]	0.008	1	0.048
Cor Total	635.869	26					A[1]B[1]	0.065	1	0.068
							A[2]B[1]	0.481	1	0.068
Std. Dev.	0.176		R-Squared	0.99			A[1]B[2]	-0.130	1	0.068
Mean	76.809		Adj R-Squared	0.99			A[2]B[2]	-0.008	1	0.068
C.V. %	0.229		Pred R-Squared	0.98						
PRESS	1.252		Adeq Precision	141.57						

The optimized predictive model (in terms of actual factors) for dispersibility of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$\text{Dispersibility (\%)} = 76.809 + 5.704 * A - 5.639 * A^2 - 1.727 * B + 8.148E-003 * B^2 + 0.065 * A * B + 0.481 * A^2 * B - 0.130 * A * B^2 - 8.148E-003 * A^2 * B^2 \quad \dots (4.50)$$

4.21 Functional properties of microencapsulated Roselle anthocyanin powder

The effect of spray drying processing conditions viz., coating materials (MD, GA and WP) and inlet air temperatures (150, 160 and 170 °C) on functional properties such as total phenolic content, antioxidant activity, total flavonoids and total anthocyanin content of microencapsulated Roselle anthocyanin powder was determined and presented hereunder.

4.21.1 Effect of different coating materials and inlet air temperatures on total phenolic content of microencapsulated Roselle anthocyanin powder

The total phenolic content of microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures are presented in Table 76. From the table, it was observed that the total phenolic content of microencapsulated Roselle anthocyanin powder using maltodextrin (MD) as a coating material was found to be in the range of 38.25 to 40.21 mg GAE.g⁻¹. Similarly, the total phenolic content of microencapsulated Roselle anthocyanin powder obtained using gum arabica (GA) as a coating material was found to be in the range of 35.05 to 37.91 mg GAE.g⁻¹ whereas, the total phenolic content of microencapsulated Roselle anthocyanin powder obtained using whey protein (WP) as a coating material was found to be in the range of 30.68 to 34.17 mg GAE.g⁻¹.

Considering the different coating materials and inlet air temperatures interaction, it is revealed that the lowest total phenolic content of 30.68 mg GAE.g⁻¹ was recorded using whey protein as a coating material at 150 °C inlet air temperature whereas, maximum of 40.21 mg GAE.g⁻¹ total phenolic content was observed in case of the microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material at inlet air temperature of 170 °C.

Table 76. Effect of different coating materials and inlet air temperatures on functional properties of microencapsulated Roselle anthocyanin powder

Treatment	Coating material	Inlet air temperature (°C)	Total phenolic content (mg GAE.g⁻¹)	Antioxidant activity (%)	Total flavonoids (mg RE.g⁻¹)	Total anthocyanin content (mg CyaG.100g⁻¹)
M1	Maltodextrin	150	38.25	70.51	45.15	912.88
M2	Maltodextrin	160	39.11	72.63	48.67	950.98
M3	Maltodextrin	170	40.21	74.87	50.25	994.99
M4	Gum arabic	150	35.05	66.09	38.65	820.51
M5	Gum arabic	160	36.26	67.36	41.32	845.32
M6	Gum arabic	170	37.91	69.59	43.85	880.32
M7	Whey protein	150	30.68	59.72	31.50	538.04
M8	Whey protein	160	32.94	62.42	34.24	569.29
M9	Whey protein	170	34.17	64.89	36.01	605.90

Table 77 shows the analysis of variance (ANOVA) and estimated coefficient values for the total phenolic content of microencapsulated Roselle anthocyanin powder. The Model (2FI) F-value of 106927.72 implies that the model was significant ($p < 0.0001$). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A, B and AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.99 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 997.75 indicates an adequate signal.

The optimized predictive model (in terms of actual factors) for total phenolic content of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$\begin{aligned} \text{Total phenolic content (mg GAE.g}^{-1}\text{)} = & 36.064 + 3.128*A + 0.341*A^2 - 1.405*B + 0.039*B^2 \\ & + 0.463*A*B + 0.050*A^2*B - 0.118*A*B^2 - 0.188 \\ & *A^2*B^2 \dots (4.51) \end{aligned}$$

4.21.2 Effect of different coating materials and inlet air temperatures on antioxidant activity of microencapsulated Roselle anthocyanin powder

The antioxidant activity of microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures are presented in Table 76. From the table, it is noticed that the antioxidant activity of microencapsulated Roselle anthocyanin powder varied from 70.51 to 74.87% using maltodextrin (MD) as a coating material and at different inlet air temperatures, whereas, using gum arabica (GA) the antioxidant activity was varied from 66.09 to 69.59% and with whey protein (WP), the antioxidant activity was varied from 59.72 to 64.89% at different inlet air temperatures.

In case of coating materials and inlet air temperatures interaction, it is revealed that the microencapsulated Roselle anthocyanin powder produced using whey protein as a coating material with 150 °C inlet air temperature showed the lowest antioxidant activity of 59.72%.

Table 77. Analysis of variance (ANOVA) of total phenolic content in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	234.449	8	29.306	106927.72	< 0.0001	Significant	Intercept	36.064	1	0.003
A-Coating material	197.462	2	98.731	360235.19	< 0.0001		A[1]	3.128	1	0.005
B-Inlet temperature	34.577	2	17.288	63078.87	< 0.0001		A[2]	0.341	1	0.005
AB	2.410	4	0.603	2198.41	< 0.0001		B[1]	-1.405	1	0.005
Pure Error	0.005	18	0.000				B[2]	0.039	1	0.005
Cor Total	234.454	26					A[1]B[1]	0.463	1	0.006
							A[2]B[1]	0.050	1	0.006
Std. Dev.	0.017		R-Squared	0.99			A[1]B[2]	-0.118	1	0.006
Mean	36.064		Adj R-Squared	0.99			A[2]B[2]	-0.188	1	0.006
C.V. %	0.046		Pred R-Squared	0.99						
PRESS	0.011		Adeq Precision	997.75						

Whereas, microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material with 170 °C inlet air temperature showed the highest antioxidant activity of 74.87%.

Table 78 shows the analysis of variance (ANOVA) and estimated coefficient values for the antioxidant activity of microencapsulated Roselle anthocyanin powder. The model (2FI) F-value of 1747.34 implies that the model was significant ($p < 0.0001$). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A, B and AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R^2 value of 0.99, whereas the optimized predictive model had an R^2 value of 0.98 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 130.20 indicates an adequate signal.

The optimized predictive model (in terms of actual factors) for antioxidant activity of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$\begin{aligned} \text{Antioxidant activity (\%)} = & 67.566 + 5.106*A + 0.115*A^2 - 2.123*B - 0.096*B^2 - 0.038*A*B \\ & + 0.535*A^2*B + 0.057*A*B^2 - 0.229*A^2*B^2 \quad \dots (4.52) \end{aligned}$$

4.21.3 Effect of different coating materials and inlet air temperatures on total flavonoid content of microencapsulated Roselle anthocyanin powder

The total flavonoid content of microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures are presented in Table 76. From the table, it was observed that the total flavonoid content of microencapsulated Roselle anthocyanin powder using maltodextrin (MD) as a coating material was found to be in the range of 45.15 to 50.25 mg RE.g⁻¹. Similarly, the total flavonoid content of microencapsulated Roselle anthocyanin powder obtained using gum arabica (GA) as a coating material was found to be in the range of 38.65 to 43.85 mg RE.g⁻¹ whereas, the total flavonoid content of microencapsulated Roselle anthocyanin powder obtained using whey protein (WP) as a coating material was found to be in the range of 31.50 to 36.01 mg RE.g⁻¹.

Table 78. Analysis of variance (ANOVA) of antioxidant activity in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	567.483	8	70.935	1747.34	< 0.0001	Significant	Intercept	67.566	1	0.039
A-Coating material	480.091	2	240.046	5913.00	< 0.0001		A[1]	5.106	1	0.055
B-Inlet temperature	84.930	2	42.465	1046.03	< 0.0001		A[2]	0.115	1	0.055
AB	2.462	4	0.616	15.16	< 0.0001		B[1]	-2.123	1	0.055
Pure Error	0.731	18	0.041				B[2]	-0.096	1	0.055
Cor Total	568.213	26					A[1]B[1]	-0.038	1	0.078
							A[2]B[1]	0.535	1	0.078
Std. Dev.	0.201		R-Squared	0.99			A[1]B[2]	0.057	1	0.078
Mean	67.566		Adj R-Squared	0.99			A[2]B[2]	-0.229	1	0.078
C.V. %	0.298		Pred R-Squared	0.98						
PRESS	1.644		Adeq Precision	130.20						

Considering the different coating materials and inlet air temperatures interaction, it is revealed that the lowest total flavonoid content of 31.50 mg RE.g⁻¹ was recorded using whey protein as a coating material at 150 °C inlet air temperature whereas, maximum of 50.25 mg RE.g⁻¹ total flavonoid content was observed in case of the microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material at inlet air temperature of 170 °C.

Table 79 shows the analysis of variance (ANOVA) and estimated coefficient values for the total flavonoid content of microencapsulated Roselle anthocyanin powder. The Model (2FI) F-value of 241458.48 implies that the model was significant (p<0.0001). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A, B and AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R² value of 0.99, whereas the optimized predictive model had an R² value of 0.99 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 1421.64 indicates an adequate signal.

The optimized predictive model (in terms of actual factors) for total flavonoid content of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$\text{Total flavonoids (mg RE.g}^{-1}\text{)} = 41.071 + 6.953*A + 0.202*A^2 - 2.638*B + 0.339*B^2 - 0.233*A*B + 0.014*A^2*B + 0.303*A*B^2 - 0.292*A^2*B^2 \quad \dots (4.53)$$

4.21.4 Effect of different coating materials and inlet air temperatures on total anthocyanin content of microencapsulated Roselle anthocyanin powder

The total anthocyanin content of microencapsulated Roselle anthocyanin powder obtained from different coating materials and inlet air temperatures are presented in Table 76. From the table, it is noticed that the total anthocyanin content of microencapsulated Roselle anthocyanin powder varied from 912.88 to 994.99 mg CyaG.100g⁻¹ using maltodextrin (MD) as a coating material and at different inlet air temperatures.

Table 79. Analysis of variance (ANOVA) of total flavonoids in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	1008.760	8	126.095	241458.48	< 0.0001	Significant	Intercept	41.071	1	0.004
A-Coating material	896.325	2	448.163	858183.92	< 0.0001		A[1]	6.953	1	0.006
B-Inlet temperature	111.218	2	55.609	106485.77	< 0.0001		A[2]	0.202	1	0.006
AB	1.216	4	0.304	582.12	< 0.0001		B[1]	-2.638	1	0.006
Pure Error	0.009	18	0.001				B[2]	0.339	1	0.006
Cor Total	1008.769	26					A[1]B[1]	-0.233	1	0.009
							A[2]B[1]	0.014	1	0.009
Std. Dev.	0.023		R-Squared	0.99			A[1]B[2]	0.303	1	0.009
Mean	41.071		Adj R-Squared	0.99			A[2]B[2]	-0.292	1	0.009
C.V. %	0.056		Pred R-Squared	0.99						
PRESS	0.021		Adeq Precision	1421.64						

Whereas, using gum arabica (GA) the total anthocyanin content was varied from 820.51 to 880.32 mg CyaG.100g⁻¹ and with whey protein (WP), the total anthocyanin content was varied from 538.04 to 605.90 mg CyaG.100g⁻¹ at different inlet air temperatures.

In case of coating materials and inlet air temperatures interaction, it is revealed that the microencapsulated Roselle anthocyanin powder produced using whey protein as a coating material with 150 °C inlet air temperature showed the lowest total anthocyanin content of 538.04 mg CyaG.100g⁻¹. Whereas, microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material with 170 °C inlet air temperature showed the highest total anthocyanin content of 994.99 mg CyaG.100g⁻¹.

Table 80 shows the analysis of variance (ANOVA) and estimated coefficient values for the total anthocyanin content of microencapsulated Roselle anthocyanin powder. The model (2FI) F-value of 2063052.49 implies that the model was significant (p<0.0001). Values of “Prob>F” less than 0.05 indicate model terms were significant. In this case, A, B and AB were significant model terms. Values greater than 0.1 indicate that the model terms were not significant. The statistical analysis indicated that the proposed models fitted the experimental data with R² value of 0.99, whereas the optimized predictive model had an R² value of 0.99 and was in reasonable agreement with the "Adj R-Squared" of 0.99. Adeq Precision ratio of more than 4 was desirable. The Adeq. Precision ratio of 3779.37 indicates an adequate signal.

The optimized predictive model (in terms of actual factors) for total anthocyanin content of microencapsulated Roselle anthocyanin powder is presented in the following equation.

$$\begin{aligned} \text{Total anthocyanin content (mg CyaG.100g}^{-1}\text{)} = & 790.916 + 162.035*A + 57.802*A^2 - 33.771 \\ & *B - 2.385*B^2 - 6.301*A*B + 5.565*A^2*B \\ & + 0.417*A*B^2 - 1.014*A^2*B^2 \quad \dots (4.54) \end{aligned}$$

Table 80. Analysis of variance (ANOVA) of total anthocyanin content in microencapsulated Roselle anthocyanin powder for different coating materials and inlet air temperatures

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F		Term	Coefficient Estimate	df	Standard Error
Model	723793.383	8	90474.173	2063052.49	< 0.0001	Significant	Intercept	790.916	1	0.040
A-Coating material	701323.238	2	350661.619	7996020.32	< 0.0001		A[1]	162.035	1	0.057
B-Inlet temperature	22080.496	2	11040.248	251747.11	< 0.0001		A[2]	57.802	1	0.057
AB	389.649	4	97.412	2221.26	< 0.0001		B[1]	-33.771	1	0.057
Pure Error	0.789	18	0.044				B[2]	-2.385	1	0.057
Cor Total	723794.172	26					A[1]B[1]	-6.301	1	0.081
							A[2]B[1]	5.565	1	0.081
Std. Dev.	0.209		R-Squared	0.99			A[1]B[2]	0.417	1	0.081
Mean	790.916		Adj R-Squared	0.99			A[2]B[2]	-1.014	1	0.081
C.V. %	0.026		Pred R-Squared	0.99						
PRESS	1.776		Adeq Precision	3779.37						

4.22 Optimization of process parameters for microencapsulated Roselle anthocyanin powder

Optimization of two variables namely, coating material and inlet air temperature combinations was performed using the general factorial design in Design Expert Software 7.7.0. Numerical optimization was performed using statistical models to find the optimum coating material and inlet air temperature. In the present investigation, the independent variables were kept within the range and dependent variables were chosen as either maximum or minimum. Table 81 shows the effect of different coating materials and inlet air temperatures on optimization of microencapsulated Roselle anthocyanin powder achieved from General Factorial in Design Expert Software 7.7.0.

4.22.1 Verification of predicted variables for microencapsulated Roselle anthocyanin powder

The suitability of the model equation for predicting the optimum response values was verified using the optimal condition. The experimental sample had the optimum process conditions of maltodextrin as a coating material and 170 °C inlet air temperature. This optimized parameter was carried out thrice and the average values of each response are presented and verification of the predicted and actual responses for optimized coating materials and inlet air temperatures is presented in Table 82.

4.23 Cost of production of microencapsulated Roselle anthocyanin powder using different coating materials and inlet air temperatures

The cost of production of microencapsulated Roselle anthocyanin powder obtained using different coating materials and inlet air temperatures is presented in Appendix II. Estimation of cost of production for 100 kg of microencapsulated anthocyanin from calyces of Roselle was computed as ₹ 1,84,200 with benefit cost ratio of 3.53. The Net returns and Gross returns of microencapsulated anthocyanin from calyces of Roselle was computed as ₹ 4,65,800 and 6,50,000, respectively. Hence, the developed process technology could be adopted for commercial production of microencapsulated Roselle anthocyanin powder.

Table 81. Effect of different coating materials and inlet air temperatures on optimization of microencapsulated Roselle anthocyanin powder

Sl. No.	Parameter	Goal	Lower limit	Upper limit
1	Coating material	is in range	Maltodextrin	Whey protein
2	Inlet air temperature (°C)	is in range	150	170
3	Powder yield (%)	maximize	63.11	92.76
4	Moisture content (%)	minimize	2.14	3.94
5	Water activity	minimize	0.21	0.31
6	pH	minimize	2.28	3.91
7	Titrateable acidity (%)	maximize	13.89	17.66
8	Loose bulk density (g.cc ⁻¹)	minimize	0.23	0.40
9	Tapped bulk density (g.cc ⁻¹)	minimize	0.28	0.53
10	Carr's index	minimize	15.91	24.00
11	Hausner ratio	minimize	1.19	1.32
12	Particle size (d.nm)	minimize	326.60	1186.70
13	L^*	minimize	64.00	70.50
14	a^*	maximize	7.00	28.90
15	b^*	minimize	2.78	8.97
16	Solubility (%)	maximize	34.79	97.06
17	Wettability (s)	minimize	187.20	629.40
18	Dispersibility (%)	maximize	69.74	84.48
19	Total phenolic content (mg GAE.g ⁻¹)	maximize	30.67	40.23
20	Antioxidant activity (%)	maximize	59.55	75.04
21	Total flavonoids (mg RE.g ⁻¹)	maximize	31.48	50.27
22	Total anthocyanin content (mg CyaG.100 g ⁻¹)	maximize	537.92	995.17

Table 82. Predicted and actual values of responses for optimized coating materials and inlet air temperatures

Sl. No.	Responses	Predicted value	Actual value	Variation %
1	Powder yield (%)	92.04	91.35	-0.028
2	Moisture content (%)	2.16	2.19	0.051
3	Water activity	0.21	0.20	-0.176
4	pH	2.29	2.31	0.032
5	Titrateable acidity (%)	17.41	17.35	-0.013
6	Loose bulk density (g.cc ⁻¹)	0.23	0.24	0.161
7	Tapped bulk density (g.cc ⁻¹)	0.28	0.29	0.132
8	Carr's index	16.03	17.24	0.279
9	Hausner ratio	1.19	1.20	0.031
10	Particle size (d.nm)	354.70	359.56	0.051
11	L^*	64.81	67.35	0.145
12	a^*	28.87	25.46	-0.437
13	b^*	2.93	2.98	0.063
14	Solubility (%)	96.75	95.88	-0.033
15	Wettability (s)	190.80	186.48	-0.084
16	Dispersibility (%)	84.30	85.67	0.060
17	Total phenolic content (mg GAE.g ⁻¹)	40.21	40.78	0.052
18	Antioxidant activity (%)	74.87	76.45	0.078
19	Total flavonoids (mg RE.g ⁻¹)	50.25	49.58	-0.049
20	Total anthocyanin content (mg CyaG.100 g ⁻¹)	994.99	991.23	-0.014
21	Desirability	0.97		

4.24 SEM analysis of microencapsulated Roselle anthocyanin powder

The analysis of the surface morphology and characteristics of powder particles obtained with optimized coating material and inlet air temperature was performed using SEM allowing three dimensional characteristics to be visualized. The powder obtained with maltodextrin at 170 °C resulted in the formation of more homogeneous capsules. The spheres had less wrinkles on their surfaces for microencapsulated Roselle anthocyanin powder produced using maltodextrin as coating material with 170 °C inlet air temperature. The poor capsule formation was observed with whey protein as a coating material at an inlet air temperature of 150 °C.

4.25 Stability of microencapsulated Roselle anthocyanin

The influence of key factors, pH and temperature on the stability of microencapsulated Roselle anthocyanin were studied and the results are presented hereunder.

4.25.1 Effect of pH on stability of microencapsulated Roselle anthocyanin

Effect of pH (2, 3, 4, 5, 6 and 7) on stability of microencapsulated Roselle anthocyanin are presented in Table 83. The highest retention of Roselle anthocyanin was observed in case of pH 2 (968.69 mg CyaG.100 g⁻¹) followed by pH 3 (898.81 mg CyaG.100 g⁻¹) after 10 days of storage. Similarly, lowest retention was observed in case of pH 6 (364.46 mg CyaG.100 g⁻¹) after 10 days of storage.

4.25.2 Effect of temperature on stability of microencapsulated Roselle anthocyanin

Effect of temperature (5, 20, 35, 50, 65 and 80 °C) on stability of microencapsulated Roselle anthocyanin are presented in Table 84. The highest retention of Roselle anthocyanin was observed in case of 5 °C (992.96 mg CyaG.100 g⁻¹) followed by 20 °C (965.30 mg CyaG.100 g⁻¹) temperature after 2 hours of storage. Similarly, lowest retention was observed in case of 80 °C (199.46 mg CyaG.100 g⁻¹) temperature after 2 hours of storage. Whereas, the Roselle anthocyanin at room temperature after 2 hours of storage was found to be 913.36 mg CyaG.100 g⁻¹.

Table 83. Effect of pH on stability of microencapsulated Roselle anthocyanin

Days	Total anthocyanin content (mg CyaG.100 g ⁻¹)						
	pH	2	3	4	5	6	7
0 (1 h)		994.87 (99.99)	993.92 (99.89)	968.75 (97.36)	884.44 (88.89)	845.26 (84.95)	814.67 (81.88)
1		994.69 (99.97)	993.26 (99.83)	959.75 (96.46)	791.65 (79.56)	778.95 (78.29)	775.91 (77.98)
2		994.10 (99.91)	992.13 (99.71)	941.03 (94.58)	761.24 (76.51)	737.92 (74.16)	736.20 (73.99)
3		993.92 (99.89)	990.34 (99.53)	918.31 (92.29)	712.82 (71.64)	666.19 (66.95)	659.39 (66.27)
4		992.90 (99.79)	988.61 (99.36)	892.07 (89.66)	630.53 (63.37)	584.08 (58.70)	576.98 (57.99)
5		991.00 (99.60)	986.58 (99.16)	869.05 (87.34)	579.19 (58.21)	570.01 (57.29)	572.75 (57.66)
6		987.95 (99.29)	970.48 (97.54)	809.48 (81.36)	541.68 (54.44)	516.52 (51.91)	528.98 (53.16)
7		981.04 (98.60)	963.27 (96.81)	784.38 (78.83)	507.39 (50.99)	459.03 (46.13)	469.59 (47.20)
8		974.84 (97.97)	938.64 (94.34)	764.28 (76.81)	473.40 (47.58)	418.49 (42.06)	437.63 (43.98)
9		970.84 (97.57)	917.17 (92.18)	743.59 (74.73)	420.69 (42.28)	385.69 (38.76)	398.33 (40.03)
10		968.69 (97.36)	898.81 (90.33)	714.79 (71.84)	382.41 (38.43)	364.46 (36.63)	376.57 (37.85)

*Values in the parenthesis represents the pigment retention per cent; Pigment retention per cent was calculated based on initial total anthocyanin content present in the optimized microencapsulated Roselle anthocyanin powder (100%)

Table 84. Effect of temperature on stability of microencapsulated Roselle anthocyanin

Time (min)	Total anthocyanin content (mg CyaG.100 g ⁻¹)							
	Temp.	5 °C	20 °C	35 °C	50 °C	65 °C	80 °C	Room temp.
0		994.87 (100)	994.87 (100)	994.87 (100)	994.87 (100)	994.87 (100)	994.87 (100)	994.87 (100)
30		994.87 (100)	991.35 (99.65)	960.05 (96.50)	822.12 (82.64)	748.72 (75.26)	505.19 (50.78)	970.48 (97.55)
60		994.45 (99.96)	988.61 (99.37)	931.54 (93.63)	753.73 (75.76)	612.52 (61.57)	393.08 (39.51)	950.69 (95.56)
90		993.68 (99.88)	983.12 (98.82)	914.97 (91.97)	714.61 (71.83)	539.12 (54.19)	292.19 (29.37)	933.99 (93.88)
120		992.96 (99.81)	965.30 (97.03)	910.97 (91.57)	675.07 (67.86)	451.64 (45.40)	199.46 (20.05)	913.36 (91.81)

*Values in the parenthesis represents the pigment retention per cent; Pigment retention per cent was calculated based on initial total anthocyanin content present in the optimized microencapsulated Roselle anthocyanin powder (100%)

*Discussion*__

V. DISCUSSION

The results of investigation on proximate composition, physico-chemical and functional properties of fresh calyces of Roselle are discussed in first section, drying behaviour and mathematical modeling for drying of fresh calyces of Roselle in different drying methods, proximate composition, physico-chemical and functional properties of calyces of Roselle powder are discussed in second section. Physico-chemical and functional properties of solvent extracted anthocyanin from calyces of Roselle are discussed in third section. Physico-chemical, reconstitution and functional properties of microencapsulated Roselle anthocyanin powder are discussed in fourth section. In the last section, stability of microencapsulated Roselle anthocyanin are discussed.

5.1 Proximate composition of fresh calyces of Roselle

The results of proximate composition viz., moisture content, crude protein, crude fat, carbohydrates, crude fibre and total ash of fresh calyces of Roselle are presented in Table 6, Chapter IV and are discussed hereunder.

5.1.1 Moisture content

In the present investigation, average moisture content of fresh calyces of Roselle was found to be $86.08 \pm 0.65\%$ (w.b.). The result was in good agreement with the literature values of Babalola *et al.* (2001), Fajar *et al.* (2012) and Langova *et al.* (2013) who reported the moisture content of fresh Roselle calyces as 86.50% (w.b.), 85.00% (w.b.) and 86.50% (w.b.), respectively. Zaman *et al.* (2017) reported the higher value of moisture content as 88.09% (w.b.) for fresh Roselle calyces.

5.1.2 Crude protein

The average crude protein content of fresh calyces of Roselle was found to be $2.95 \pm 0.14\%$. The results obtained were lower than the earlier studies reported by Zaman *et al.* (2017), who reported the protein content of 10.28% for fresh Roselle calyces. Mahadevan *et al.* (2009) reported the lower value of protein content as 1.46% for fresh Roselle calyces.

5.1.3 Crude fat

The average crude fat content of fresh calyces of Roselle was found to be $0.90 \pm 0.02\%$. The results obtained were less than the earlier studies of Mahadevan *et al.* (2009), who reported the fat content of 2.61% for fresh Roselle calyces.

5.1.4 Carbohydrates

The average carbohydrate content of the fresh calyces of Roselle was found to be $6.21 \pm 0.21\%$. The result obtained was in close agreement with the literature value of Fasoyiro *et al.* (2005), who reported that the fresh Roselle calyces had carbohydrate content of 6.30%.

5.1.5 Crude fibre

The average crude fibre content of the fresh fresh calyces of Roselle was found to be $2.59 \pm 0.27\%$. The result obtained was more than the findings of Gautam (2004), who reported the crude fibre content of fresh Roselle calyces as 1.58%.

5.1.6 Total ash

The data pertaining to total ash content of fresh calyces of Roselle was observed as $1.09 \pm 0.02\%$. This result is lower than the earlier studies of Mahadevan *et al.* (2009), who reported the total ash content of 6.90% for fresh Roselle calyces.

The variation in the proximate composition of fresh calyces of Roselle procured from the M/s 'Herbs n Health' Gadag, Karnataka for the present investigation in comparison with the earlier studies might be due to the varietal difference, maturity stage, geographical area of cultivation and cultivation practices.

5.2 Physico-chemical properties of fresh calyces of Roselle

The results of physico-chemical properties of fresh calyces of Roselle viz., pH, water activity, L^* value, a^* value, b^* value, ascorbic acid and titratable acidity are presented in Table 7, Chapter IV and are discussed hereunder.

5.2.1 pH

The average pH value of fresh calyces of Roselle was found to be 2.16 ± 0.02 . The result obtained was in close agreement with the literature value of Zaman *et al.* (2017), who reported that the fresh Roselle calyces had pH value of 2.07.

5.2.2 Water activity

The average water activity of fresh calyces of Roselle was found to be 0.75 ± 0.03 .

5.2.3 L^* value

The average L^* value of fresh calyces of Roselle was found to be 32.44 ± 0.26 . The result obtained was more than the literature value of Zaman *et al.* (2017), who reported that the fresh Roselle calyces had L^* value of 28.76.

5.2.4 a^* value

The results on a^* value of fresh calyces of Roselle was found to be 26.83 ± 0.05 . The result obtained was more than the literature value of Zaman *et al.* (2017), who reported that the fresh Roselle calyces had a^* value of 18.82.

5.2.5 b^* value

The average b^* value of fresh calyces of Roselle was found to be 9.38 ± 0.18 . The result obtained was more than the literature value of Zaman *et al.* (2017), who reported that the fresh Roselle calyces had b^* value of 3.22.

5.2.6 Ascorbic acid

The average ascorbic content of fresh calyces of Roselle was found to be 46.36 ± 0.49 mg.100g⁻¹. The result obtained was less than the literature value of Zaman *et al.* (2017), who reported that the fresh Roselle calyces had ascorbic content of 66.52 mg.100g⁻¹.

5.2.7 Titratable acidity

The average titratable acidity of fresh calyces of Roselle was found to be $20.12 \pm 0.80\%$. The result obtained was in good agreement with the literature value of

Zaman *et al.* (2017), who reported that the fresh Roselle calyces had titratable acidity of 20.22%.

The variation in the physico-chemical properties of fresh calyces of Roselle procured from the M/s 'Herbs n Health' Gadag, Karnataka for the present investigation in comparison with the earlier studies might be due to the varietal difference, maturity stage, geographical area of cultivation and cultivation practices.

5.3 Functional properties of fresh calyces of Roselle

The results of functional properties of fresh calyces of Roselle viz., total phenolic content, antioxidant activity, total flavonoids and total anthocyanin content is presented in Table 8, Chapter IV and are discussed hereunder.

5.3.1 Total phenolic content

The average total phenolic content of fresh calyces of Roselle was found to be 20.85 ± 0.08 mg GAE.g⁻¹. The result obtained was more than the literature value of Zaman *et al.* (2017), who reported that the fresh Roselle calyces had total phenolic content of 10.18 mg GAE.g⁻¹.

5.3.2 Antioxidant activity

The average antioxidant activity of fresh calyces of Roselle was found to be $92.23 \pm 0.77\%$. The result obtained was in close agreement with the literature value of Zaman *et al.* (2017), who reported that the fresh Roselle calyces had antioxidant activity of 92.12%.

5.3.3 Total flavonoids

The average total flavonoid content of fresh calyces of Roselle was found to be 38.34 ± 0.11 mg RE.g⁻¹. The result obtained was more than the literature value of Zaman *et al.* (2017), who reported that the fresh Roselle calyces had total flavonoid content of 3.26 mg RE.g⁻¹.

5.3.4 Total anthocyanin content

The average total anthocyanin content of fresh calyces of Roselle was found to be 718.72 ± 1.19 mg CyaG.100g⁻¹. The result obtained was more than the literature value of

Zaman *et al.* (2017), who reported that the fresh Roselle calyces had total anthocyanin content of 326.12 mg CyaG.100g⁻¹.

The variation in the functional properties of fresh calyces of Roselle procured from the M/s 'Herbs n Health' Gadag, Karnataka for the present investigation in comparison with the earlier studies might be due to the varietal difference, maturity stage, geographical area of cultivation and cultivation practices.

5.4 Drying behaviour of calyces of Roselle

Removal of moisture takes place by high pressure or high temperature conditions. Drying removes the volatile materials present inside solid matter. Drying is carried out to bring down the moisture content to a pre-determined level. The change in moisture content during successive post harvest stages is dependent on the initial moisture content of product and atmospheric conditions. The information on moisture content is necessary because it tells us whether the product is suitable for storage or for any other processing job. For some period, if the moisture content of product increases to a fixed critical value, under such conditions chances of metabolic reactions inside the product become higher. Due to such reactions, drying becomes necessary at higher moisture contents which were reported by Manjula *et al.* (2013).

The results of drying characteristics namely, reduction in moisture content, drying rate and moisture ratio of fresh calyces of Roselle dried under three different drying methods are discussed hereunder.

5.4.1 Effect of different drying methods on reduction in moisture content of calyces of Roselle

The variations in moisture content of calyces of Roselle dried under solar tunnel drying, shade drying and dehumidified air drying methods are depicted in Fig. 4. From the figure, it is observed that the drying time was significantly influenced by the drying methods used for drying of fresh calyces of Roselle.

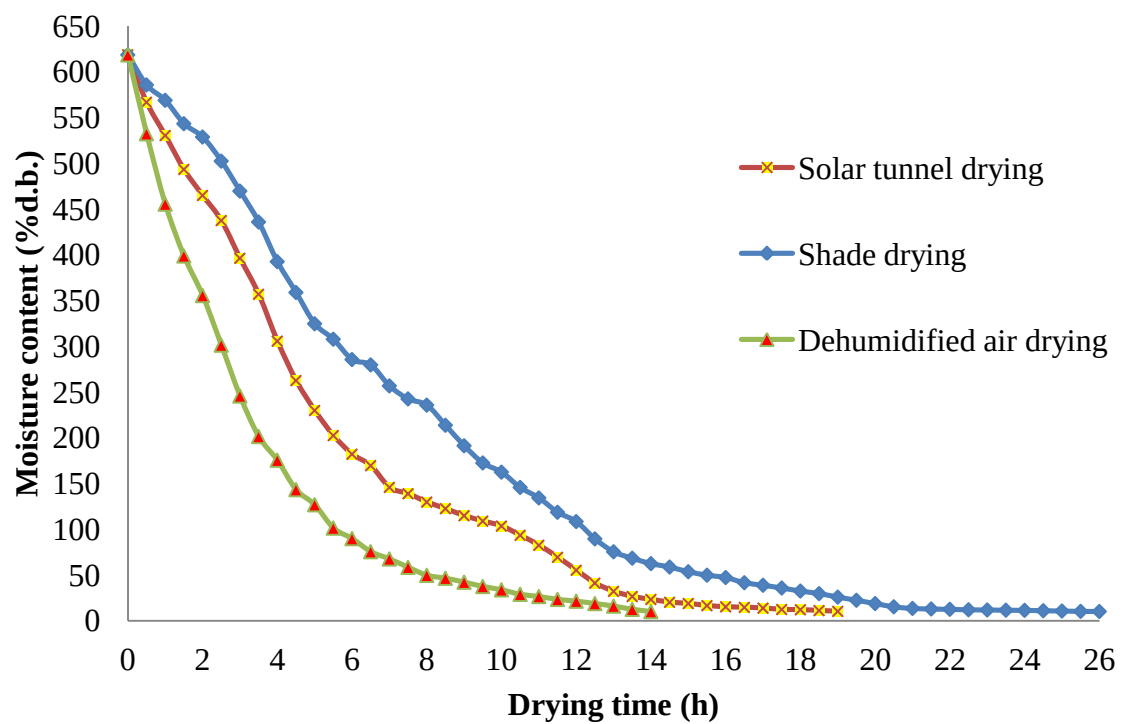


Fig. 4. Effect of different drying methods on reduction in moisture content of calyces of Roselle

Considering the effect of selected drying methods, it can be observed that shade drying (SD) took highest drying time followed by solar tunnel drying (STD) and dehumidified air drying (DD) methods. This might be due to the lower drying air temperature prevailed during the drying process and more relative humidity fluctuations in the atmosphere in case of shade drying, similar result were recorded for shade drying of Isparta rose flowers (Boyar *et al.*, 2013). Shorter drying period in dehumidified air drying might be due to less relative humidity of air maintained inside the drying chamber, similar results were recorded for drying of whole leaf Aloe vera in dehumidified air dryer (Pattali *et al.*, 2015). Whereas, the solar tunnel drying of Roselle calyces took lesser time in relating to shade drying this might be due to the higher temperature attained inside the STD and less relative humidity might have absorbed larger quantity of moisture in lesser time. Similar results were recorded for drying of Byadagi chilli and onion slices in solar tunnel dryer by Manjula *et al.* (2013) and Gouda *et al.* (2014), respectively.

5.4.2 Effect of different drying methods on drying rate of calyces of Roselle

The variations in drying of calyces of Roselle dried under solar tunnel drying, shade drying and dehumidified air drying methods are depicted in Fig. 5. This figure shows the influence of drying air temperature on drying rate with respect to drying time. It can be seen that, the drying process mainly consisted of three drying periods *i.e.*, heating up, constant rate and falling rate period.

From the figure, it is noticed that the drying rate was significantly influenced by the drying methods. The lowest drying rate was noticed in case of SD followed by STD and DD methods. This might be due to the lower drying air temperature prevailed during the drying process and more relative humidity fluctuations in the atmosphere in case of shade drying, similar result were recorded for shade drying of Isparta rose flowers (Boyar *et al.*, 2013). Shorter drying period in dehumidified air drying might be due to less relative humidity of air maintained inside the drying chamber, similar result were recorded for drying of whole leaf Aloe vera in dehumidified air dryer (Pattali *et al.*, 2015). Whereas, the solar tunnel drying of Roselle calyces took lesser time in relating to shade drying this might be due to the higher temperature attained inside the STD and less relative humidity might have absorbed larger quantity of moisture in lesser time resulting in higher drying rate.

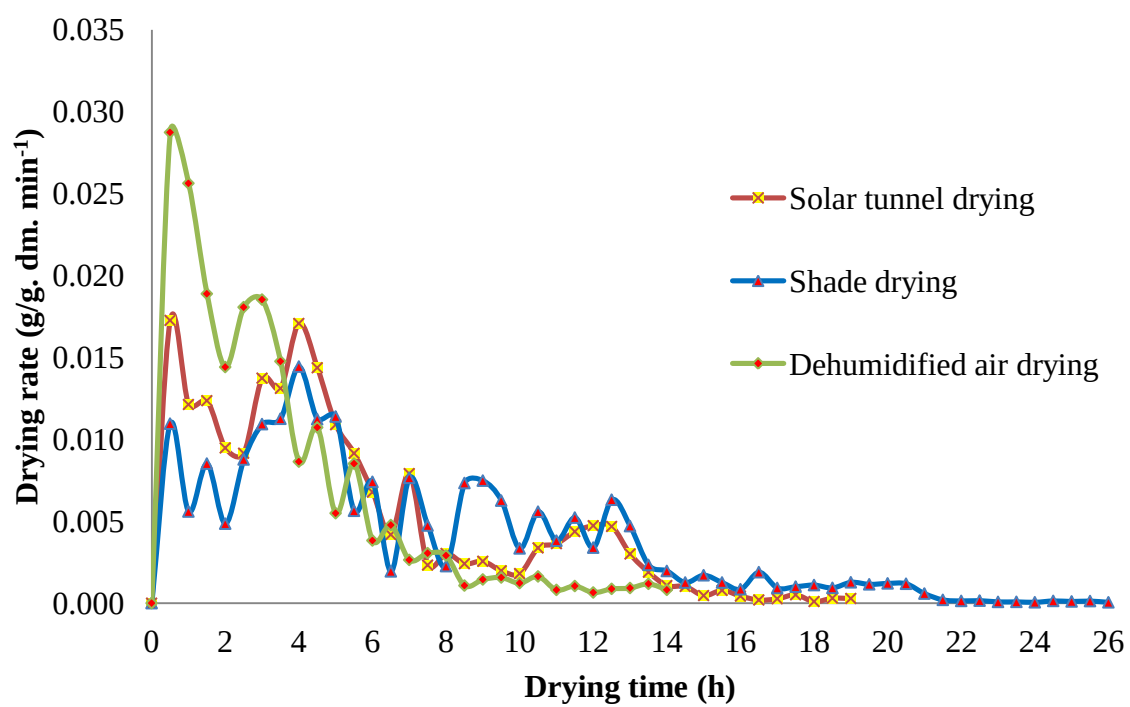


Fig. 5. Effect of different drying methods on drying rate of calyces of Roselle

Similar results were recorded for drying of Byadagi chilli and onion slices in solar tunnel dryer by Manjula *et al.* (2013) and Gouda *et al.* (2014), respectively.

From the data obtained during investigation, it was observed that, the constant rate period of drying was absent during the entire period of drying and the drying took place only under the falling rate period in all the three drying methods. Similar types of results were observed in the case of dill and parsley leaves dried in laboratory hot air cabinet drier (Doymaz *et al.*, 2006), Roselle dried in tray drier (Fajar *et al.*, 2012), Isparta rose flowers dried in shade drying (Boyar *et al.*, 2013) and osmo air drying of Roselle calyces (Undre *et al.*, 2015).

5.4.3 Effect of different drying methods on moisture ratio of calyces of Roselle

The variations in moisture ratio of calyces of Roselle dried under shade drying, solar tunnel drying and dehumidified air drying methods are depicted in Fig. 6. From the figure, it can be observed that the moisture ratio was significantly influenced by the drying methods. The lowest moisture ratio was noticed in case of SD followed by STD and DD methods. This might be due to the lower drying air temperature prevailed during the drying process and more relative humidity fluctuations in the atmosphere in case of shade drying, similar result were recorded for shade drying of Isparta rose flowers (Boyar *et al.*, 2013). Shorter drying period in dehumidified air drying might be due to less relative humidity of air maintained inside the drying chamber, similar result were recorded for drying of whole leaf Aloe vera in dehumidified air dryer (Pattali *et al.*, 2015). Whereas, the solar tunnel drying of Roselle calyces took lesser time in relating to shade drying this might be due to the higher temperature attained inside the STD and less relative humidity might have absorbed larger quantity of moisture in lesser time. Similar results were recorded for drying of Byadagi chilli and onion slices in solar tunnel dryer by Manjula *et al.* (2013) and Gouda *et al.* (2014), respectively.

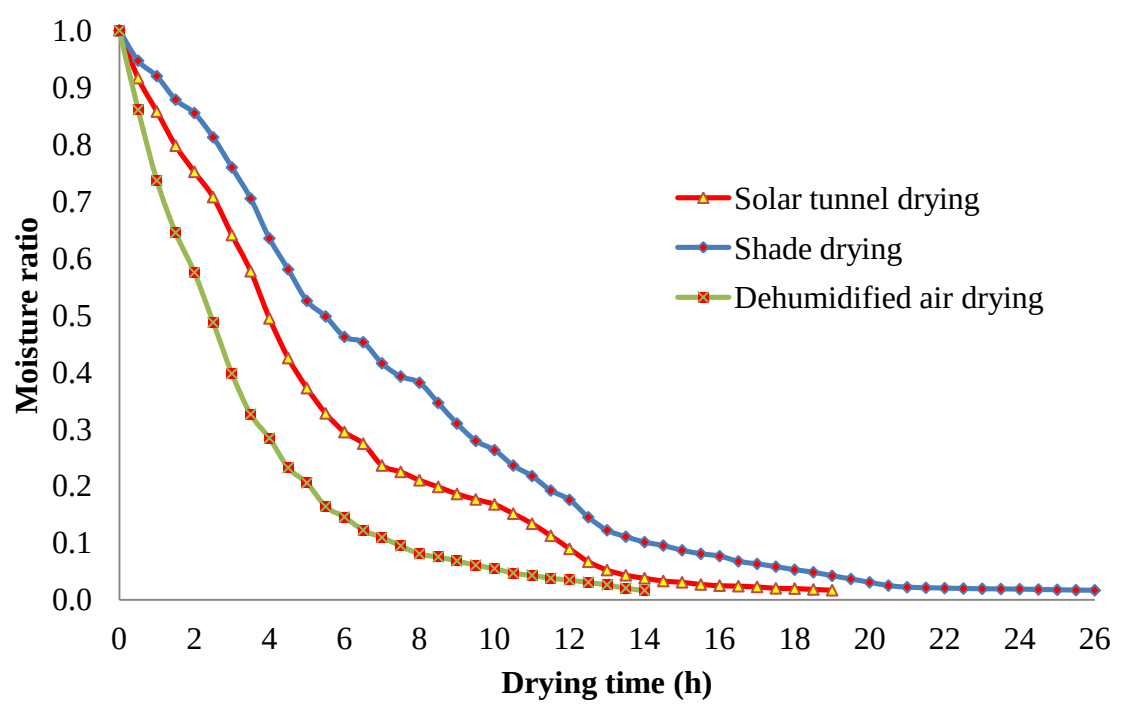


Fig. 6. Effect of different drying methods on moisture ratio of calyces of Roselle

5.4.4 Mathematical modeling of drying of calyces of Roselle in different drying methods

The drying data obtained during thin layer drying was fitted into six different drying models viz., Newton, Page, Modified Page, Henderson-Pabis, Modified Henderson-Pabis and Logarithmic models. The values of moisture ratio were determined for all drying methods such as shade drying, solar tunnel drying and dehumidified air drying. The Page model was successfully described the relationship between moisture ratio and drying time than the other models irrespective of drying methods. The variations of experimental and predicted moisture ratio values with drying time are depicted in Fig. 7.

Among the different drying methods, the Page model was best fitted with the samples dried under dehumidified air dryer with the highest R^2 value of 0.9983. This might be due to the RH and temperature of drying air influenced k and n values in the Page model. The result obtained was in good agreement with the results reported by Gunhan *et al.* (2005) for drying of bay leaves in laboratory dryer with R^2 value of 0.9993, Upadhyaya *et al.* (2012) for hot air drying of spinach leaves with R^2 value of 0.9911, Ali *et al.* (2014) for solar dried seaweed with R^2 value of 0.9903 and Pattali *et al.* (2015) for dehumidified air drying of whole leaf aloe vera powder with R^2 value of 0.9764. Whereas, Fajar *et al.* (2012) reported Newton model was best fit model for tray drying of Roselle calyces with R^2 value of 0.9963.

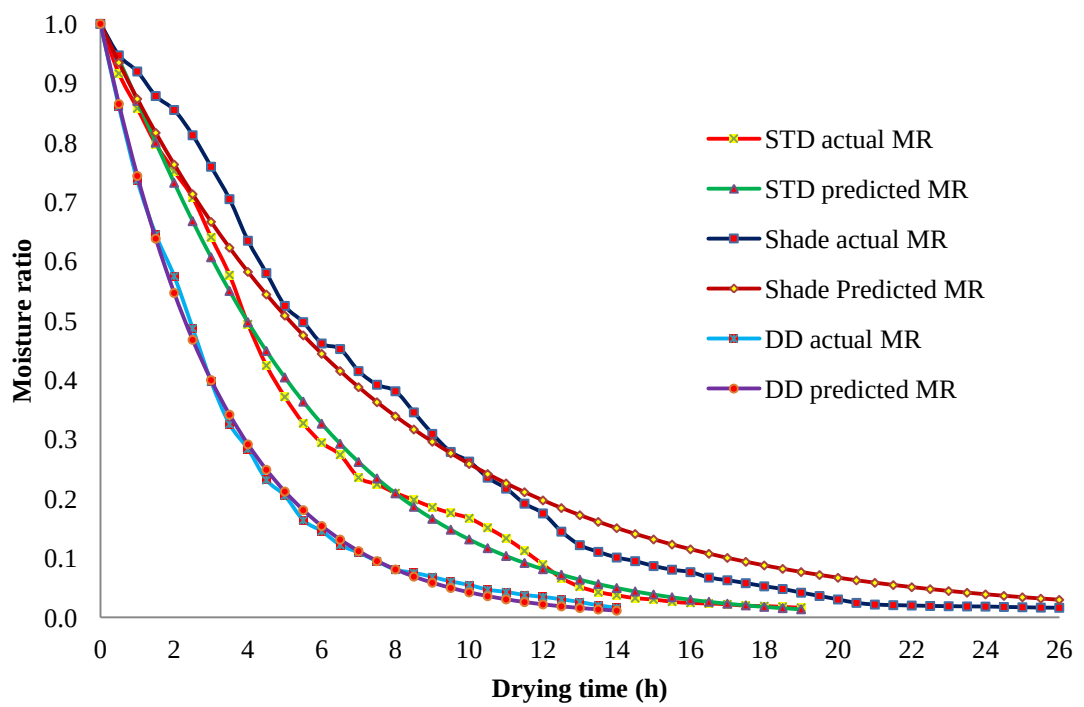


Fig. 7. Effect of different drying methods on experimental and Page model predicted moisture ratio of calyces of Roselle

5.5 Effect of different drying and milling methods on proximate composition of calyces of Roselle powder

The proximate composition of calyces of Roselle powder viz., moisture content, crude protein, crude fat, carbohydrates, crude fibre and total ash content of calyces of Roselle powder in different drying and milling methods were analyzed and discussed hereunder.

5.5.1 Moisture content

The moisture content represents the water composition in a food system, it plays a key role in all food material and it is one of the most commonly measured properties of food materials. The information on moisture content is necessary because it tells us whether the product is suitable for storage or for further processing. The variation in moisture content of calyces of Roselle powder obtained during different drying and milling methods are depicted in Fig. 8. The treatment DM9 recorded 4.45% lower moisture content compared to DM1.

From the figure, it is seen that the moisture content was significantly influenced by the drying methods and milling methods as well. Considering the effect of selected drying methods, it is clear that solar tunnel drying resulted in higher moisture content compared to shade drying and dehumidified drying irrespective of milling methods. The lowest moisture content was observed in case of dehumidified air dried samples, this might be due to the dehumidification affected the vapour pressure sufficiently to increase the driving force for drying of samples. Similar results were reported by Djaeni *et al.* (2015) for drying of carrageenan and Sankalpa *et al.* (2016a) for drying of sweet orange peel.

From the figure, it can also be observed that lower moisture content was noticed in case of calyces of Roselle powder obtained by LN₂ cooled pulverizer followed by water cooled pulverizer and normal pulverizer. The similar results were reported (Sankalpa *et al.*, 2016a) for milling of sweet orange peel powder. The dehumidified air dried sample had the lower moisture content, it might be due to the shielding effect that the equipment has on the sample, preventing rehydration by relative humidity in air. Similar results were also reported by Abioye *et al.* (2014) for solar drying of baobab leaves.

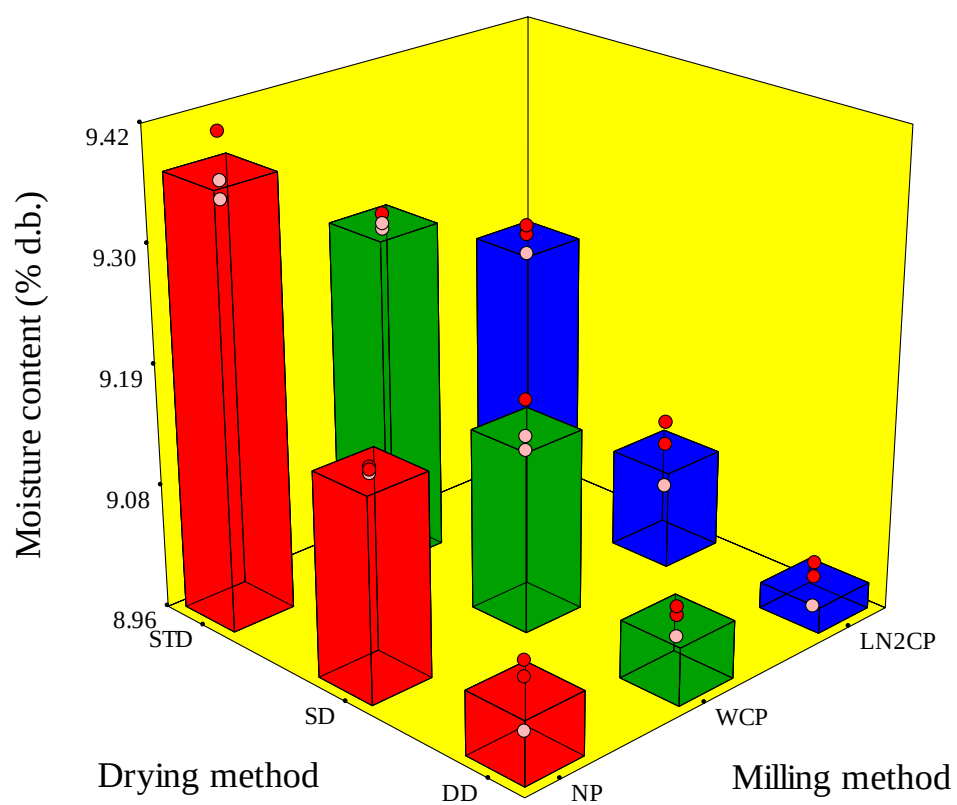


Fig. 8. Effect of drying and milling methods on moisture content of calyces of Roselle powder

5.5.2 Crude protein

The crude protein content of calyces of Roselle powder obtained by different drying and milling methods were determined and presented graphically in Fig. 9. From the figure, it can be observed that the highest crude protein content of calyces of Roselle powder was noticed in case of DD followed by SD and STD methods. The treatment DM9 recorded 19.91% higher crude protein content compared to DM1. The increase in crude protein was due to the increase in dry matter contents and concentration of soluble solids and decrease in moisture content in the sample (Zaman *et al.*, 2017). The similar results were reported by Wijewardane *et al.* (2015) for drying of hibiscus. The loss of protein in solar tunnel drying might be due to the denaturation or changes in solubility during drying and subsequent milling. Another possible cause was the release of amino acids from the proteins following denaturation, which could react with other compounds such as sugars to produce dark brown coloured polymers (Lee and Shibamoto, 2002; Perera, 2005). The loss in protein could also be attributed to the mild heating effect associated with all the drying conditions which could result in the unzipping of hydrophobic forces leading to a partial distribution of the primary, secondary, tertiary and quaternary structure of the protein molecule (Abioye *et al.*, 2014).

From the figure, it can also be depicted that the highest crude protein content of calyces of Roselle powder was observed in case of LN₂CP followed by WCP and NP milling methods. This might be due to the normal milling in pulverizer at ambient condition generates heat which is detrimental to the product and results in loss of quality *i.e.* high temperature reduces the volatile components and heat sensitive constituents present in dried calyces of Roselle. Whereas, LN₂ cooled pulverizer does not damage or alter the chemical composition of the material (Singh and Goswami, 1999) since the process was done at low temperature. Also Maillard reaction could be responsible for losses of protein as this also depends on the intensity of heat and temperature (Ogunlakin *et al.*, 2012). Because of these reasons, protein content was higher in calyces of Roselle powder obtained by dehumidified air drying and LN₂ cooled pulverized milling methods.

5.5.3 Crude fat

The crude fat content of calyces of Roselle powder obtained by different drying and milling methods is depicted graphically in Fig. 10.

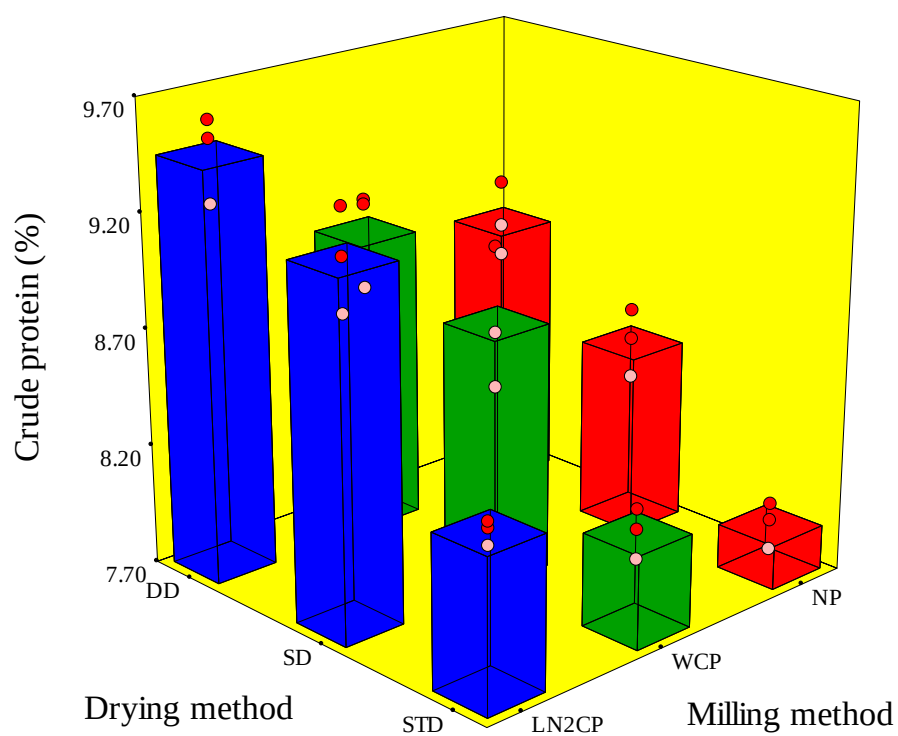


Fig. 9. Effect of drying and milling methods on crude protein content of calyces of Roselle powder

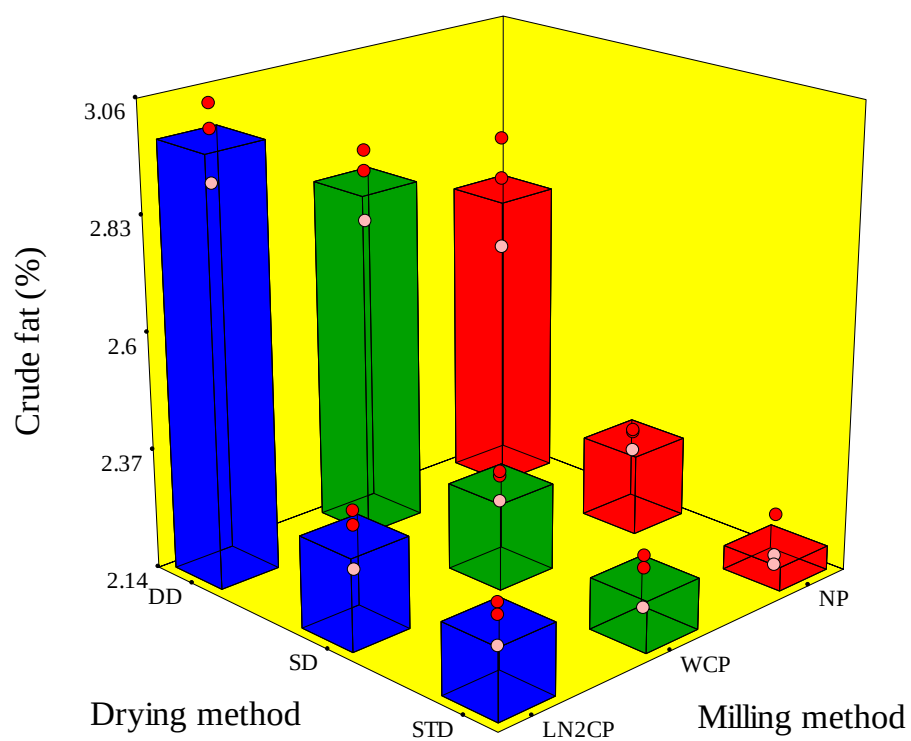


Fig. 10. Effect of drying and milling methods on crude fat content of calyces of Roselle powder

From the figure, it is seen that the highest crude fat content was observed in case of DD followed by SD and STD methods. The treatment DM9 recorded 36.38% higher crude fat content compared to DM1. This might be due to the reduction of moisture content during drying resulted in increased nutrient content of the dried samples. Similar results were reported by Wijewardane *et al.* (2015) for drying of hibiscus, Zaman *et al.* (2017) for oven and sun drying of Roselle calyces and Abioye *et al.* (2014) for shade drying of baobab leaves. The lower fat content observed in shade and solar tunnel drying samples could be associated with the oxidation of fat during the period of drying. Similar results were reported by Agoreyo *et al.* (2011) for sun and solar drying of cocoyam. Similarly from the figure, it can also be depicted that the highest fat content of calyces of Roselle powder was observed in case of LN₂CP followed by WCP and NP milling methods. LN₂ cooled pulverizer does not damage or alter the chemical composition of the material (Singh and Goswami, 1999) since the process was done at low temperature.

5.5.4 Carbohydrates

The results of carbohydrate content of calyces of Roselle powder obtained by different drying and milling methods are depicted graphically in Fig. 11. From the figure, it can be observed that the highest carbohydrate content was observed in case of STD followed by SD and DD methods. The treatment DM9 recorded 9.72% lower carbohydrate content compared to DM1. The decreases in the carbohydrate content of the dehumidified air dried samples on dry weight basis could be linked to the increases in the other components. Similar results were reported by Zaman *et al.* (2017) for oven and sun drying of Roselle calyces and Abioye *et al.* (2014) for shade drying of baobab leaves. Lower carbohydrate content contribute to the lower calorific value, due to lower calorific value, it can be used as dietary fibre. Decrease in carbohydrate content probably occurred as a result of Maillard reaction; which results in complex changes in food due the reaction between carbohydrate and protein (Boumendjel and Boutebba, 2003; Wiriya *et al.*, 2009; Agoreyo *et al.*, 2011) . This might be due to the conversion of starch into dextrin during drying, which drove off the water, whilst the sugar caramelised quickly and then burnt. The amount lost during drying may be the low molecular weight carbohydrates. Similar results were reported by Boateng (2013) for drying of Basil. Similarly from the figure, it can also be depicted that the highest carbohydrate content of calyces of Roselle powder was observed in case of NP followed by WCP and LN₂CP milling methods. This might be due to increase in other components in the LN₂CP milled sample.

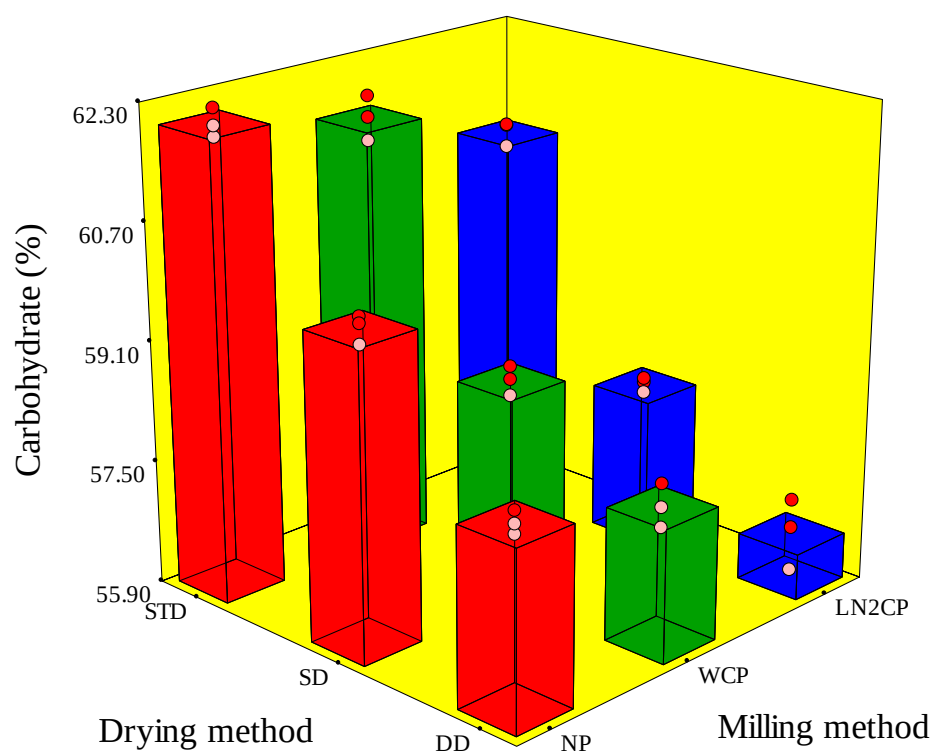


Fig. 11. Effect of drying and milling methods on carbohydrate content of calyces of Roselle powder

5.5.5 Crude fibre

The variations in crude fibre content of calyces of Roselle powder dried under solar tunnel drying, shade drying and dehumidified air drying methods are depicted in Fig. 12. The crude fibre content measures the cellulose, hemicelluloses and lignin contents of food (Ogunlakin *et al.*, 2012). From the figure, it is clear that the highest crude fibre content was observed in case of DD followed by SD and STD methods. The treatment DM9 recorded 14.91% higher crude fibre content compared to DM1. Processes involving heat treatment may affect the fibre in different ways. An increased temperature leads to breakage of weak bonds between polysaccharide chains. Also glycosidic linkages in the dietary fibre polysaccharides may be broken, lead to the loss in fibre content (Peerajith *et al.*, 2012). The apparent increase in fibre content observed in this study could be due to removal of moisture which tends to increase the concentration of nutrients (Morris *et al.*, 2004; Agoreyo *et al.*, 2011; Yarkwan and Uvir, 2015).

Similarly from the figure, it can also be depicted that the highest crude fibre content of calyces of Roselle powder was observed in case of LN₂CP followed by WCP and NP milling methods. Temperature mainly effects the changes in fibre content. With the use of LN₂ cooled pulverizer and dehumidified air drying, less or negligible heat is produced. Hence it might be no change in the chemical composition of calyces of Roselle powder.

5.5.6 Total ash

The variations in total ash content of calyces of Roselle powder dried under solar tunnel drying, shade drying and dehumidified air drying methods are depicted in Fig. 13. The ash content is a measure of the total amount of minerals present within a food (Zaman *et al.*, 2017). From the figure, it is seen that the highest ash content was noticed in case of DD followed by SD and STD methods. The treatment DM9 recorded 33.49% higher total ash content compared to DM1. This might be due to the reduction in moisture content increased the nutrient composition of the food materials. In addition, it is known that loss of moisture increased nutrient density of foods of which ash is among the nutrients. Similar results were reported by Singh *et al.* (2006) for drying of Amaranth, curry leaves, drumstick leaves, fenugreek (methi) and spinach (palak) and Wijewardane *et al.* (2015) for drying of hibiscus.

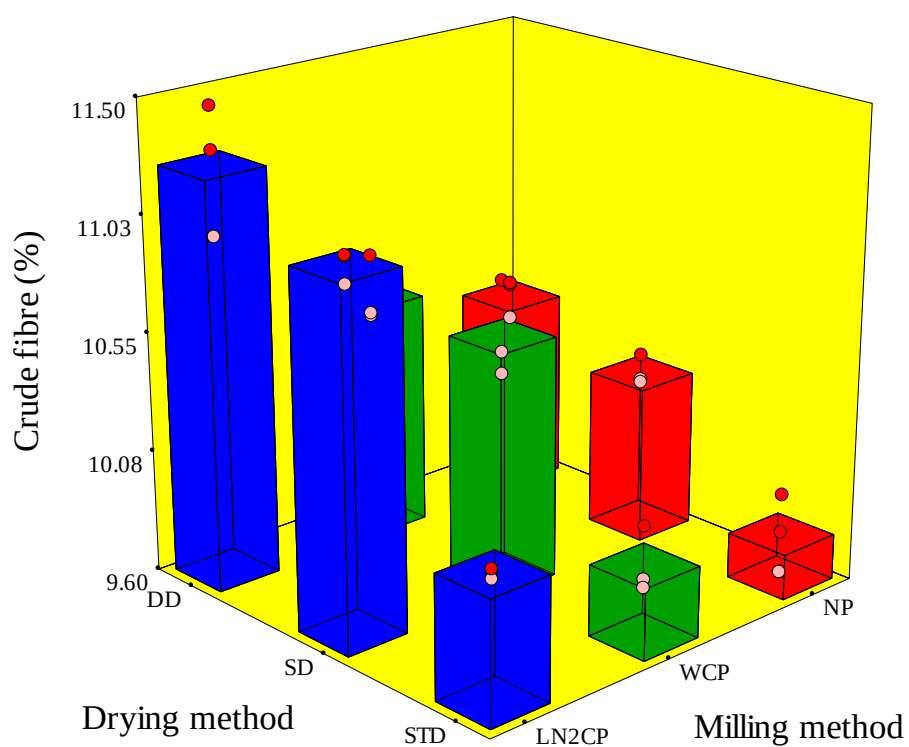


Fig. 12. Effect of drying and milling methods on crude fibre content of calyces of Roselle powder

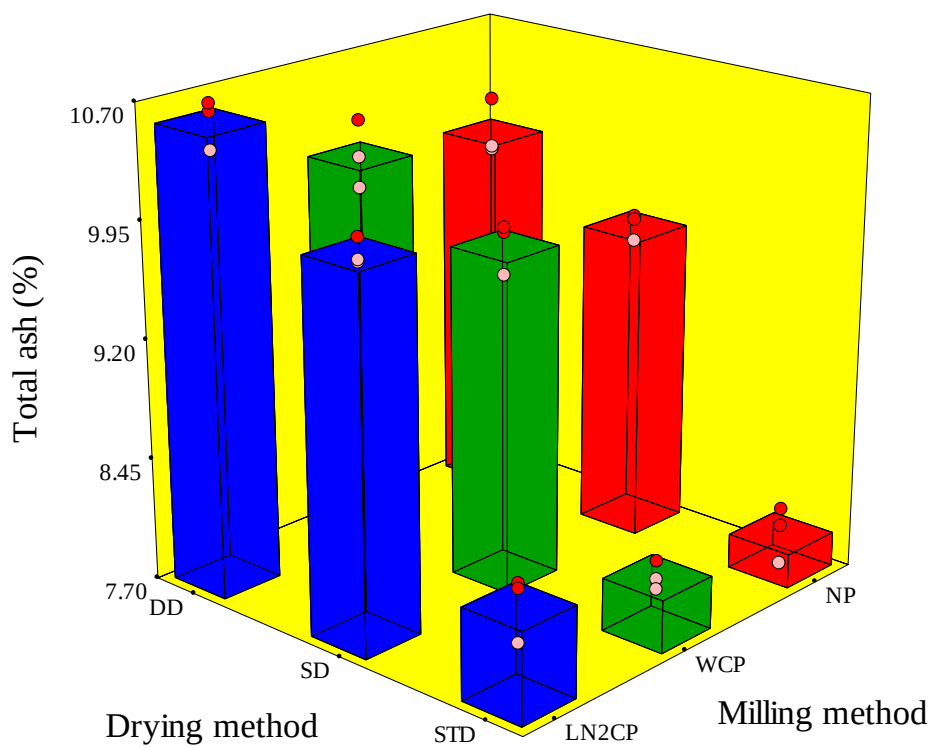


Fig. 13. Effect of drying and milling methods on total ash content of calyces of Roselle powder

Similarly from the figure, it can also be depicted that the highest total ash content of calyces of Roselle powder was observed in case of LN₂CP followed by WCP and NP milling methods. The apparent increase in ash content observed in this study could be as a result of the removal of moisture which tends to increase the concentration of nutrients (Morris *et al.*, 2004; Agoreyo *et al.*, 2011; Yarkwan and Uvir, 2015).

The significance differences observed in the nutrient composition of the calyces of Roselle powder may be due to the increased activities of the microorganisms due to chance of fermentation resulting in the extracellular enzymatic secretion and production of organic acids from available nutrients as described by Ashaye *et al.* (2008) and Bolade *et al.* (2009). Also varietal characteristics, soil nutrient and climatic condition may be responsible for these observations (Hsien *et al.*, 2005; Ashaye, 2013).

5.6 Physico-chemical properties of calyces of Roselle powder

Physico-chemical properties viz., L^* , a^* , b^* , loose bulk density, tapped bulk density, Carr's index, Hausner ratio, pH, water activity, titratable acidity, ascorbic acid, solubility index and particle size of calyces of Roselle powder obtained from different drying and milling methods are discussed hereunder.

5.6.1 Effect of different drying and milling methods on colour L^* value of calyces of Roselle powder

The variation in L^* value of calyces of Roselle powder obtained during different drying and milling methods are depicted in Fig. 14. From the figure, it can be noticed that the L^* value was significantly influenced by the drying and milling methods. The treatment DM9 recorded 22.23% lower L^* value compared to DM1. Considering the effect of selected drying methods, it is clear that DD resulted in lower L^* value compared to SD and STD samples irrespective of milling methods. This might be due more retention of red colour in the dehumidified air dried sample as compared to other two drying methods which resulted in lesser L^* value. Similar results were reported by Arab *et al.* (2011) for shade drying of Roselle and Ramallo and Mascheroni (2012) for hot air oven drying of pineapple fruit. From the figure, it can also be observed that NP had highest L^* value followed by WCP and LN₂CP milling methods. This might be due more retention of red colour in the LN₂CP milled sample as compared to other two milling methods which resulted in less L^* value.

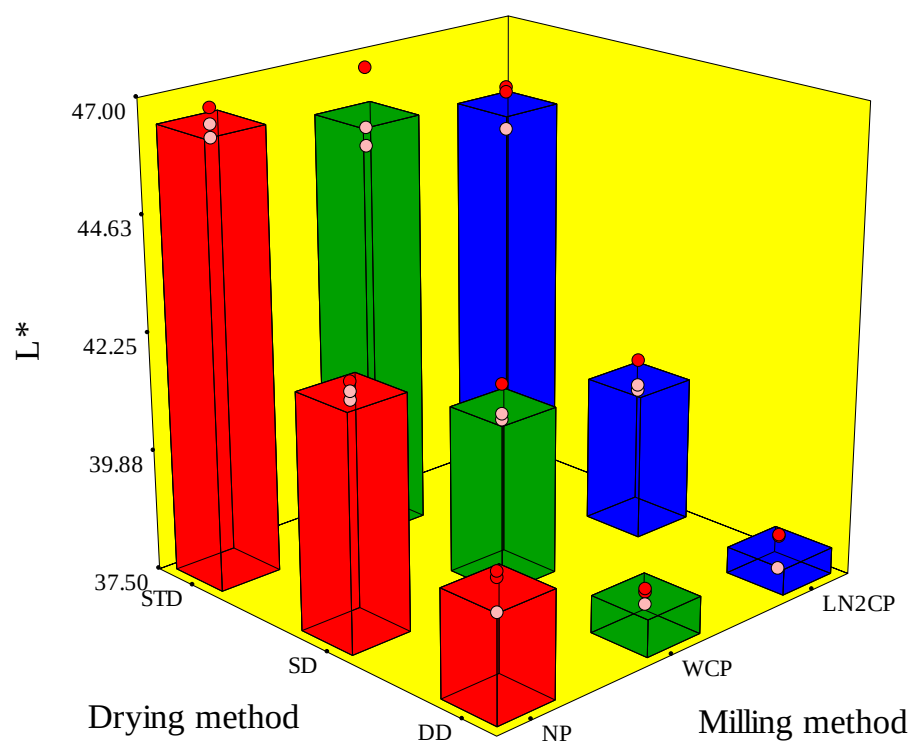


Fig. 14. Effect of drying and milling methods on L^* value of calyces of Roselle powder

5.6.2 Effect of different drying and milling methods on colour a^* value of calyces of Roselle powder

The variation in a^* value of calyces of Roselle powder obtained during different drying and milling methods are depicted in Fig. 15. From the figure, it can be noticed that the a^* value was significantly influenced by the drying and milling methods. The treatment DM9 recorded 33.61% higher a^* value compared to DM1. Considering the effect of selected drying methods, it is clear that DD resulted in higher a^* value compared to SD and STD samples irrespective of milling methods. The longer drying time required during shade drying and solar tunnel drying and convective heat transfer process and high temperatures involved in solar tunnel drying might lead to reductions in the redness of the samples. Similar results were reported by Arslan and Ozcan, (2011) for oven drying of tomato slices and Gamli (2011) for conventional drying of tomato puree.

From the figure, it can also be observed that NP had lower a^* value followed by WCP and LN₂CP milling methods. This might be due more retention of red colour in the LN₂CP milled sample as compared to other two milling methods which resulted in higher a^* value. Low temperature milling resulted in better retention of red colour.

5.6.3 Effect of different drying and milling methods on colour b^* value of calyces of Roselle powder

The variation in b^* value of calyces of Roselle powder obtained during different drying and milling methods are depicted in Fig. 16. From the figure, it can be noticed that the b^* value was significantly influenced by the drying and milling methods. The treatment DM9 recorded 22.11% lower b^* value compared to DM1. Considering the effect of selected drying methods, it is clear that DD resulted in lower b^* value compared to SD and STD samples irrespective of milling methods. This might be due more retention of red colour in the dehumidified air dried sample as compared to other two drying methods which resulted in lesser b^* value. Similar results were reported by Arab *et al.* (2011) for shade drying of Roselle and Ramallo and Mascheroni (2012) for hot air oven drying of pineapple fruit. From the figure, it can also be observed that NP had highest b^* value followed by WCP and LN₂CP milling methods. This might be due more retention of red colour in the LN₂CP milled sample as compared to other two milling methods which resulted in less b^* value.

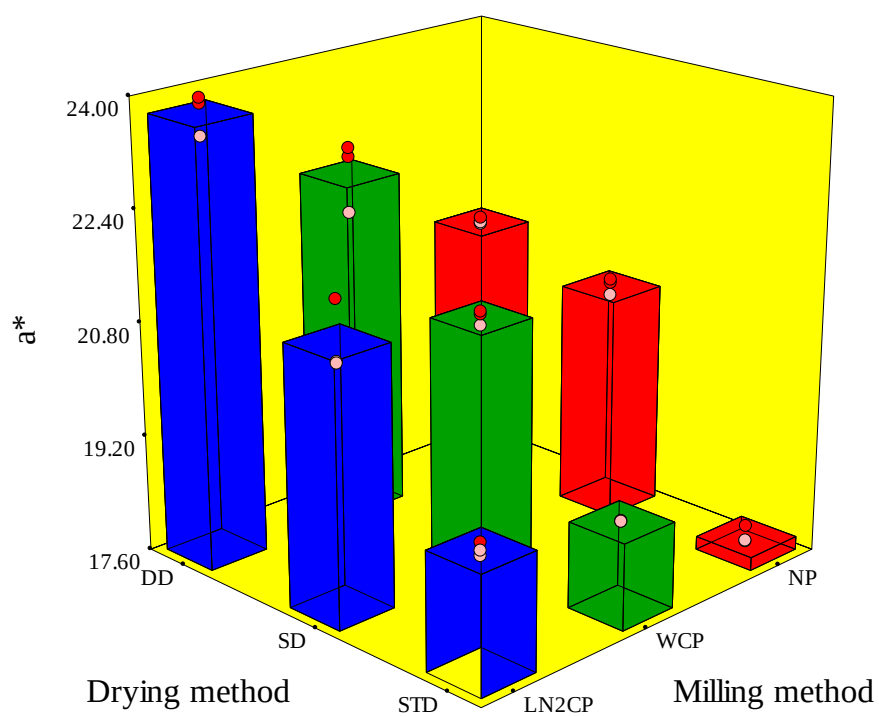


Fig. 15. Effect of drying and milling methods on a^* value of calyces of Roselle powder

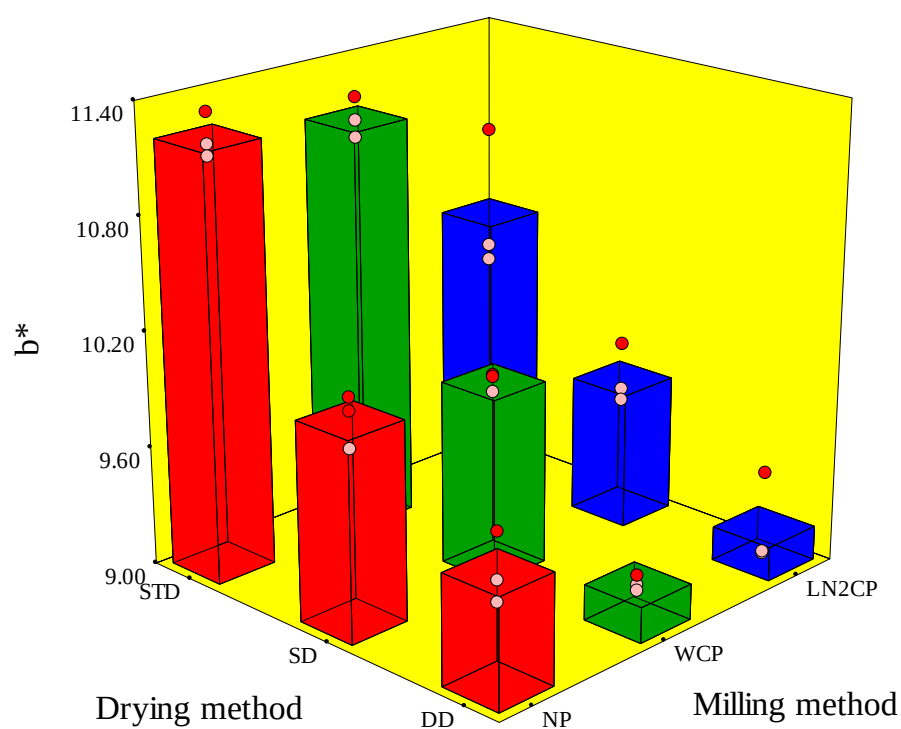


Fig. 16. Effect of drying and milling methods on b^* value of calyces of Roselle powder

5.6.4 Effect of different drying and milling methods on loose bulk density and tapped bulk density of calyces of Roselle powder

The variations in loose bulk density and tapped bulk density of calyces of Roselle powder obtained from different drying and milling methods are depicted in Fig. 17 and Fig. 18. From the figure, it is seen that the lower loose bulk density and tapped bulk density was noticed in case of DD with LN₂CP milled Roselle powder. The treatment DM9 recorded 27.66% and 32.73% lower loose and tapped bulk density, respectively compared to DM1. The bulk density provides a perspective from the packing and arrangement of the particles and the compaction profile of a material. The drying process significantly influenced the bulk density of the powder. The bulk density depends on the attractive inter-particle forces, particle size and number of contact positions. The bulk density of the powder is primarily dependent on particle size, particle size distribution and particle shape, this might be the reason for the significant changes in the bulk density of powder (Mirhosseini and Amid, 2013). These variations might be due to variation observed in the infinite compressibility achieved during tapping, and reduced effect of particle rearrangement due to particle size (Chevanan *et al.*, 2010). The decrease in value could be attributed to the increase in pore spaces which became proportionally greater on moisture absorption, resulting to a decrease in bulk density. Similar trend was observed in case of bulk density of Roselle seeds reported by Bamgboye and Adejumo (2009).

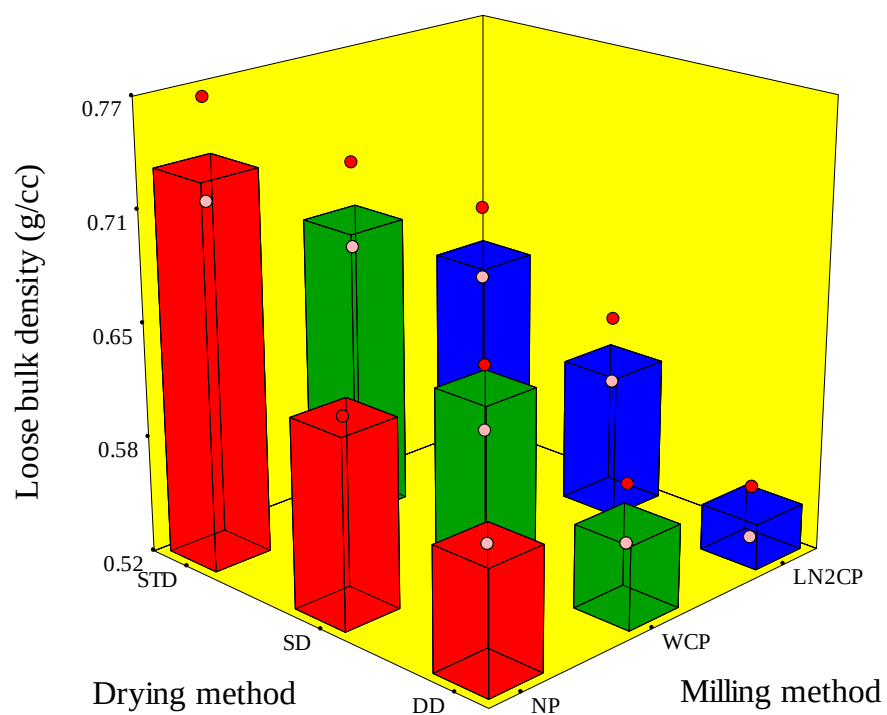


Fig. 17. Effect of drying and milling methods on loose bulk density of calyces of Roselle powder

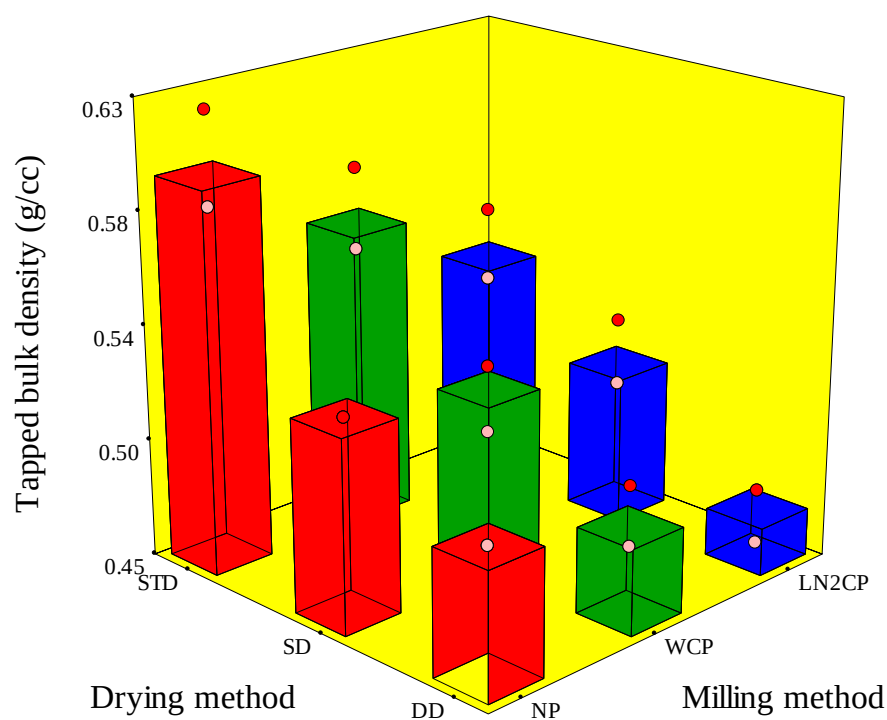


Fig. 18. Effect of drying and milling methods on tapped bulk density of calyces of Roselle powder

5.6.5 Effect of different drying and milling methods on Carr's index of calyces of Roselle powder

Carr's index (CI) is a measure of powder bridge strength and stability and the Hausner ratio (HR) is a measure of the interparticulate friction (Jan *et al.*, 2015). Carr's index or compressibility index is the indirect measure of various powder characteristics viz., bulk density, size and shape, surface area, moisture content and cohesiveness of the material (Singh and Kumar, 2012). The variations in Carr's index of calyces of Roselle powder obtained from different drying and milling methods are depicted in Fig. 19. From the figure, it can be observed that the lower Carr's index was noticed in case of DD with LN₂CP milled Roselle powder. The treatment DM9 recorded 28.05% lower Carr's index compared to DM1. This can be related to the particle size of the milled sample. As the particle size increased, the flowability and cohesiveness of powder was expected to decrease (Jinapong *et al.*, 2008). However, for date palm powder the smaller particles produced larger surface area per unit mass of powder, created more cohesive forces and more frictional forces to resist against the flow. Moisture content had a significant effect on the flowability and cohesiveness of powder. Liquid bridges and capillary forces acting between powder particles reduced flowability and increased the cohesiveness of powder. In addition, moisture content plasticizes the powder material, especially the water soluble constituents, results in deformation of the powder (Kim *et al.*, 2009; Moreira *et al.*, 2009).

5.6.6 Effect of different drying and milling methods on Hausner ratio of calyces of Roselle powder

The variations in Hausner ratio of calyces of Roselle powder obtained from different drying and milling methods are depicted in Fig. 20. From the figure, it can be observed that the lower Hausner ratio was noticed in case of DD with LN₂CP milled Roselle powder. The treatment DM9 recorded 5.17% lower Hausner ratio compared to DM1. This can be related to the particle size of the milled sample. The reason behind this flowability at small particle sizes was due to the large surface area per unit mass of powder. There was more contact surface area between powder particles available for cohesive forces, in particular and frictional forces to resist flow (Fitzpatrick *et al.*, 2004; Kim *et al.*, 2002). Teunou *et al.* (1999) also stated that the presence of water in a powder could significantly affect its flowability, sticking and caking properties.

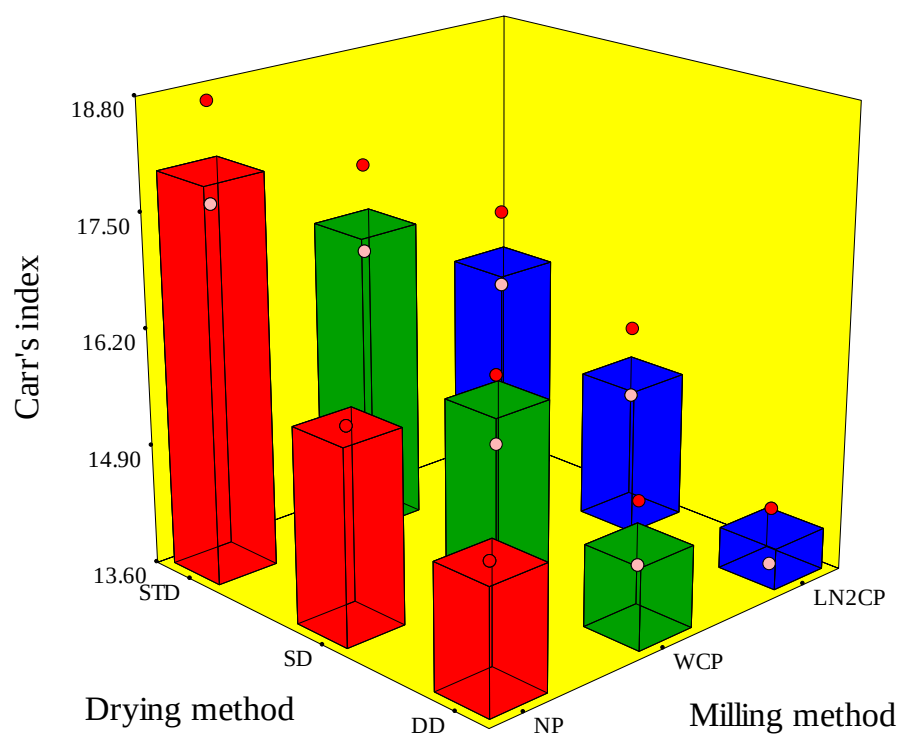


Fig. 19. Effect of drying and milling methods on Carr's index of calyces of Roselle powder

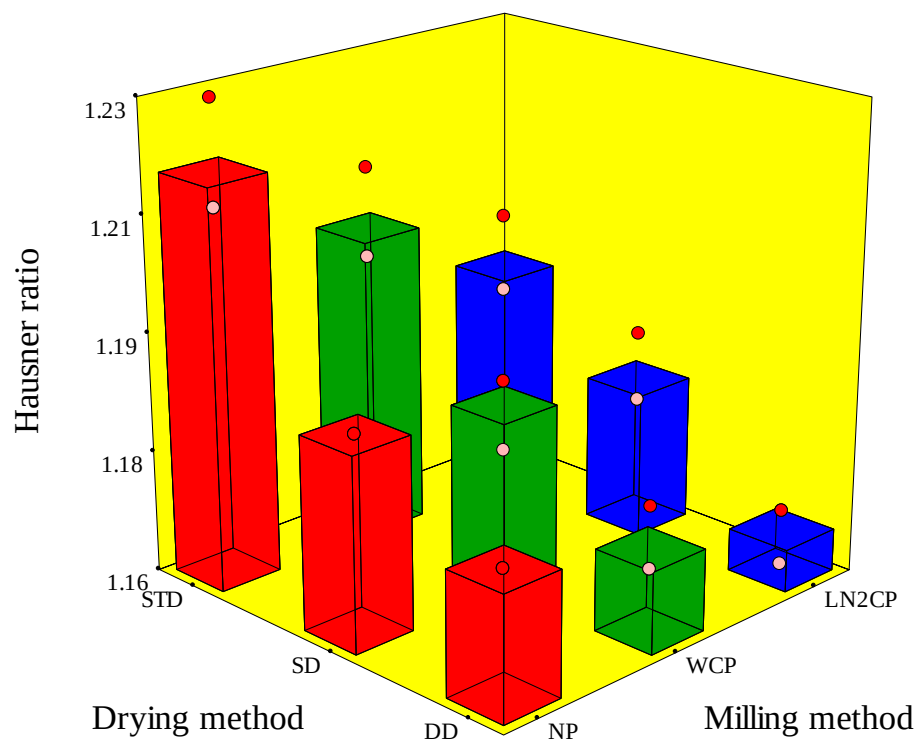


Fig. 20. Effect of drying and milling methods on Hausner ratio of calyces of Roselle powder

In general, the greater the water content of a powder, the more cohesive the powder becomes and the more difficult to flow.

5.6.7 Effect of different drying and milling methods on pH of calyces of Roselle powder

The variation in pH of calyces of Roselle powder obtained during different drying and milling methods are depicted in Fig. 21. From the figure, it can be noticed that the pH was significantly influenced by the drying and milling methods. The treatment DM9 recorded 18.05% lower pH compared to DM1. Considering the effect of selected drying methods, it is clear that DD resulted in lower pH compared to SD and STD samples irrespective of milling methods. This might be attributed to high drying rate which did not allow for the completion of the needed oxidative reaction and acid diffusion process. The pH is expected to increase with the increase in drying temperature. Similar trend was explained by Lasisi (2014) for oven drying of cocoa beans, Adejumo (2012) for oven and sun drying of tomato and Sahoo *et al.* (2015) for solar and cabinet drying of banana powder. Arab *et al.* (2011) reported pH of 2.73 for calyces of Roselle, it depends on the concentration of free H ions or mirrored the changes in total organic acids. The free state of H ions was due to dissociation of H ions from the carboxylic group (-COOH) of organic acid. From the figure, it can also be observed that LN₂CP had lower pH followed by WCP and LN₂CP milling methods. This might be due to low temperature milling retained higher acidity which in turn resulted in lower pH value. This can be correlated to the higher ascorbic acid content in the LN₂CP milled Roselle powder.

5.6.8 Effect of different drying and milling methods on water activity of calyces of Roselle powder

The results of water activity of calyces of Roselle powder obtained by different drying and milling methods are depicted graphically in Fig. 22. The treatment DM9 recorded 22.86% lower water activity compared to DM1. From the figure, it can be observed that the highest water activity was observed in case of STD followed by SD and DD methods. The water activity was decreased due to reduction in moisture content during drying. From the figure, it can also be observed that LN₂CP had lower water activity followed by WCP and LN₂CP milling methods. This might be due to lower moisture content in the LN₂CP milled Roselle powder. The water activity of calyces of Roselle powder obtained from different drying and milling method was less than 0.6.

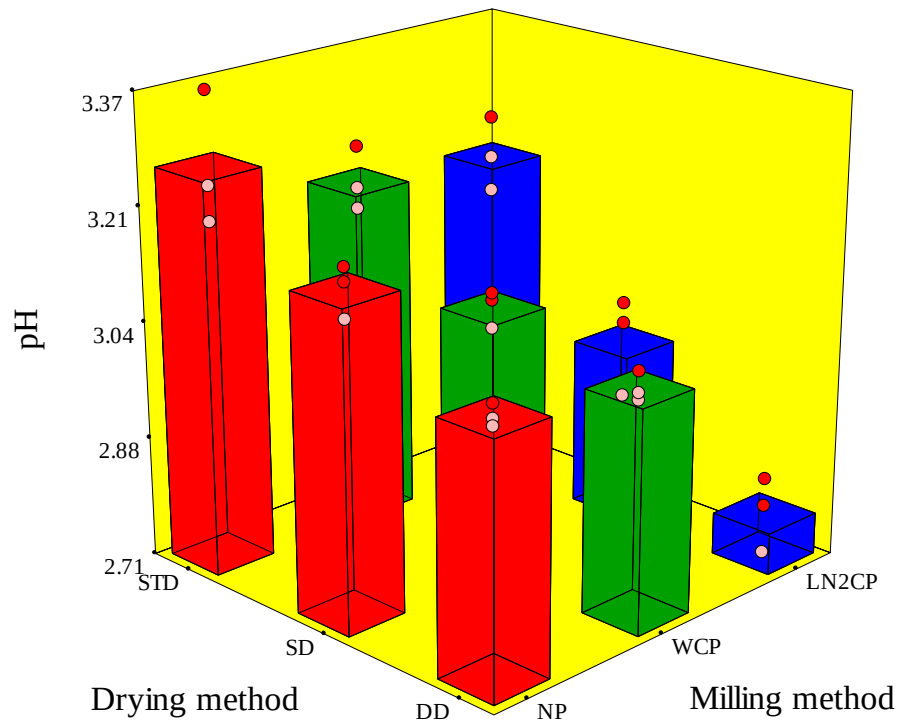


Fig. 21. Effect of drying and milling methods on pH of calyces of Roselle powder

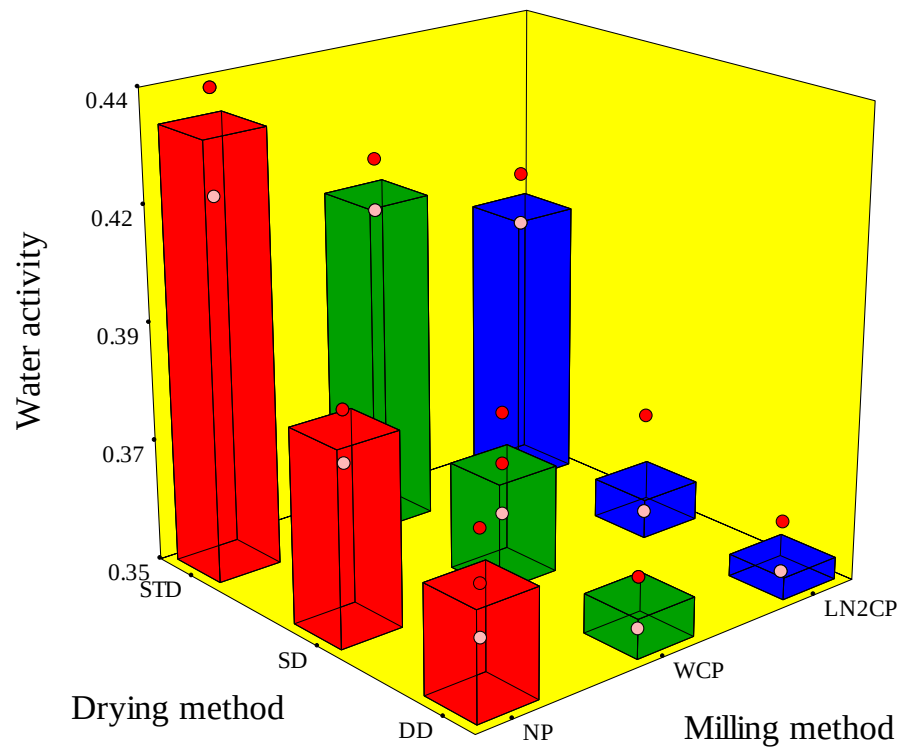


Fig. 22. Effect of drying and milling methods on water activity of calyces of Roselle powder

Reducing a_w below 0.6 prevents microbiological spoilage; however, other deteriorative reactions, such as enzymatic activity, non-enzymatic browning and lipid oxidation, can be prevented in a dried food with a_w values near 0.3 (Ramaswamy and Marcotte, 2006; Pinzon *et al.*, 2013).

5.6.9 Effect of different drying and milling methods on titratable acidity of calyces of Roselle powder

The variation in titratable acidity of calyces of Roselle powder obtained during different drying and milling methods are depicted in Fig. 23. From the figure, it can be noticed that the titratable acidity was significantly influenced by the drying and milling methods. The treatment DM9 recorded 21.82% higher titratable acidity compared to DM1. Considering the effect of selected drying methods, it is clear that DD resulted in higher titratable acidity compared to SD and STD samples irrespective of milling methods. This might be due to lower drying time taken during dehumidified air drying of Roselle calyces and controlled drying condition in DD. Similar results were reported by Sahoo *et al.* (2015) for solar and cabinet drying of banana powder and Mechlouch *et al.* (2012) for sun and solar drying of tomato slice. Arab *et al.* (2011) reported titratable acidity of 18.85% for calyces of Roselle. From the figure, it can also be observed that LN₂CP had higher titratable acidity followed by WCP and LN₂CP milling methods. This might be attributed to the lower pH of LN₂CP milled Roselle calyces.

5.6.10 Effect of different drying and milling methods on ascorbic content of calyces of Roselle powder

The results of ascorbic content of calyces of Roselle powder obtained by different drying and milling methods are depicted graphically in Fig. 24. The treatment DM9 recorded 58.73% higher ascorbic acid compared to DM1. From the figure, it can be observed that the highest ascorbic content was observed in case of DD followed by SD and STD methods. The variable losses of ascorbic acid under different drying methods were due to sensitivity to high temperature. Similar results for ascorbic acid contents were reported by Osunde and Makama (2007) in sun drying of okra, tomato and sweet pepper and Reis *et al.* (2013) for conventional drying of pepper. The more severe drying conditions in solar tunnel drier caused higher losses of ascorbic acid.

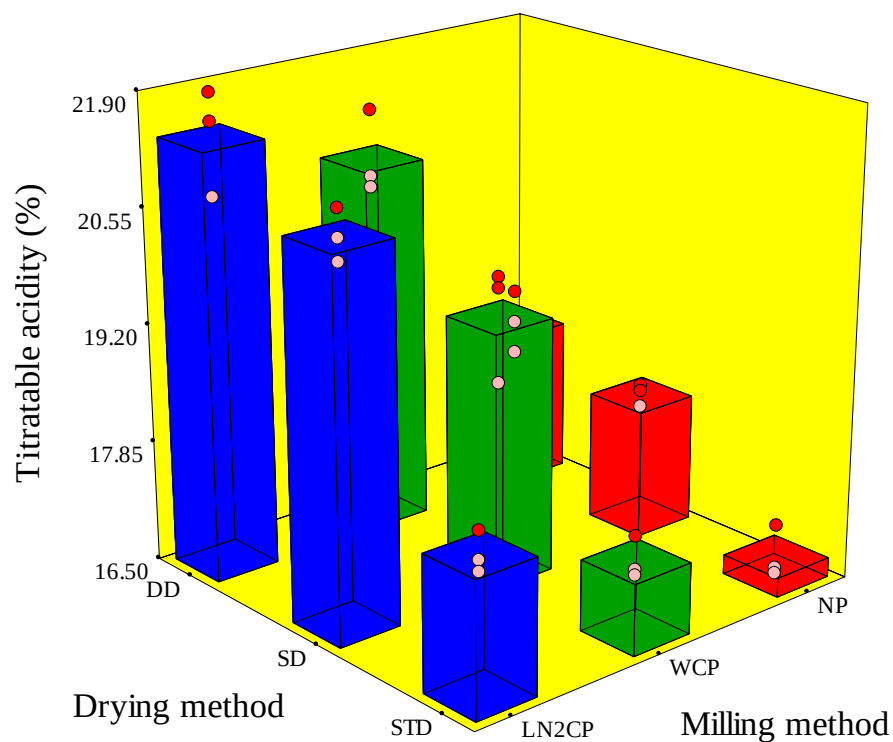


Fig. 23. Effect of drying and milling methods on titratable acidity of calyces of Roselle powder

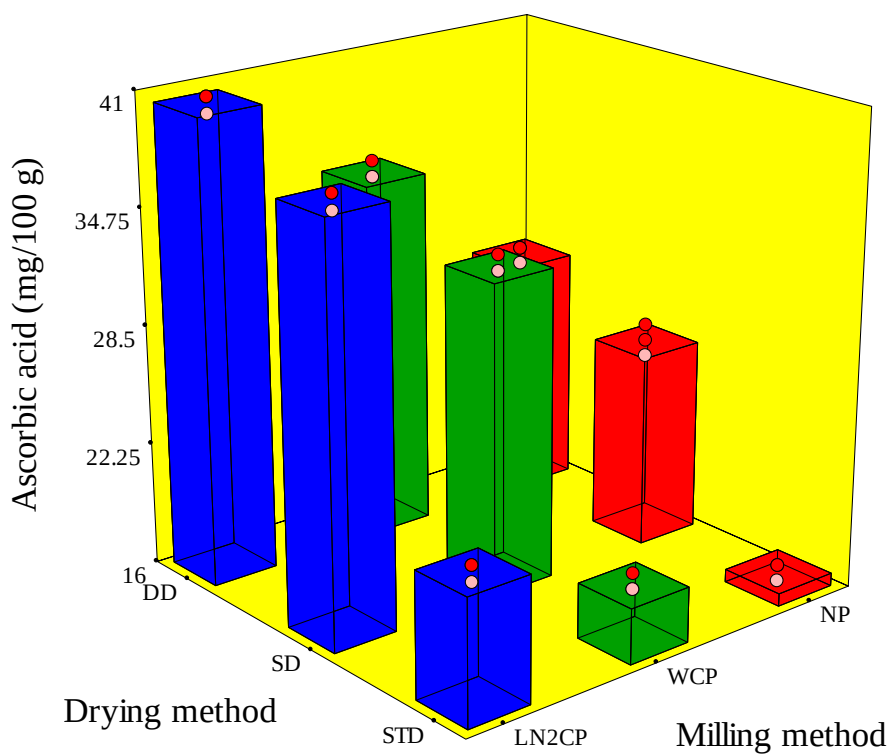


Fig. 24. Effect of drying and milling methods on ascorbic acid of calyces of Roselle powder

Loss of ascorbic acid in solar tunnel and shade drying might be attributed to the fact that ascorbic acid was oxidized by the light, oxygen and high temperature during drying leading to the formation of L-dehydroascorbic acid and a wide variety of carbonyl and other unsaturated compounds. Also this variation might be due to the leaching of the vitamin being water soluble and oxidation due to longer period of drying especially the conventional dried samples (Manjula and Ramachandra, 2014). From the figure, it can also be observed that LN₂CP had higher ascorbic content followed by WCP and NP milling methods. This might be attributed to the lower temperature during LN₂CP milling of Roselle calyces.

5.6.11 Effect of different drying and milling methods on solubility index of calyces of Roselle powder

Solubility of Roselle powder may be a function of processing and reconstitution. The higher the solubility, the more the Roselle powder may reconstitute well in water. The variation in solubility index of calyces of Roselle powder obtained during different drying and milling methods are depicted in Fig. 25. From the figure, it can be observed that the solubility index was significantly influenced by the drying and milling methods. The treatment DM9 recorded 21.21% higher solubility compared to DM1. Considering the effect of selected drying methods, it is clear that DD resulted in higher solubility index compared to SD and STD samples irrespective of milling methods. This could be explained by the fact that different drying techniques resulted in different molecular weights, thus varying the solubility (Mirhosseini and Amid, 2013). The structure of protein significantly affects its reaction with water molecules. It is hypothesized that the drying process significantly affects the position of hydrophobic and hydrophilic amino acids in the interior or exterior layer of the protein molecule, thus affecting the solubility of the protein fraction (Alberts *et al.*, 2007). Higher temperature and non uniform drying also affects the solubility index to be lower (Roongruangsri and Bronlund, 2016).

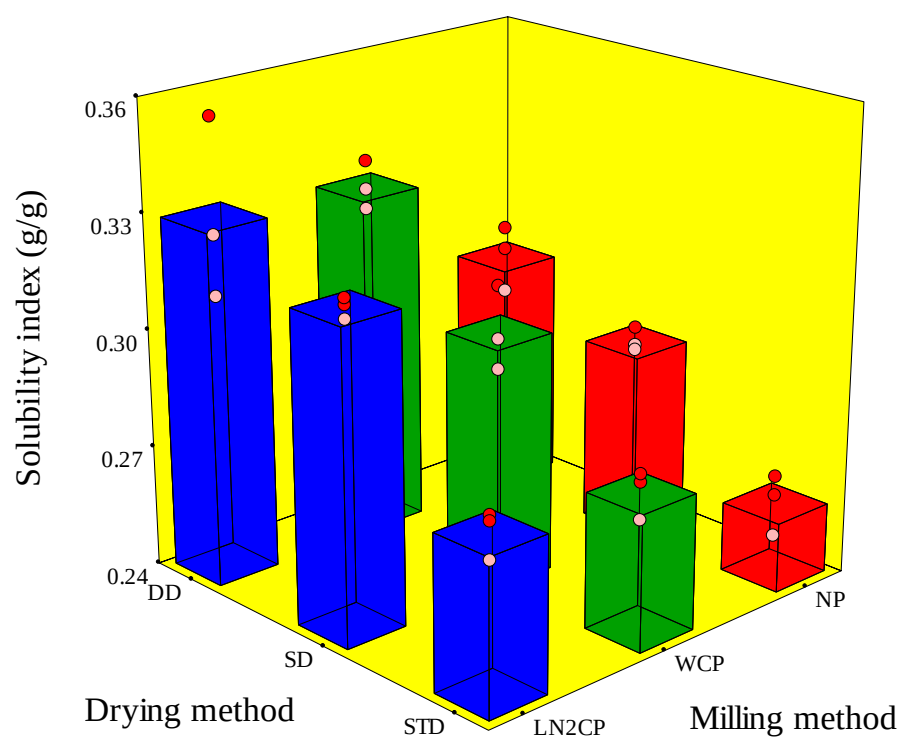


Fig. 25. Effect of drying and milling methods on solubility index of calyces of Roselle powder

From the figure, it can also be depicted that LN₂CP had higher solubility index followed by WCP and NP milling methods. This could be also due to the significant size reduction, more particle size uniformity and complete conversion of particle to more soluble amorphous form in case of LN₂CP milled Roselle powder compared to WCP and NP milling methods. The high solubility of LN₂CP milled Roselle powder could be also due to their low bulk density or high porosity. Similar findings were reported by Mirhosseini and Amid (2013) for spray and freeze drying of durian seed gum and Corrigan *et al.* (2003) for spray drying of polyethylene glycol.

5.6.12 Effect of different drying and milling methods on particle size of calyces of Roselle powder

The results of particle size of calyces of Roselle powder obtained by different drying and milling methods are depicted graphically in Fig. 28. The treatment DM9 recorded 50.88% lower particle size compared to DM1. From the figure, it can be observed that the lowest particle size was observed in case of DD (Fig.26) followed by SD and STD methods. From the figure, it can also be depicted that LN₂CP had lower particle size followed by WCP and NP (Fig.27) milling methods. In a milling operation, particle size reduction ceases at a practical limit beyond which the material becomes progressively difficult to comminute even when milling time is prolonged (Loh *et al.*, 2015). LN₂CP had lower particle size, this might be due to when material is exposed to liquid nitrogen results in the “embrittlement” of the material which facilitates crack propagation, reducing the specific energy required for milling and potentially shortening the milling duration resulted in lower particle size (Ye and Schoenung, 2004). Apart from chilling the chamber, the liquid nitrogen also prevents the plastic deformation of the material and improves the particle fracture process (Saleem and Smyth, 2010). The latter stems from the low surface tension and viscosity of liquid nitrogen which allows it to penetrate into inter and intra-particulate void spaces and micropores of the particles, forming a physical barrier which prevents particle agglomeration (Sugimoto *et al.*, 2012). Cryogenic-cogrinding also led to higher levels of amorphization than cogrinding at room temperature. This is because milling at cryogenic temperatures effectively ‘traps’ the milled material in its amorphous state by removing the thermal energy required for re-crystallization to occur (Willart and Descamps, 2008).

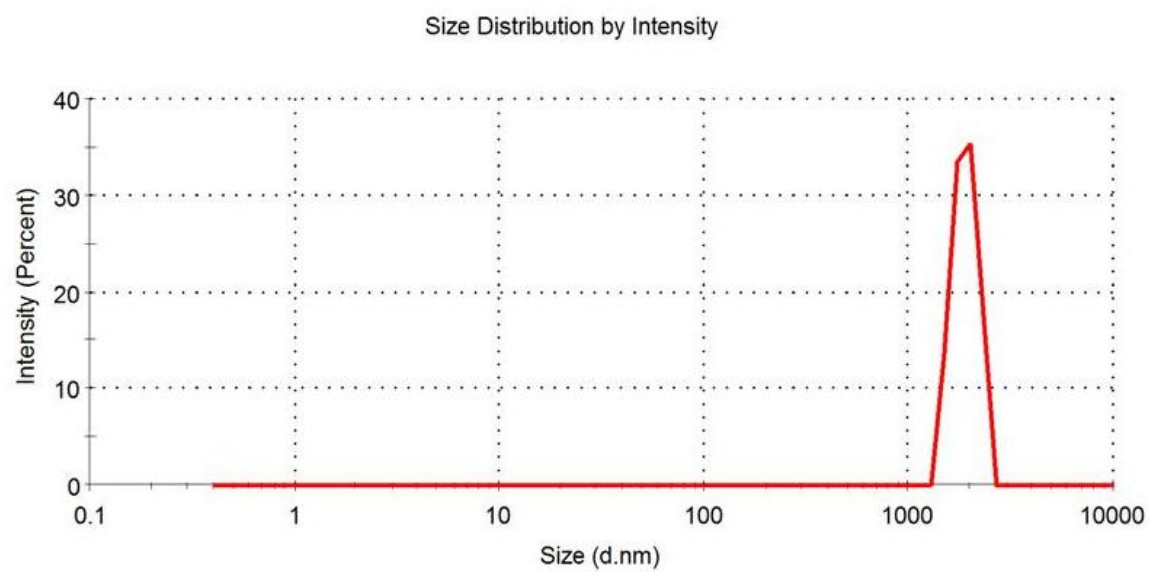


Fig. 26. Z average particle size (d.nm) of DM9 treatment

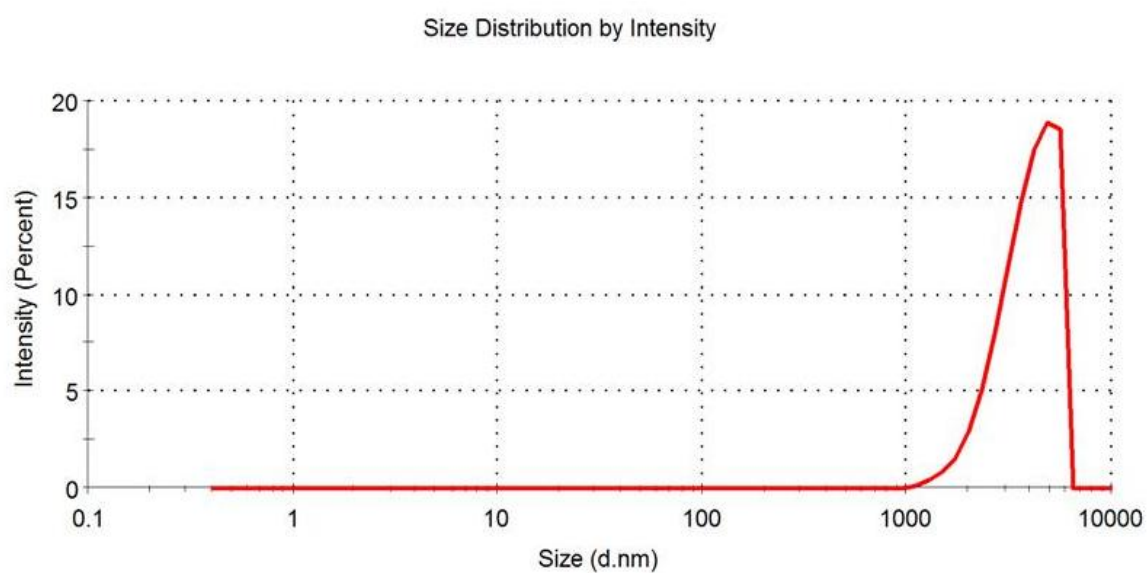


Fig. 27. Z average particle size (d.nm) of DM1 treatment

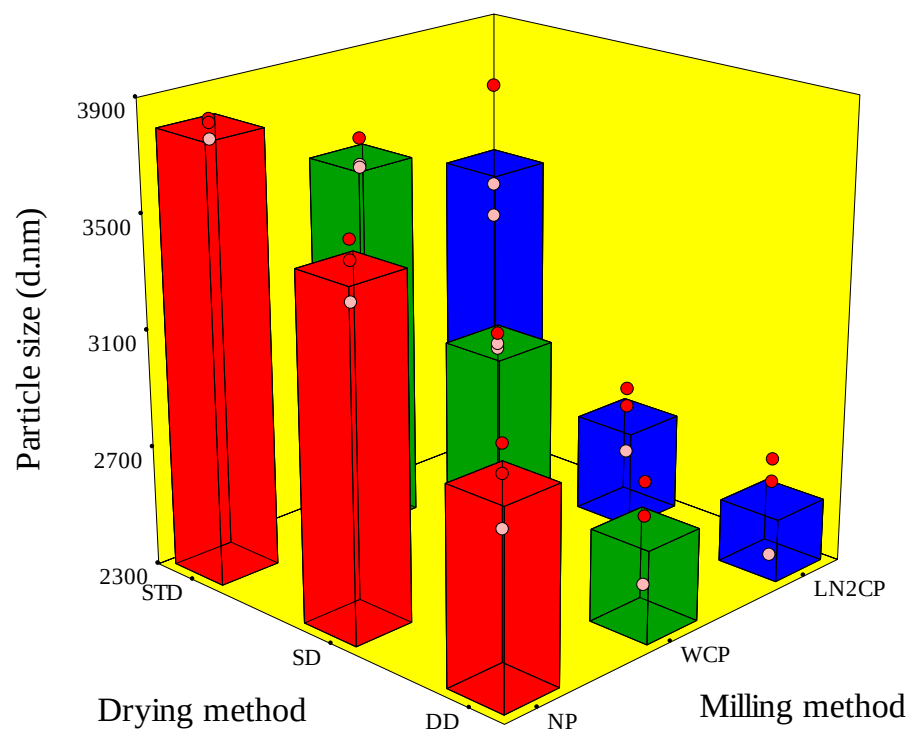


Fig. 28. Effect of drying and milling methods on particle size of calyces of Roselle powder

5.7 Functional properties of calyces of Roselle powder

Functional properties viz., total phenolic content, antioxidant activity, total flavonoids and total anthocyanin content of calyces of Roselle powder obtained from different drying and milling methods are discussed hereunder.

5.7.1 Effect of different drying and milling methods on total phenolic content of calyces of Roselle powder

The effect of different drying and milling methods on total phenolic content of calyces of Roselle powder are presented graphically in Fig. 29. From the figure, it can be observed that the total phenolic content was significantly influenced by the drying and milling methods. The treatment DM9 recorded 40.93% higher total phenolic content compared to DM1. Considering the effect of selected drying methods, it is clear that DD resulted in higher total phenolic content compared to SD and STD samples irrespective of milling methods. With increase in drying time, there was decrease in phenol content in Roselle calyces, shorter the drying times preserves the maximum phenol content (Ghanem *et al.*, 2012). Similar observation was found by Chantro *et al.* (2008) for dehydration of carrot peel, higher loss of phenolic content was observed in higher drying temperature due to more degradation of phenolic content. Similar results were reported by Ji *et al.* (2012) for sun and hot air drying of black locust flowers and Zaman *et al.* (2017) for sun and oven drying of Roselle. Since particularly high temperatures in a prolonged drying process can destroy some phenolic compounds, a higher content of total phenols was found at the lower temperature assessed (Daniel *et al.*, 2012). From the figure, it can also be depicted that LN₂CP had higher total phenolic content followed by WCP and NP milling methods. Volatile compounds and heat sensitive constituents present in Roselle calyces were loss during normal milling due to generation of heat which is detrimental to powder quality. This might be due to the cryogenic milling technique using liquid nitrogen provides the refrigeration needed for pre-cooling the herbs and spices and to maintain the desired low temperature by absorbing the heat generated during the milling operation and thus retained the total phenolic content in the herbs and spices. Similar results were reported by Sharma *et al.* (2014) for cryogenic grinding of cumin. LN₂CP milling process does not harm or alter the chemical components present in calyces of Roselle powder like phenols (Singh and Goswami, 1999).

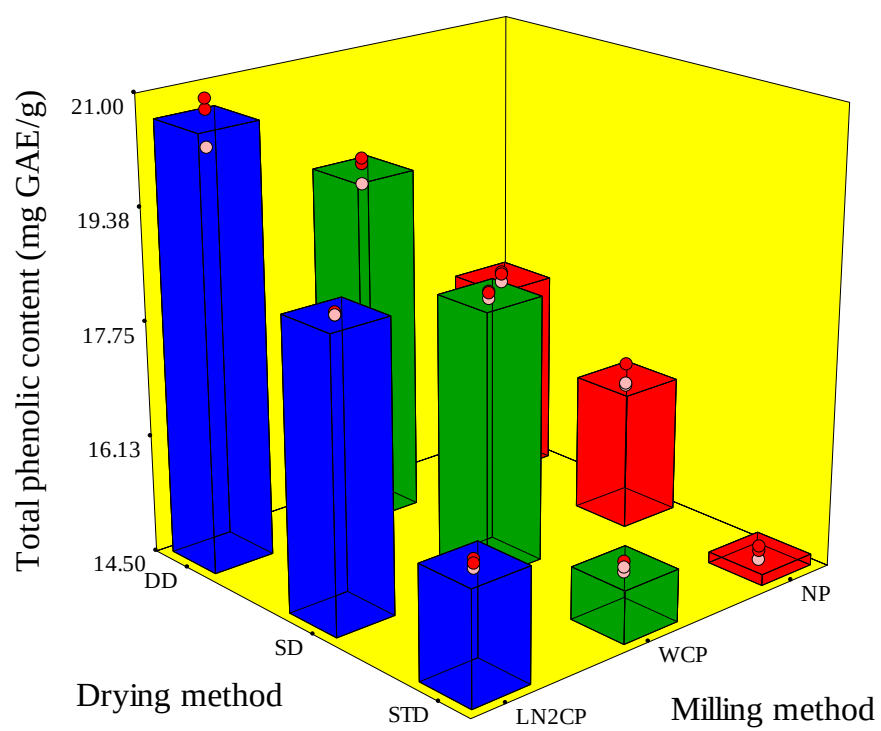


Fig. 29. Effect of drying and milling methods on total phenolic content of calyces of Roselle powder

5.7.2 Effect of different drying and milling methods on antioxidant activity of calyces of Roselle powder

The variation in antioxidant activity of calyces of Roselle powder obtained during different drying and milling methods are depicted in Fig. 30. From the figure, it can be observed that the antioxidant activity was significantly influenced by the drying and milling methods. The treatment DM9 recorded 109.24% higher antioxidant activity compared to DM1. Considering the effect of selected drying methods, it is clear that DD resulted in higher antioxidant activity compared to SD and STD samples irrespective of milling methods. Drying affects the antioxidant activity of plant materials. Increased temperature and water removal generally cause a loss of chemical compounds with antioxidant properties (Manzocco *et al.*, 2000; Zaman *et al.*, 2017). Antioxidant activity depends on presence of phenolic, Vitamin C, anthocyanin and flavonoid compounds. Phenolic compounds are known to present antioxidant activity that inhibits oxidative damage and may consequently prevent inflammatory conditions, ageing and neurodegenerative diseases (Oliveira *et al.*, 2013). Drying process, particularly high temperature and long drying time, might destroy some of the phenolic compounds (Li *et al.*, 2006; Arslan and Ozcan, 2011), thus resulted in lower antioxidant activity. Similar results were reported by Ji *et al.* (2012) for sun and hot air drying of black locust flowers and Zaman *et al.* (2017) for sun and oven drying of Roselle.

From the figure, it can also be depicted that LN₂CP had higher antioxidant activity followed by WCP and NP milling methods. During milling, the temperature of the product rises to a high level which depends upon the moisture content, but it loses a significant fraction of its volatile compounds due to this temperature rise (Singh and Goswami., 1999), so milling with liquid nitrogen preserves these components that results in high bio-active component retention in LN₂CP milled Roselle powder.

5.7.3 Effect of different drying and milling methods on total flavonoid content of calyces of Roselle powder

Flavonoids are one group of phenolics, which consists of two aromatic rings linked by 3 carbons that usually in an oxygenated heterocycle ring (Ivanisova *et al.*, 2012). The results of total flavonoid content of calyces of Roselle powder obtained by different drying and milling methods are depicted graphically in Fig. 31.

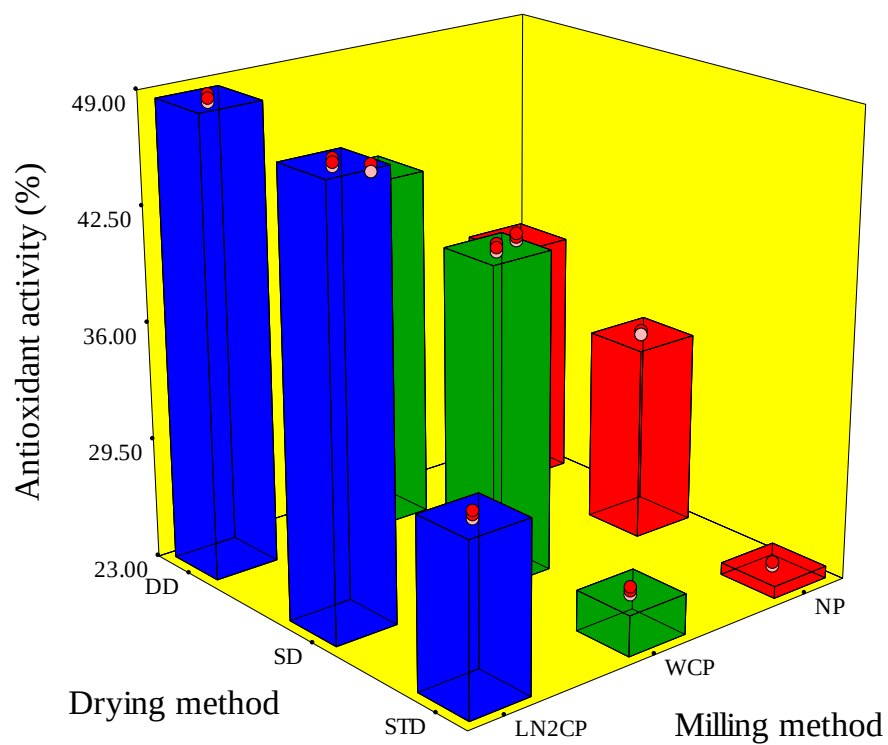


Fig. 30. Effect of drying and milling methods on antioxidant activity of calyces of Roselle powder

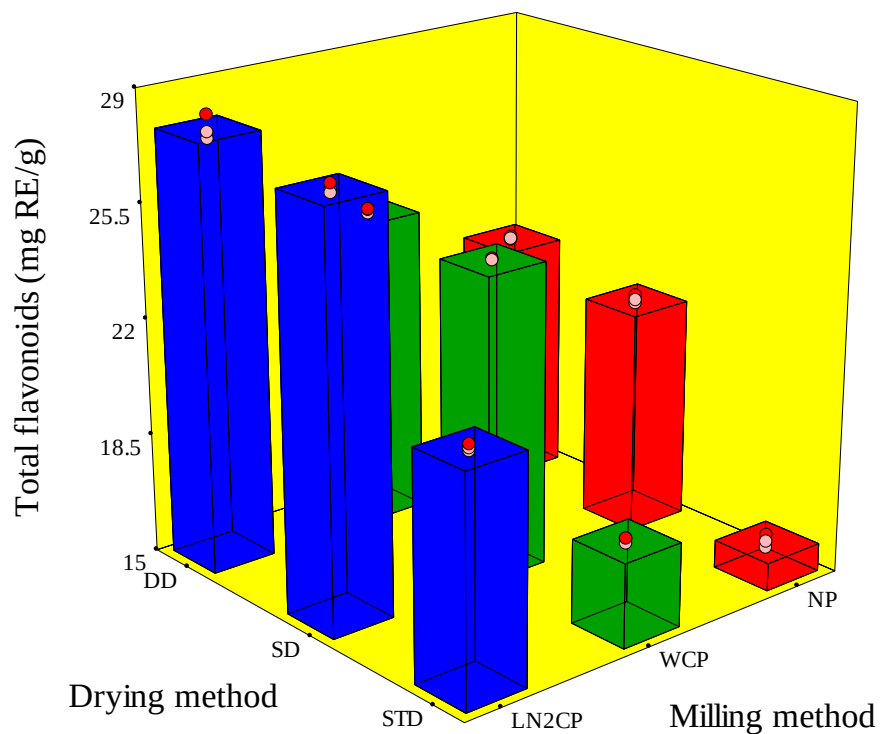


Fig. 31. Effect of drying and milling methods on total flavonoid content of calyces of Roselle powder

The treatment DM9 recorded 75.71% higher total flavonoid content compared to DM1. From the figure, it can be observed that the highest total flavonoid content was observed in case of DD followed by SD and STD methods. Phenolics and flavonoids have been considered as great antioxidants and proved to be more effective than Vitamin C, E and carotenoids (Dai and Mumper, 2010; Ghasemzadeh and Ghasemzadeh, 2011). The loss of flavonoid content in STD and SD Roselle calyces might be due to higher temperature, uneven drying and long duration of drying. The degradation of flavonoids not only depends on temperature and magnitude of heating; it also depends on other parameters such as pH, phytochemicals, structure and even the presence or absence of oxygen (Macias *et al.*, 2013; Zaman *et al.*, 2017). Drying processes particularly, high temperature drying and long drying time might destroy flavonoid compound and these bioactive compounds usually oxidized at high temperature (Garau *et al.*, 2007). Similar findings were reported by Zaman *et al.* (2017) for sun and oven drying of Roselle.

From the figure, it can also be depicted that LN₂CP had higher total flavonoid content followed by WCP and NP milling methods. Low temperature milling with LN₂CP process did not alter the chemical components present in calyces of Roselle powder like flavonoids and phenols. Similar results were also reported by Singh and Goswami (1999) for black pepper.

5.7.4 Effect of different drying and milling methods on total anthocyanin content of calyces of Roselle powder

The effect of different drying and milling methods on total anthocyanin content of calyces of Roselle powder are presented graphically in Fig. 32. From the figure, it can be observed that the total anthocyanin content was significantly influenced by the drying and milling methods. The treatment DM9 recorded 55.01% higher total anthocyanin content compared to DM1. Considering the effect of selected drying methods, it is clear that DD resulted in higher total anthocyanin content compared to SD and STD samples irrespective of milling methods. This might be due to anthocyanins are more sensitive to heat than colourless phenolic compounds. The lower content of anthocyanin in STD and SD might be due to higher drying temperature and drying time. Drying at higher temperatures can lead to loss of anthocyanins because these pigments are heat sensitive and are unstable during thermal treatment.

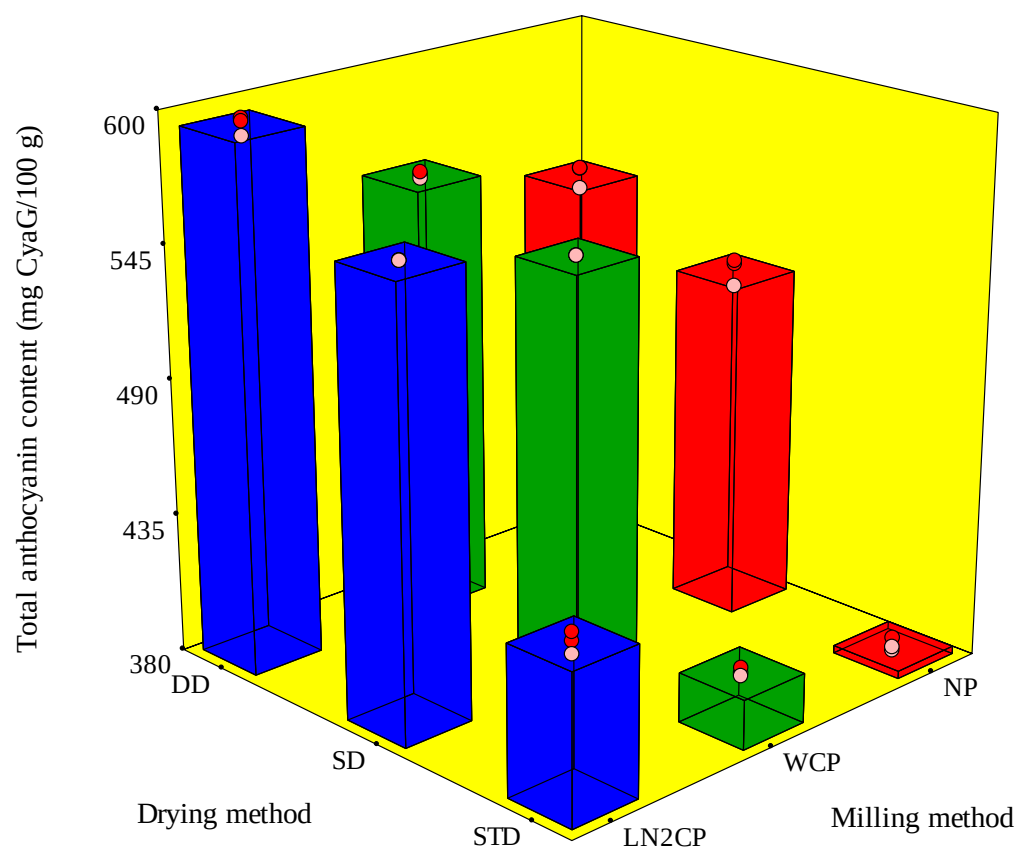


Fig. 32. Effect of drying and milling methods on total anthocyanin content of calyces of Roselle powder

As the intensity and duration of the heat treatment increases, the anthocyanins become more unstable and susceptible to degradation (Mitra *et al.*, 2013). The loss of anthocyanins was attributed to many factors or combination of factors such as pH and acidity, phenolic compounds, sugar and sugar degradation products, oxygen and ascorbic acid (Zaman *et al.*, 2017). Anthocyanin content was significantly affected by drying method and drying temperature reported by Yilmaz *et al.*, (2015) for drying of pomegranate. Similar findings were reported by Zaman *et al.* (2017) for sun and oven drying of Roselle.

From the figure, it can also be depicted that LN₂CP had higher total anthocyanin content followed by WCP and NP milling methods. Mathew and Sreenarayanan (2007) reported that there was a better retention of monoterpenes (pinene, myrcene and limonene) for low temperature and ultra low temperature samples compared to ambient condition samples of black pepper. The presence of oxygen can also accelerate the destruction of anthocyanin by direct chemical oxidation or through the effects of oxidative enzymes (Kwok *et al.*, 2004; Patras *et al.*, 2010; Mitra *et al.*, 2013). Therefore, LN₂CP milling provides oxygen free environment for the milling of Roselle calyces resulted in better retention of anthocyanin content.

5.8 Optimization of process parameters for drying and milling of calyces of Roselle

The experimental run and optimization work were designed for drying of calyces of Roselle using factorial design method in Design-Expert 7.7.0 software. The values of all the responses at operating conditions were converted to a desirability function. The desirability values of the minimum and maximum were configured as 0 and 1, respectively. The maximum desirability function obtained was taken as the optimum method for drying of calyces of Roselle (Krishnaiah *et al.*, 2012). Optimization of the two process variables namely; drying methods and milling methods was performed using the general factorial method in Design Expert.

The experimental sample had the optimum conditions of dehumidified air drying and liquid nitrogen cooled pulverizer milling with a desirability of 0.93 was depicted in Fig. 33.

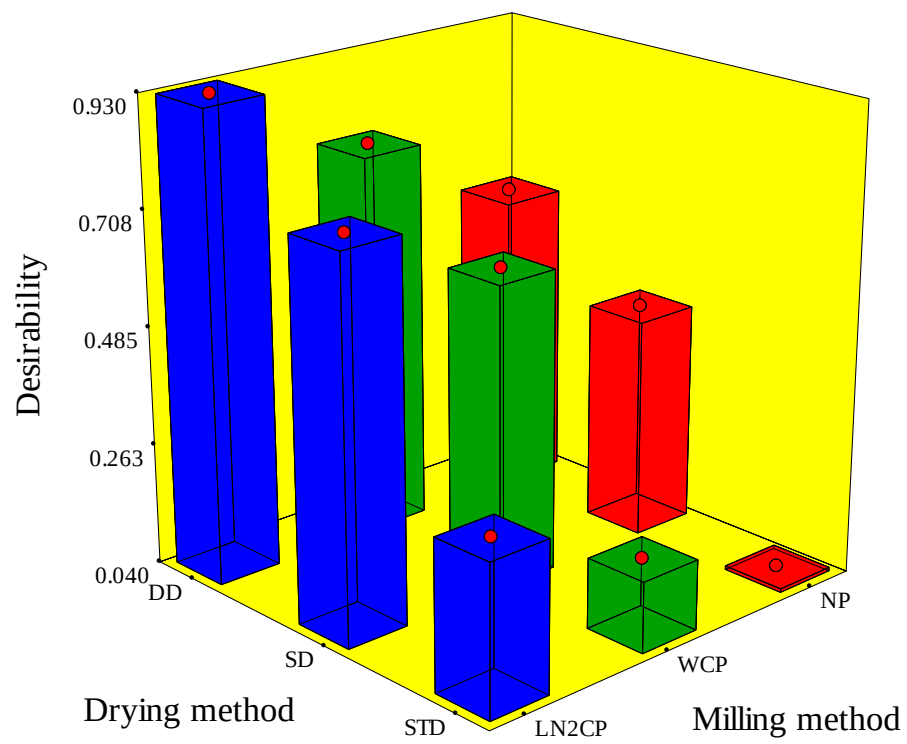


Fig. 33. Desirability graph for optimization of drying and milling methods of calyces of Roselle

5.9 Extraction of anthocyanin from calyces of Roselle

Several solid to solvent ratios and concentration of citric acid (%) were compared in order to use the most effective one for extracting the pigments for calyces of Roselle powder. The optimized dried and milled calyces of Roselle powder was subjected to solvent extraction of anthocyanin using three different solid to solvent ratio (1:5, 1:10 and 1:15) and concentration of citric acid (1, 2 and 3%) and physico-chemical and functional properties of Roselle anthocyanin extract are discussed hereunder.

5.10 Physico-chemical properties of solvent extracted anthocyanin from calyces of Roselle

The physico-chemical properties of solvent extracted anthocyanin from calyces of Roselle viz., extraction yield, pH, TSS, colour (L^* , a^* and b^*) and titratable acidity are discussed hereunder.

5.10.1 Effect of solid to solvent ratios and concentration of citric acid (%) on extraction yield of solvent extracted anthocyanin from calyces of Roselle

The extraction yield of solvent extracted anthocyanin from calyces of Roselle recovered with the different solid to solvent ratios and concentration of citric acid (%) are presented in Fig. 34. From the figure, it is seen that the extraction yield was significantly influenced by the solid to solvent ratios and concentration of citric acid (%) as well. The extraction yield increased 99.98% in treatment E5 followed by 95.87% increase in treatment E6 compared to control. Considering the effect of selected solid to solvent ratios, it is clear that the 1:10 solid to solvent ratio resulted in higher extraction yield followed by 1:15 and 1:5 solid to solvent ratio irrespective of concentration of citric acid (%). This might be due to increasing the solid to solvent ratio (increasing solvent proportion) increased the anthocyanin extraction yield. Indeed, solids are completely submerged by the solvent in the ratio 1:10. These conditions allowed speeding up the mass transfers between both phases. Indeed, increasing of solvent level improves the concentration gradient and thus the rate of diffusion of the compounds from the solid to the solvent (Diessana *et al.*, 2015).

Further increase in solid to solvent ratio resulted in decrease in extraction yield, this might be due to the fact that at higher solid to solvent ratio there is a decrease in the

concentration of anthocyanin yield due to water soluble nature of anthocyanin and it might be due to change of polarity nature of ethanol at higher solvent concentration. The results are in support with Diessana *et al.* (2015) for aqueous extraction of Roselle calyces and Liu *et al.* (2015) for ethanol extraction of total flavonoids from Baical skullcap. Furthermore decrease in extraction yield at higher solid to solvent ratio may be due to the saturation phenomenon. In fact when the solvent is saturated on bioactive components, the cellular phenomenon of diffusion stops and there has stabilization rate of extracted compounds or decreased (Nyamien *et al.*, 2013).

Ethanol is another good solvent for polyphenol extraction and is safe for human consumption (Shil *et al.*, 2005). In preparing anthocyanin rich phenolic extracts from plant materials, an acidified organic solvent, most commonly methanol or ethanol is used. This solvent system denatures the cell membranes, simultaneously dissolves the anthocyanins, and stabilizes them. However, care should be taken to avoid addition of excess acid which can hydrolyze labile, acyl and sugar residues during concentration steps (Arab *et al.*, 2011). To obtain the best yield of anthocyanin extraction, weak organic acids, such as formic acid, acetic acid, citric acid, tartaric acid and phosphoric acid, and low concentrations of strong acids, such as 0.5-3.0% of trifluoroacetic acid and < 1.0% of hydrochloric acid are recommended (Nicoue *et al.*, 2007).

From the figure, it can also be depicted that 2% citric acid had higher extraction yield followed by 3% and 1% citric acid. This might be due to formation of flavylum cation at higher concentration of acid in ethanol (2% citric acid), which is red and stable in a highly acid medium. However at 3% citric acid the extraction yield decreased, this might be due to hydrolysis of the acyl moieties in acylated anthocyanins at higher concentration of acid in solvent medium (Arab *et al.*, 2011). However, control sample resulted in lower extraction yield, this might be due to lower polarity of 70% ethanol compared to other solvent mixtures. Bridgers *et al.* (2010) reported that extraction of anthocyanin from purple-fleshed sweet potato was more effective with acidified methanol and ethanol than non-acidified solvents.

5.10.2 Effect of solid to solvent ratios and concentration of citric acid (%) on pH of solvent extracted anthocyanin from calyces of Roselle powder

The effect of solid to solvent ratios and concentration of citric acid (%) on pH of solvent extracted anthocyanin from calyces of Roselle is presented graphically in Fig. 35.

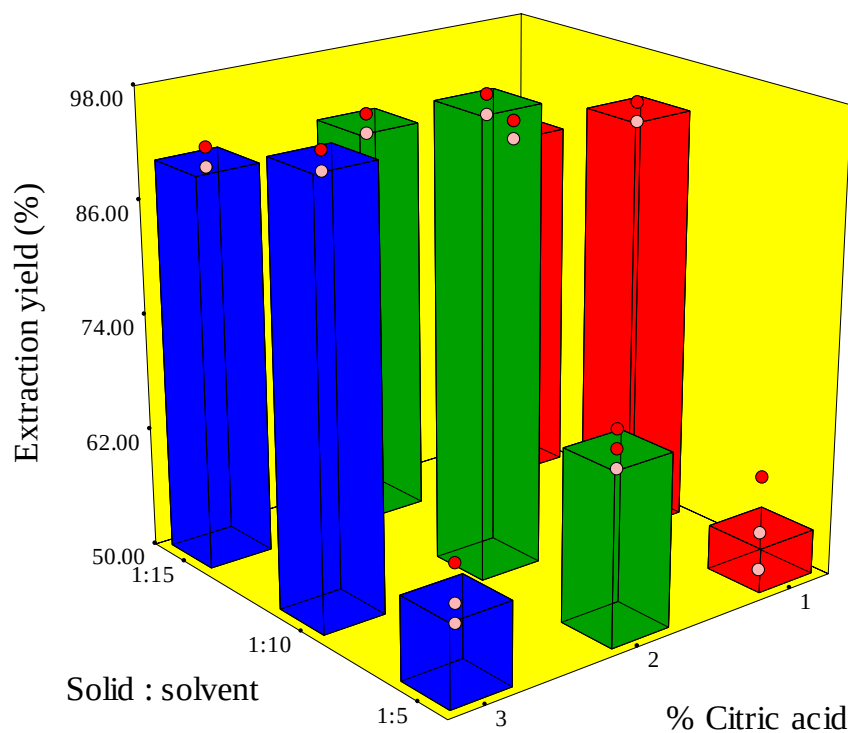


Fig. 34. Effect of solid to solvent ratios and concentration of citric acid (%) on extraction yield of solvent extracted anthocyanin from calyces of Roselle

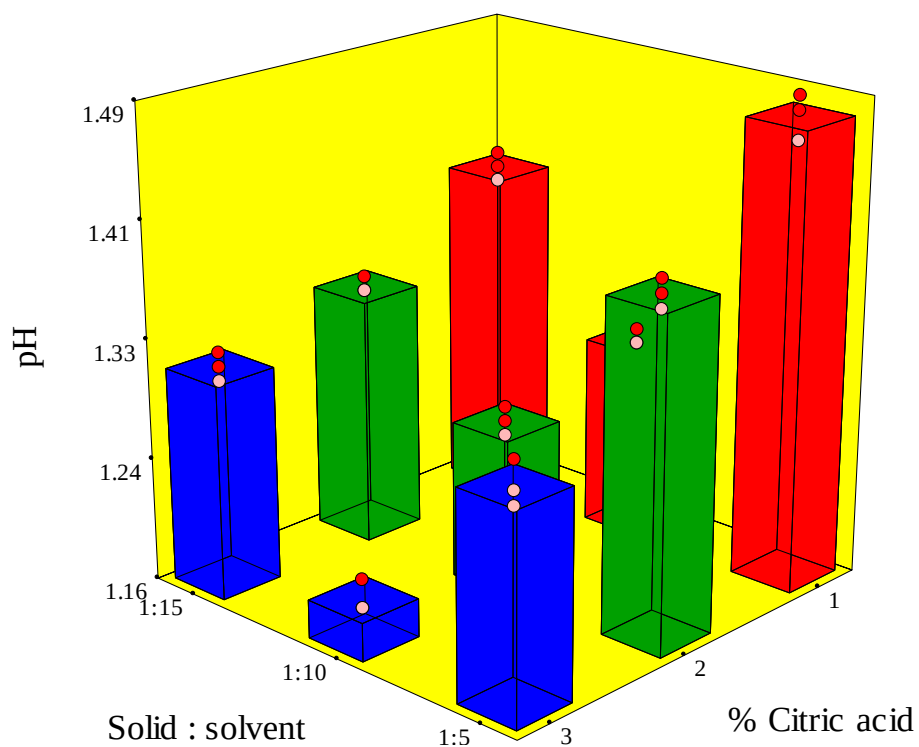


Fig. 35. Effect of solid to solvent ratios and concentration of citric acid (%) on pH of solvent extracted anthocyanin from calyces of Roselle

Considering the effect of selected solid to solvent ratios, it is clear that 1:10 solid to solvent ratio resulted in lower pH compared to 1:5 and 1:15 solid to solvent ratio irrespective of % citric acid. The pH decreased 28.57% in treatment E6 followed by 20.47% decrease in treatment E5 compared to control. As the solid to solvent ratio increased, the pH of the extract decreased firstly and then increased. Similar results were reported by Gartaula and Karki (2010) for the extraction of anthocyanins from Roselle in aqueous medium and Chumsri *et al.* (2008) for the water extraction of Roselle. This might be due to increase in alcohol content in the extracting solvent. Youssef *et al.* (2015) reported that the pH value depends on the concentration of free H ions or mirrored the changes in total organic acids. The free state of H ions is due to dissociation of H ions from the carboxylic group (-COOH) of organic acid. The high acidity in the roselle calyces extracts is given by organic acids such as citric, malic, tartaric, hibiscus, succinic, oxalic and ascorbic acid (Suliman *et al.*, 2011).

From the figure, it can also be noticed that with the increase in citric acid percentage in the extracting solvent, the pH value decreased. This decrease in pH may be due to the extraction of anthocyanin by higher percent of citric acid. It is known that citric acid is comparatively less corrosive than HCl but would still act to stabilize the anthocyanin structure in the cationic form by maintaining a low acidic pH (Heidari *et al.*, 2006; Joshi and Devi, 2015). Similar results were reported by Arab *et al.* (2011) for the extraction of natural pigments from calyces of Roselle. It can also be observed that the control sample yielded higher pH, this might be due to absence of citric acid in the extracting medium.

5.10.3 Effect of solid to solvent ratios and concentration of citric acid (%) on TSS of solvent extracted anthocyanin from calyces of Roselle powder

The results of TSS of solvent extracted anthocyanin from calyces of Roselle obtained by different solid to solvent ratios and concentration of citric acid (%) are depicted graphically in Fig. 36. The TSS increased 49.51% in treatment E5 followed by 42.85% increase in treatment E8 compared to control. From the figure, it can be observed that the highest TSS content was observed in case of 1:10 solid to solvent ratio compared to 1:5 and 1:15 solid to solvent ratio. With the increase in solvent content, the TSS content also increased first and then decreased. This might be due to the increase in water and alcohol portion in the extracting solvent and also TSS depends on the extraction

yield, where in extraction yield decreased at higher solid to solvent ratio. Similar findings were reported by Chumsri *et al.* (2008) for the extraction and concentration of Roselle extract and Arab *et al.* (2011) for the extraction of natural pigment from Roselle calyces.

From the figure, it can also be depicted that 2% citric acid had higher TSS followed by 3% and 1% citric acid. This might be due to formation of flavylum cation at higher concentration of acid in ethanol (2% citric acid), which is red and stable in a highly acid medium resulted in higher TSS. However, at 3% citric acid the TSS decreased, this might be due to hydrolysis of the acyl moieties in acylated anthocyanins at higher concentration of acid in solvent medium resulted in lower extraction yield with lower TSS content (Arab *et al.*, 2011). However, control sample resulted in lower TSS this might be due to lower polarity of 70% ethanol compared to other solvent mixtures which extracted lower total soluble solids from the Roselle.

5.10.4 Effect of solid to solvent ratios and concentration of citric acid (%) on L^* value of solvent extracted anthocyanin from calyces of Roselle powder

The L^* value of solvent extracted anthocyanin from calyces of Roselle recovered with the different solid to solvent ratio and concentration of citric acid (%) are presented in Fig. 37. From the figure, it is seen that the L^* value was significantly influenced by the solid to solvent ratios and concentration of citric acid (%) as well. The L^* value decreased 228.57% in treatment E9 followed by 200.00% decrease in treatment E8 compared to control. Considering the effect of selected solid to solvent ratio, it is clear that the 1:5 solid to solvent ratio resulted in higher L^* value followed by 1:10 and 1:15 solid to solvent ratio irrespective of % citric acid. This might be due to change in pH of the extracting solvent. The colour of anthocyanins depends essentially on the chemical structure of different forms in which they can be found, these structures being strongly related to the pH value of the solution. For example, the flavylum cation I (red colour) is the predominant species at pH = 1, while at pH values between 2 and 4, the quinoidal blue base II predominates (Moldovan *et al.* 2012). Similar results were reported by Chumsri *et al.* (2008) for the extraction and concentration of Roselle extract.

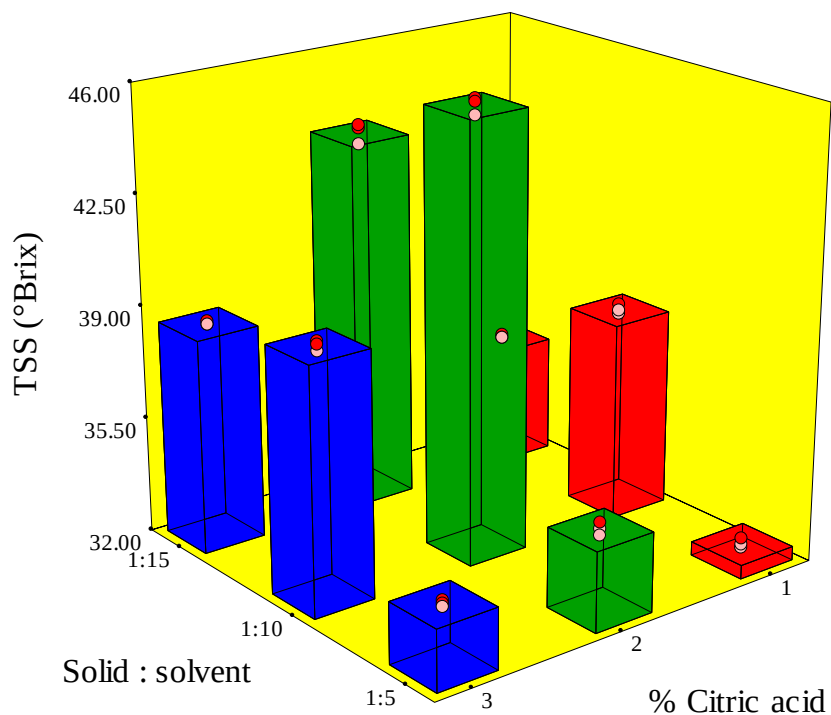


Fig. 36. Effect of solid to solvent ratios and concentration of citric acid (%) on TSS of solvent extracted anthocyanin from calyces of Roselle

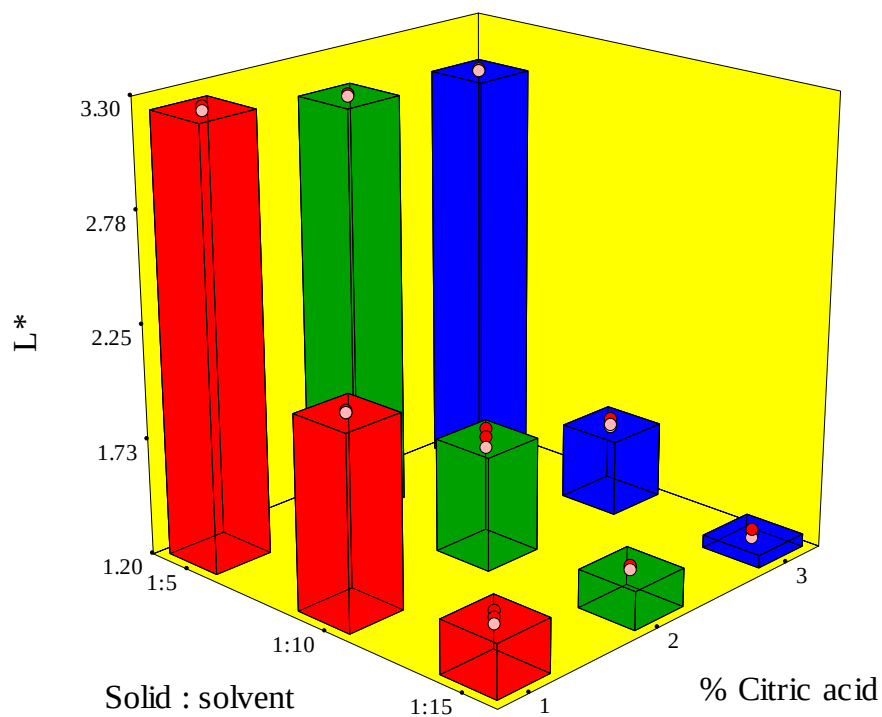


Fig. 37. Effect of solid to solvent ratios and concentration of citric acid (%) on L^* value of solvent extracted anthocyanin from calyces of Roselle

From the figure, it can also be depicted that 1% citric acid had higher L^* value followed by 2% and 3% citric acid. Greater the Roselle extracted by citric acid solution (Lower pH), the more the red colour intensity observed, which resulted in decrease in L^* value at higher concentration of citric acid. Similar results were reported by Arab *et al.* (2011) for the extraction of natural pigment from Roselle calyces. However, control sample resulted in higher L^* value was due to higher pH which resulted in less red colour intensity.

5.10.5 Effect of solid to solvent ratios and concentration of citric acid (%) on a^* value of solvent extracted anthocyanin from calyces of Roselle

The a^* value of solvent extracted anthocyanin from calyces of Roselle recovered with the different solid to solvent ratios and concentration of citric acid (%) are presented in Fig. 38. From the figure, it is seen that the a^* value was significantly influenced by the solid to solvent ratios and concentration of citric acid (%) as well. The a^* value increased 200.00% in treatment E5 followed by 164.00% decrease in treatment E6 compared to control. Considering the effect of selected solid to solvent ratio, it is clear that the 1:10 solid to solvent ratio resulted in higher a^* value followed by 1:15 and 1:5 solid to solvent ratio irrespective of % citric acid. This might be due to the colour of anthocyanins depends essentially on the chemical structure of different forms in which they can be found, these structures being strongly related to the pH value of the solution. For example, the flavilium cation I (red colour) is the predominant species at pH = 1, while at pH values between 2 and 4, the quinoidal blue base II predominates (Moldovan *et al.* 2012). Similar results were reported by Chumsri *et al.* (2008) for the extraction and concentration of Roselle extract.

From the figure, it can also be depicted that 2% citric acid had higher a^* value followed by 3% and 1% citric acid. This might be due to greater the Roselle extracted by 2% citric acid solution the more the red colour intensity observed. Similar results were reported by Arab *et al.* (2011) for the extraction of natural pigment from Roselle calyces. Natural constituents of organic acid in Roselle calyxes such as malic, citric and 3-indlyl acetic acid played an important role in giving brilliant red colour of sample extract (Youssef *et al.*, 2015). It was demonstrated that in acidic media, four anthocyanin structures, including the flavylium cation, the quinonoidol base, the carbinol pseudobase and the chalcone, exist in equilibrium.

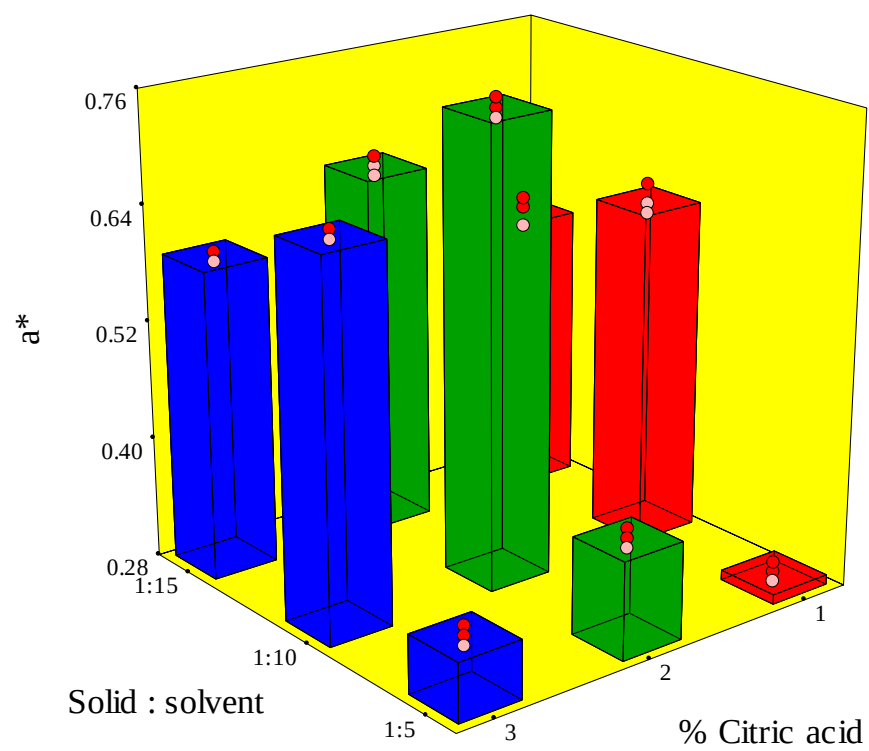


Fig. 38. Effect of solid to solvent ratios and concentration of citric acid (%) on a^* value of solvent extracted anthocyanin from calyces of Roselle

And at pHs below 2, the anthocyanin exists primarily in the form of the red flavylum cation (Chumsri *et al.*, 2008). However control sample resulted in lower a^* value this might be due to absence of citric acid (high pH) resulted in less red colour intensity.

5.10.6 Effect of solid to solvent ratios and concentration of citric acid (%) on b^* value of solvent extracted anthocyanin from calyces of Roselle

The b^* value of solvent extracted anthocyanin from calyces of Roselle recovered with the different solid to solvent ratios and concentration of citric acid (%) are presented in Fig. 39. From the figure, it is seen that the b^* value was significantly influenced by the solid to solvent ratios and concentration of citric acid (%) as well. The b^* value decreased 216.67% in treatment E5 followed by 111.11% decrease in treatment E6 compared to control. Considering the effect of selected solid to solvent ratio, it is clear that the 1:15 solid to solvent ratio resulted in higher b^* value followed by 1:5 and 1:10 solid to solvent ratio irrespective of % citric acid. This might be due to change in pH of the extracting solvent. The colour of anthocyanins depends essentially on the chemical structure of different forms in which they can be found, these structures being strongly related to the pH value of the solution. For example, the flavylum cation I (red colour) is the predominant species at pH = 1, while at pH values between 2 and 4, the quinoidal blue base II predominates (Moldovan *et al.* 2012). Similar results were reported by Chumsri *et al.* (2008) for the extraction and concentration of Roselle extract.

From the figure, it can also be depicted that 2% citric acid had lower b^* value followed by 3% and 1% citric acid. Greater the Roselle extracted by citric acid solution (Lower pH), the more the red colour intensity observed, which resulted in decrease in b^* value at higher concentration of citric acid. Similar results were reported by Arab *et al.* (2011) for the extraction of natural pigment from Roselle calyces. However, control sample resulted in higher b^* value this might be due to higher pH which resulted in less red colour intensity.

5.10.7 Effect of solid to solvent ratios and concentration of citric acid (%) on titratable acidity of solvent extracted anthocyanin from calyces of Roselle

The effect of solid to solvent ratios and concentration of citric acid (%) on titratable acidity of solvent extracted anthocyanin from calyces of Roselle is presented graphically in Fig. 40.

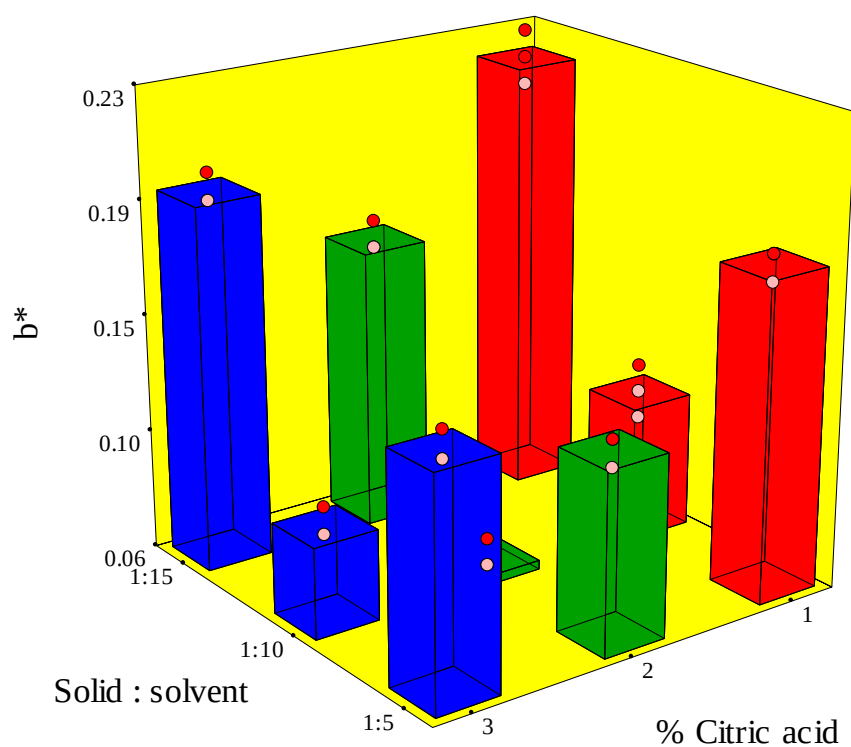


Fig. 39. Effect of solid to solvent ratios and concentration of citric acid (%) on b^* value of solvent extracted anthocyanin from calyces of Roselle

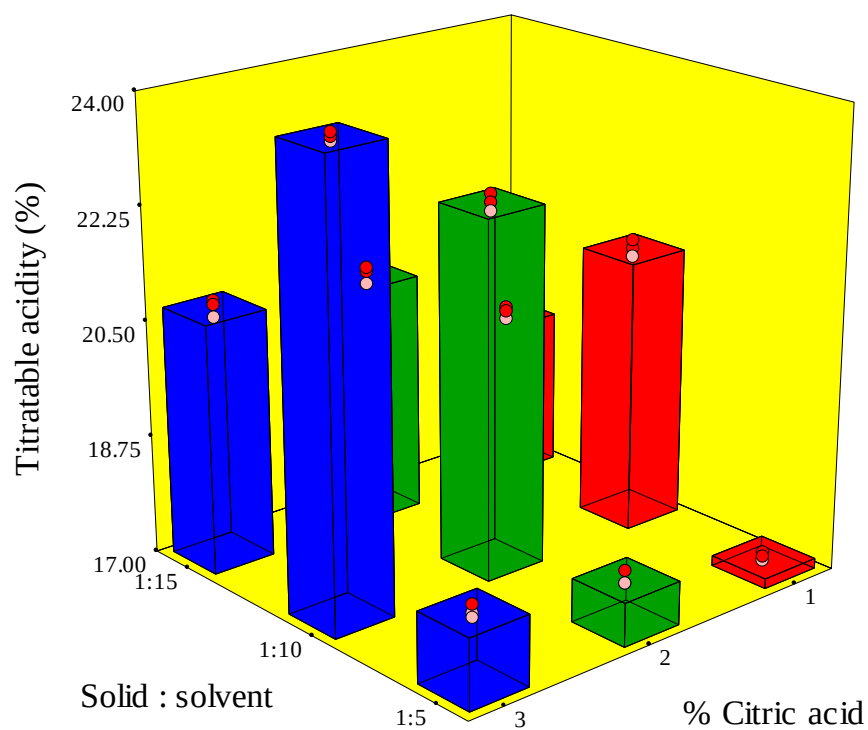


Fig. 40. Effect of solid to solvent ratios and concentration of citric acid (%) on titratable acidity of solvent extracted anthocyanin from calyces of Roselle

The titratable acidity increased 52.68% in treatment E6 followed by 43.24% increase in treatment E5 compared to control. Considering the effect of selected solid to solvent ratios, it is clear that 1:10 solid to solvent ratio resulted in higher titratable acidity compared to 1:5 and 1:15 solid to solvent ratio irrespective of % citric acid. The extraction by 2% citric acid solution exhibited high acidity, this increase in acidity may be due to the extraction of anthocyanin by citric acid (Arab *et al.*, 2011). As the solid to solvent ratio increased, the acidity of the extract increased firstly and then decreased. Similar results were reported by Gartaula and Karki (2010) for the extraction of anthocyanins from Roselle in aqueous medium and Chumsri *et al.* (2008) for the water extraction of Roselle. The high acidity in the roselle calyces extracts was given by organic acids such as citric, malic, tartaric, hibiscus, succinic, oxalic and ascorbic acid (Suliman *et al.*, 2011).

From the figure, it can also be noticed that with the increase in citric acid percentage in the extracting solvent, the titratable acidity increased. This increase in titratable acidity may be due to the extraction of anthocyanin by higher percent of citric acid. It is known that citric acid is comparatively less corrosive than HCl but would still act to stabilize the anthocyanin structure in the cationic form by maintaining a low acidic pH (Heidari *et al.*, 2006; Joshi and Devi, 2015). Similar results were reported by Arab *et al.* (2011) for the extraction of natural pigments from calyces of Roselle. It can also be observed that the control sample yielded lower titratable acidity, this was due to absence of citric acid in the extracting medium.

5.11 Functional properties of solvent extracted anthocyanin from calyces of Roselle

The functional properties of solvent extracted anthocyanin from calyces of Roselle viz., total phenolic content, antioxidant activity, total flavonoids and total anthocyanin content are discussed hereunder.

5.11.1 Effect of solid to solvent ratios and concentration of citric acid (%) on total phenolic content of solvent extracted anthocyanin from calyces of Roselle

Phenolic compounds including hydroxyl benzoic acids, caffeoylquinic acids, flavonols, phenolic acids and anthocyanins are known to be responsible for antioxidant activities in fruits and fruits with higher phenolic contents generally show stronger antioxidant activities such as Roselle fruit (Cabrera *et al.*, 2013; Youssef *et al.*, 2015). The results of total phenolic content of solvent extracted anthocyanin from calyces of

Roselle powder obtained by different solid to solvent ratios and concentration of citric acid (%) are depicted graphically in Fig. 41. The total phenolic content increased 62.55% in treatment E5 followed by 55.25% increase in treatment E8 compared to control. From the figure, it can be observed that the highest total phenolic content was observed in case of 1:10 solid to solvent ratio compared to 1:5 and 1:15 solid to solvent ratio. As the polarity of the solvent increases, higher extraction yields of total extractable phenolic compounds were obtained (Esa *et al.*, 2010; Arab *et al.*, 2011). The extraction of phenolic compounds from plant materials may also be influenced by solvent-to-solid ratio of the sample. Increasing solvent-to-solid ratio was found to work positively for enhancing phenol yields. However, an equilibrium between the use of high and low solvent-to-solid ratios, involving a balance between high costs and solvent wastes and avoidance of saturation effects, respectively, should be worked out to obtain an optimized value (Cacace *et al.*, 2003; Pinelo *et al.*, 2005; Dai and Mumper, 2010). The results are in agreement with the Shil *et al.* (2005), who reported that the ethanol is good solvent for polyphenol extraction and is safe for human.

From the figure, it can also be depicted that 2% citric acid had higher total phenolic content followed by 3% and 1% citric acid. The difference is probably due to the characteristic of the solvent; this could affect which compounds are extracted from the plant matrix. This phenomenon can be explained by a change in polarity of the antioxidant compound due to the particular solvent used for extraction (Jafarian *et al.*, 2014). Similar results were reported by Arab *et al.* (2011) for the extraction of natural pigments from calyces of Roselle. In preparing anthocyanin rich phenolic extracts from plant materials, an acidified organic solvent, most commonly ethanol or methanol is used. This solvent system denatures the cell membranes, simultaneously dissolves the anthocyanins, and stabilizes them. However, care should be taken to avoid addition of excess acid which can hydrolyze labile, acyl and sugar residues during concentration steps (Shil *et al.* 2005; Nicoue *et al.*, 2007; Dai and Mumper, 2010). However, control sample resulted in lower total phenolic content, this might be due to lower solvation power of ethanol (Boeing *et al.*, 2014). An important aspect to note is that the binary solvent systems are more efficient for the extraction of these compounds than mono-solvent systems, very probably due to their relative polarity (Ortega and Beltran, 2014).

5.11.2 Effect of solid to solvent ratios and concentration of citric acid (%) on antioxidant activity of solvent extracted anthocyanin from calyces of Roselle

The antioxidant activity of solvent extracted anthocyanin from calyces of Roselle recovered with the different solid to solvent ratios and concentration of citric acid (%) are presented in Fig. 42. The antioxidant activity increased 41.80% in treatment E5 followed by 39.28% increase in treatment E8 compared to control. From the figure, it is seen that the antioxidant activity was significantly influenced by the solid to solvent ratios and concentration of citric acid (%) as well. Considering the effect of selected solid to solvent ratios, it is clear that the 1:10 solid to solvent ratio resulted in higher antioxidant activity followed by 1:15 and 1:5 solid to solvent ratio irrespective of % citric acid. Phenolic compounds are usually mainly responsible for the antioxidant properties of fruits and vegetables and most of these compounds are classified as hydrophilic antioxidants (Boeing *et al.*, 2014). The antioxidant capacities of the extracts have a strong relationship with the solvent employed, mainly due to the different antioxidant potential of compounds with different polarities (Moure *et al.*, 2001; Boeing *et al.*, 2014). As the polarity of the solvent increases, higher extraction yields antioxidant compounds (Esa *et al.*, 2010).

From the figure, it can also be depicted that 2% citric acid had higher antioxidant activity followed by 3% and 1% citric acid. Christian and Jackson (2009) reported that the high antioxidant activity observed in the Roselle could be due to the high ascorbic acid content. Preferably formed under acidic conditions, these weak chemical associations can augment anthocyanin stability and increase antioxidant properties (Aubert *et al.*, 2001). The antioxidant activity of Roselle extract is also pH dependent, the activity decreased as pH increases. The antioxidant properties of herbs may be attributed to the plant pigments that are the main components of each herbal extract. The red pigment presented in the flowers of Hibiscus species are anthocyanins (cyanidin-3-glucoside and delphinidin-3- glucoside) which may act as an antioxidant (Arab *et al.*, 2011; Pacome *et al.*, 2014). *Hibiscus sabdariffa* L. which contained high phenolic compounds exhibited high antioxidant activity when determined by DPPH assays. It thus confirms that phenolic compounds have an important role in antioxidant activities. However, control sample resulted in lower total phenolic content which intern reduced the antioxidant activity, this might be due to lower salvation power of ethanol (Boeing *et al.*, 2014).

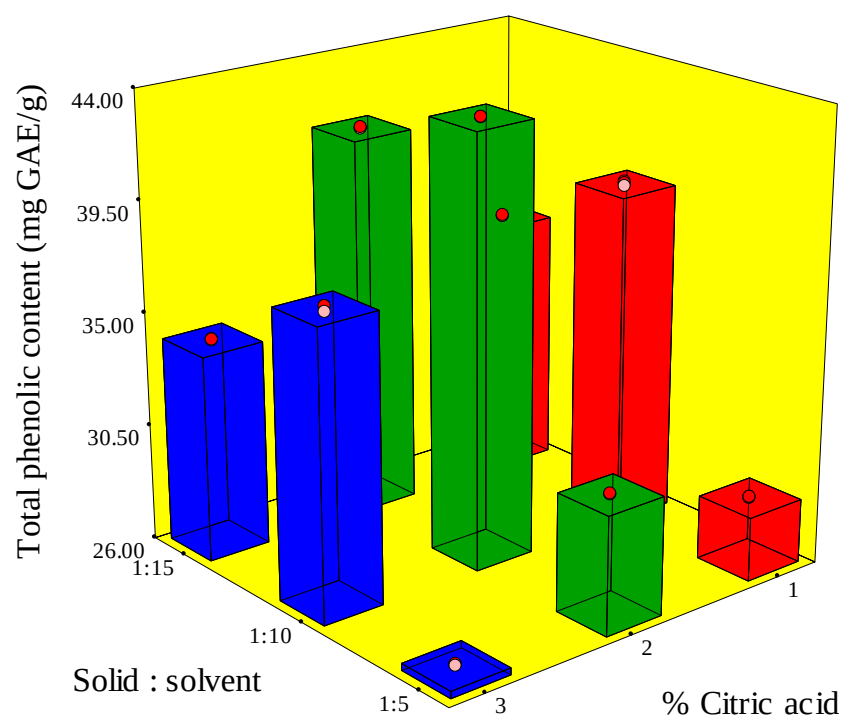


Fig. 41. Effect of solid to solvent ratios and concentration of citric acid (%) on total phenolic content of solvent extracted anthocyanin from calyces of Roselle

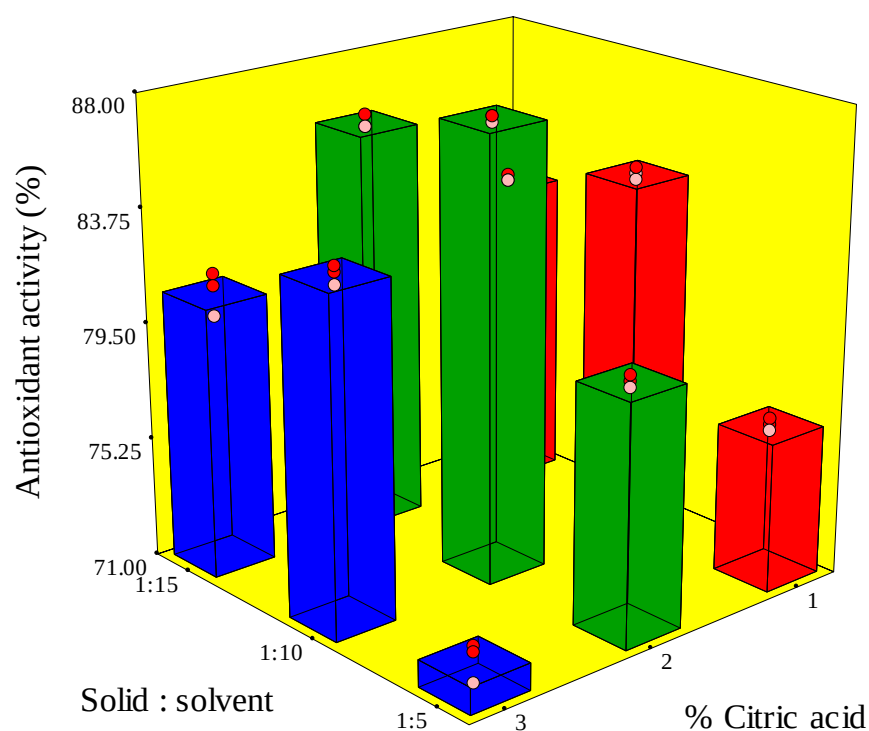


Fig. 42. Effect of solid to solvent ratios and concentration of citric acid (%) on antioxidant activity of solvent extracted anthocyanin from calyces of Roselle

5.11.3 Effect of solid to solvent ratios and concentration of citric acid (%) on total flavonoid content of solvent extracted anthocyanin from calyces of Roselle

The results of total flavonoid content of solvent extracted anthocyanin from calyces of Roselle obtained by different solid to solvent ratios and concentration of citric acid (%) are depicted graphically in Fig. 43. The total flavonoid content increased 58.34% in treatment E5 followed by 54.93% increase in treatment E8 compared to control. From the figure, it can be observed that the highest total flavonoid content was observed in case of 1:10 solid to solvent ratio compared to 1:5 and 1:15 solid to solvent ratio. This might be due to low concentration of organic solvents could access to cells, but high concentration would cause protein denaturation, preventing the dissolution of polyphenols and then influencing the extraction rate of total flavonoids (Chen *et al.*, 2013). Total flavonoids in a material could be completely extracted when the ratio of solvent to material is at an appropriate extent (Jing *et al.*, 2015). The result shows that a suitable volume of solvent contributed to the yield of flavonoids and antioxidant compounds. It was found that the lower the ratio of liquid to solid resulted in lower yield of total flavonoid content. This may be attributable to the suitable volume of solvent which could fully dissolve out the flavonoids from the material. When the ratio exceeded 1:10, more impurities were dissolved out which hindered the dissolution of flavonoids (Zou *et al.*, 2014). Similar results were reported by Jing *et al.* (2015) for the ultrasound-assisted extraction of flavonoid compounds and antioxidants from alfalfa.

From the figure, it can also be depicted that 2% citric acid had higher total flavonoid content followed by 3% and 1% citric acid. This might be due to the extraction yield of phenolic and flavonoid content is greatly depending on the solvent polarity (Lapornik *et al.*, 2005; Turkmen *et al.*, 2006; Ghasemzadeh *et al.*, 2011). The anthocyanins are major class of flavanoids in plants. It is known that citric acid is comparatively less corrosive than HCl but would still act to stabilize the anthocyanin structure in the cationic form by maintaining a low acidic pH (Heidari *et al.*, 2006; Joshi and Devi, 2015). This solvent system denatures the cell membranes, simultaneously dissolves the anthocyanins, and stabilizes them. However, care should be taken to avoid addition of excess acid which can hydrolyze labile, acyl, and sugar residues during concentration steps (Shil *et al.* 2005; Nicoue *et al.*, 2007; Dai and Mumper, 2010). However, control sample resulted in lower total flavonoid content, this might be due to lower salvation power of ethanol.

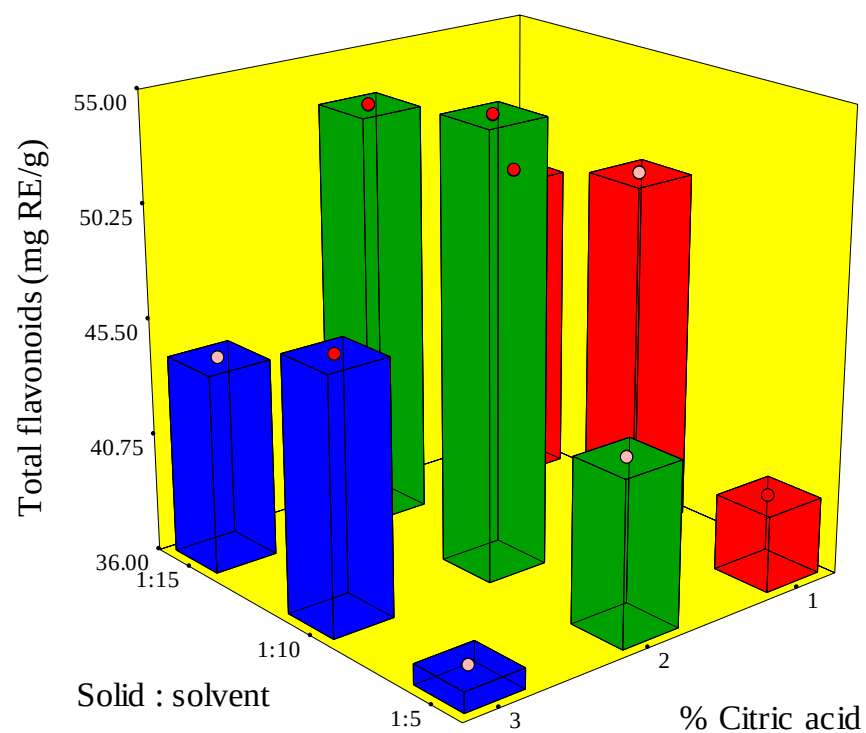


Fig. 43. Effect of solid to solvent ratios and concentration of citric acid (%) on total flavonoid content of solvent extracted anthocyanin from calyces of Roselle

5.11.4 Effect of solid to solvent ratios and concentration of citric acid (%) on total anthocyanin content of solvent extracted anthocyanin from calyces of Roselle

The total anthocyanin content of solvent extracted anthocyanin from calyces of Roselle recovered with the different solid to solvent ratios and concentration of citric acid (%) are presented in Fig. 44. From the figure, it is seen that the total anthocyanin content was significantly influenced by the solid to solvent ratios and concentration of citric acid (%) as well. The total anthocyanin content increased 108.80% in treatment E5 followed by 100.35% increase in treatment E8 compared to control. Considering the effect of selected solid to solvent ratio, it is clear that the 1:10 solid to solvent ratio resulted in higher total anthocyanin content followed by 1:15 and 1:5 solid to solvent ratio irrespective of % citric acid. Extraction of anthocyanins is commonly carried out under cold conditions with methanol or ethanol containing a small amount of acid with the objective of obtaining the flavylum cation form, which is red and stable in a highly acid medium. However, acid may cause partial hydrolysis of the acyl moieties in acylated anthocyanins, especially in anthocyanins acylated with dicarboxylic acids such as malonic acid (Arab *et al.*, 2011). Ethanol is another good solvent for polyphenol extraction and is safe for human consumption (Shil *et al.*, 2005). Water and ethanol are preferred as a solvent in the food industry and recommended by the US Food and Drug Administration for extraction purposes (Tabaraki *et al.*, 2012; Chen *et al.*, 2015). Similar results were reported by Chumsri *et al.* (2008) for extraction and concentration of Roselle extract.

From the figure, it can also be depicted that 2% citric acid had higher total anthocyanin content followed by 3% and 1% citric acid. In preparing anthocyanin-rich phenolic extracts from plant materials, an acidified organic solvent, most commonly methanol or ethanol is used. This solvent system denatures the cell membranes, simultaneously dissolves the anthocyanins, and stabilizes them. However, care should be taken to avoid addition of excess acid which can hydrolyze labile, acyl and sugar residues during concentration steps (Nicoue *et al.*, 2007; Arab *et al.*, 2011). The extraction of anthocyanins using ethanol acidified with citric acid instead of hydrochloric acid was reported by ethanol would be preferred for food use to avoid the toxicity of methanolic solutions. Citric acid is less corrosive than hydrochloric acid, chelates metals, maintains a low pH and may have a protective effect during processing (Arab *et al.*, 2011). The

extracting solution should be slightly acidic to maintain the flavylum cation form, which is red and stable in highly acidic medium, but not too acidic to cause partial hydrolysis of the acyl moieties in acylated anthocyanins (Andersen and Markham, 2006).

It has been demonstrated that in acidic media, four anthocyanin structures, including the flavylum cation, the quinonoidol base, the carbinol pseudobase and the chalcone, exist in equilibrium. pH below 2, the anthocyanin exists primarily in the form of the red flavylum cation (Chumsri *et al.*, 2008). The addition of citric acid to ethanol increased the efficiency of anthocyanins extraction compared with the control (70% ethanol) in all circumstances. It could be concluded that the addition of acid to the extraction medium have a great effect in stabilizing anthocyanins, thus leading to an increase in extraction yield. Acidification serves to maintain low pH, thereby providing a favourable medium for the formation of flavylim chloride salts from anthocyanins and improving the efficiency of anthocyanins extractions (Tawaiah *et al.*, 2016).

5.12 Optimization of process parameters for solvent extraction of anthocyanin from calyces of Roselle

Optimization of two process variables namely, solid to solvent ratio (1:5, 1:10 and 1:15) and concentration of citric acid (1, 2 and 3%) combinations were performed using the general factorial design in Design Expert Software 7.7.0. Numerical optimization was performed using statistical models to find the optimal process parameters of solvent extracted anthocyanin from calyces of Roselle powder. In the present investigation, the independent variables were kept within the range and dependent variables were chosen as maximum and minimum.

The experimental sample had the optimum process conditions of 1:10 solid to solvent ratio and 2% citric acid with desirability of 0.92 as depicted in Fig. 45.

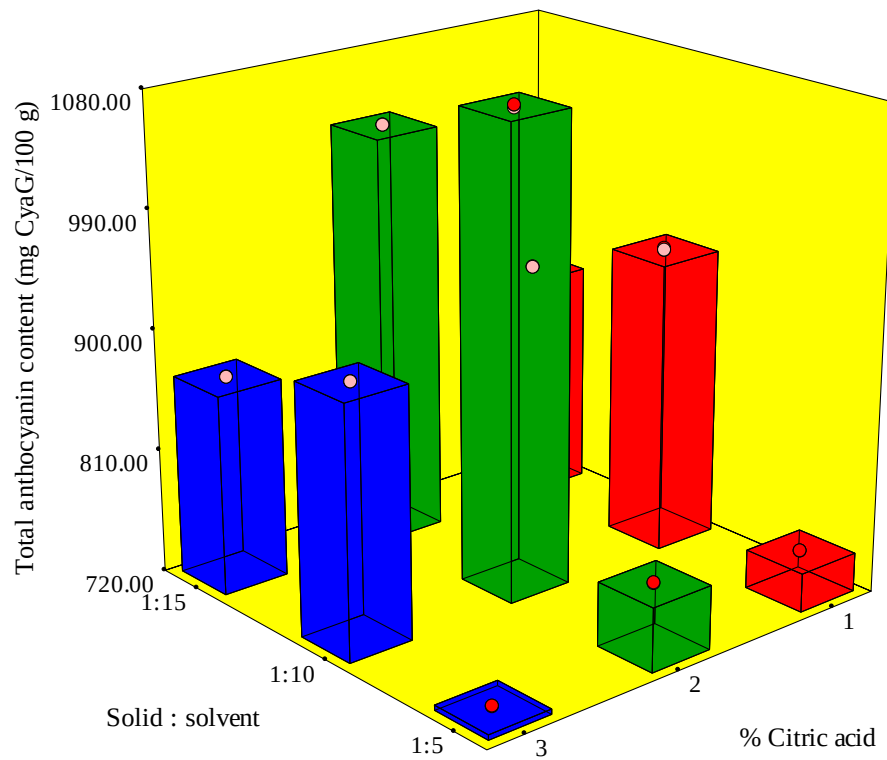


Fig. 44. Effect of solid to solvent ratios and concentration of citric acid (%) on total anthocyanin content of solvent extracted anthocyanin from calyces of Roselle

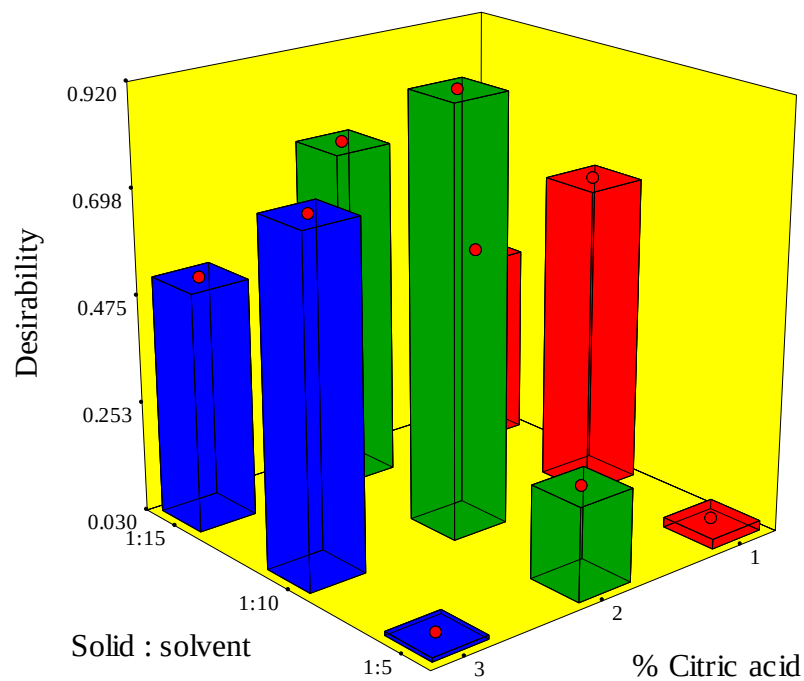


Fig. 45. Desirability graph for optimization of solvent extraction of anthocyanin from calyces of Roselle

5.13 Microencapsulation of Roselle anthocyanin

Encapsulation of anthocyanin extracts using spray drying may enhance their stability for them to be used as food colourants. The main objective of the encapsulation process is to build a barrier between the component in the particle and the environment. The barrier is made of compounds with chains that create a network. Its protection or controlled release efficiency depends on the composition and structure of the established wall but also on the operating conditions during the production and storage of these particles (Fuchs *et al.*, 2006; Aguilar *et al.*, 2011).

The quality of the microencapsulated Roselle anthocyanin powder was evaluated based on its physico-chemical properties, reconstitution properties and functional properties of powder and the results are discussed hereunder.

5.14 Physico-chemical properties of microencapsulated Roselle anthocyanin powder

The physico-chemical properties of microencapsulated Roselle anthocyanin powder viz., powder yield, moisture content, water activity, pH, titratable acidity, loose bulk density, tapped bulk density, Carr's index, Hausner ratio, particle size and colour (L^* , a^* and b^*) were determined and discussed here under.

5.14.1 Effect of different coating materials and inlet air temperatures on powder yield of microencapsulated Roselle anthocyanin powder

The variations in powder yield of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 46. The treatment M3 recorded 45.38% higher powder yield compared to M7. From the figure, it can be noticed that the powder yield was found highest using maltodextrin (MD) as coating material followed by gum arabica (GA) and whey protein (WP). This might be due to an additive such as MD and GA have been used to induce physical changes in the product, consequently reducing stickiness and wall deposition in spray drying. The addition of these carriers into the feed improves powder recovery (Truong *et al.*, 2005; Shrestha *et al.*, 2007; Sharifi *et al.*, 2015). The decrease of product yield for spray dried samples is connected with the fact that only some part of powder was sent to the receiver; while the rest remained on the walls of the

drier and cyclone. Some amount of powder was lost due to the fact that it was not separated in cyclone and was blown with air and remained on the filter (Wilkowska *et al.*, 2016).

From the figure, it can also be observed that the powder yield of the microencapsulated Roselle anthocyanin powder increased with increase in inlet air temperature. The inlet air temperature showed a positive effect on process yield, which can be attributed to the greater efficiency of heat and mass transfer processes and decreasing the probability of hitting the inadequate drying particles to the drying chamber wall when higher inlet air temperatures are used. The similar results were reported by Tonon *et al.* (2008) for spray drying of acai, Goula and Adamopoulos (2005) for spray drying of tomato pulp, Tee *et al.* (2012) for spray drying of Sirih leaves extract, Sharifi *et al.* (2015) for spray drying of barberry extract powder, Bazaria and Kumar (2016) for spray drying of beetroot juice powder, Shishir *et al.* (2016) for spray drying of pink guava powder and Shrestha *et al.* (2007) for spray drying of orange juice powder.

5.14.2 Effect of different coating materials and inlet air temperatures on moisture content of microencapsulated Roselle anthocyanin powder

The variations in moisture content of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 47. The treatment M3 recorded 80.56% lower moisture content compared to M7. From the figure, it can be noticed that the moisture content was found lowest using maltodextrin (MD) as coating material followed by gum arabica (GA) and whey protein (WP). This might be due to the maltodextrin was a powder with low moisture that exhibited good solubility and added no flavour or odour to the final product (Kenyon, 1995; Wang and Wang, 2000; Avila *et al.*, 2015). Similar findings were reported by Aliakbarian *et al.* (2015) for olive pomace polyphenols rich extracts. Righetto and Netto (2005) verified that maltodextrin was more effective in the reduction of moisture content of acerola powder produced by spray drying.

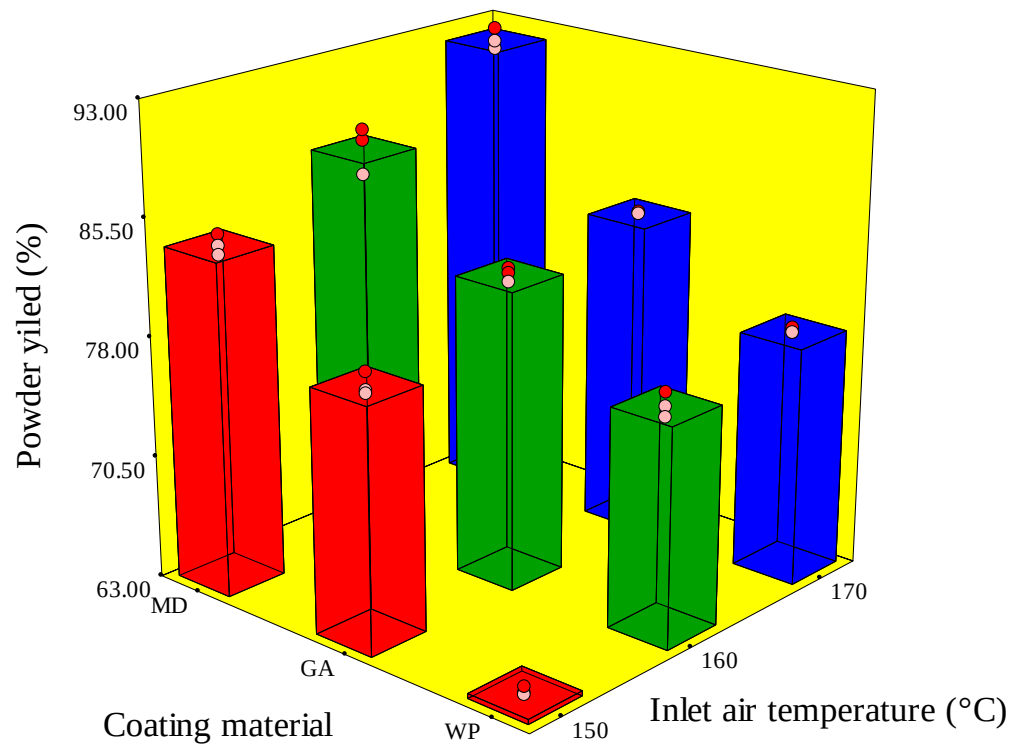


Fig. 46. Effect of different coating materials and inlet air temperatures on powder yield of microencapsulated Roselle anthocyanin powder

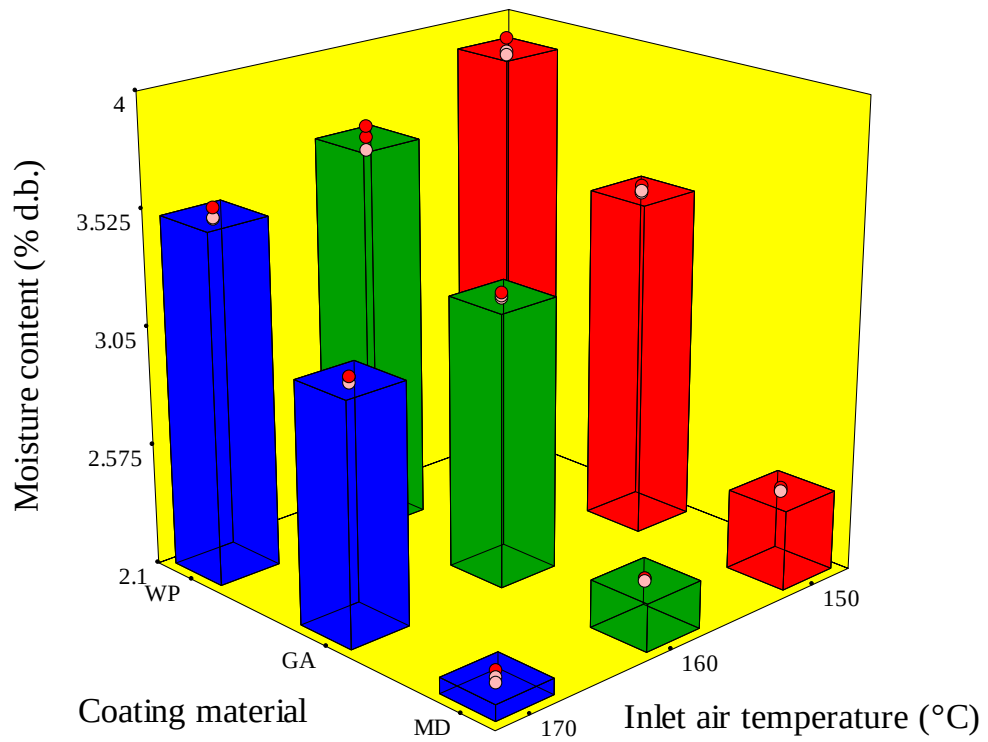


Fig. 47. Effect of different coating materials and inlet air temperatures on moisture content of microencapsulated Roselle anthocyanin powder

This behaviour was probably due to the differences between the chemical structures of both carrier agents, because gum arabic is a complex heteropolysaccharide with a highly ramified structure, containing shorter chains and more hydrophilic groups (Ferrari *et al.*, 2012). Similar results were reported by Quek *et al.* (2007) for spray drying of watermelon powder.

From the figure, it can also be observed that the moisture content of the microencapsulated Roselle anthocyanin powder decreased with an increase in inlet air temperature. This can be explained by the fact that, the greater the temperature difference between the drying medium and the particles, the greater will be the rate of heat transfer into the particles, which provides the driving force for moisture removal. When the drying medium is air, temperature plays a second important role. As water is driven from the particles in the form of water vapour, it must be carried away, or the moisture will create a saturated atmosphere at the particle surface. This will slow down the rate of subsequent water removal. The hotter the air, the more moisture it will hold before becoming saturated. Thus, high temperature air in the vicinity of the drying particles will take up the moisture being driven from the food to a greater extent than with cooler air (Goula and Adamopoulos, 2005a). It can be explained that high inlet air temperature provides the good driving force for moisture removal which increased the rate of heat transfer from hot air to the particle (Goula and Adamopoulos, 2008; Asun *et al.*, 2016). These results are in consistent with Fazaeli *et al.* (2012) who reported that the moisture content of the black mulberry powder decreased with the increase of inlet air temperature. Similar results were reported by Asun *et al.* (2016) for spray drying of coconut sugar powder, Costa *et al.* (2015) for spouted bed drying of acai powder and Farimin and Nordin (2009) for spray drying of Roselle-pineapple juice.

5.14.3 Effect of different coating materials and inlet air temperatures on water activity of microencapsulated Roselle anthocyanin powder

Water activity (a_w) is a very important index for spray-dried powder as it determines the shelf-life of powder produced. Water activity measures the activity of free water in a food system which is responsible for any biochemical reactions. Higher water activity indicates that there is more free water available for biochemical reactions and thus the shelf-life is shorter. Basically, food with a_w less than 0.6 is microbiologically stable (Farimin and Nordin, 2009).

The variations in water activity of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 48. The treatment M3 recorded 47.62% lower water activity compared to M7. From the figure, it can be noticed that the water activity was found lowest using maltodextrin (MD) as coating material followed by gum arabica (GA) and whey protein (WP). This might be due to the ability of maltodextrin to reduce reactant mobility. Moreover, adding maltodextrin might prevent the change of the state from powder to a 'sorption gel' (Chronakis, 1998). Nurhadi *et al.* (2012) reported that the water activity of the honey powder spray dried with maltodextrin was lower while the arabic gum as a carrier showed highest water activity. Similar results were reported by Duangmal *et al.* (2008) for freeze drying of Roselle extract, Samborska *et al.* (2015) for spray drying of honey and Quek *et al.* (2007) for spray dried watermelon powder.

From the figure, it can also be observed that the water activity of the microencapsulated Roselle anthocyanin powder decreased with an increase in inlet air temperature. Moisture content and water activity of the powder decreased with an increase of inlet air temperature. It can be explained that high inlet air temperature provides the good driving force for moisture removal which increased the rate of heat transfer from hot air to the particle (Goula and Adamopoulos, 2008; Asun *et al.*, 2016). Generally, the greater the temperature difference between the particles, the greater the evaporation rate (Grabowski *et al.*, 2006) which results in lower water activity. Similar results were reported by Goula *et al.* (2004) for spray dried tomato paste, Abadio *et al.* (2004) for spray dried pineapple juice, Chegini and Ghobadian (2007) for spray dried orange juice and Sarabandi *et al.* (2014) for spray dried grape syrup.

5.14.4 Effect of different coating materials and inlet air temperatures on pH of microencapsulated Roselle anthocyanin powder

The variations in pH of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 49. The treatment M3 recorded 70.31% lower pH compared to M7.

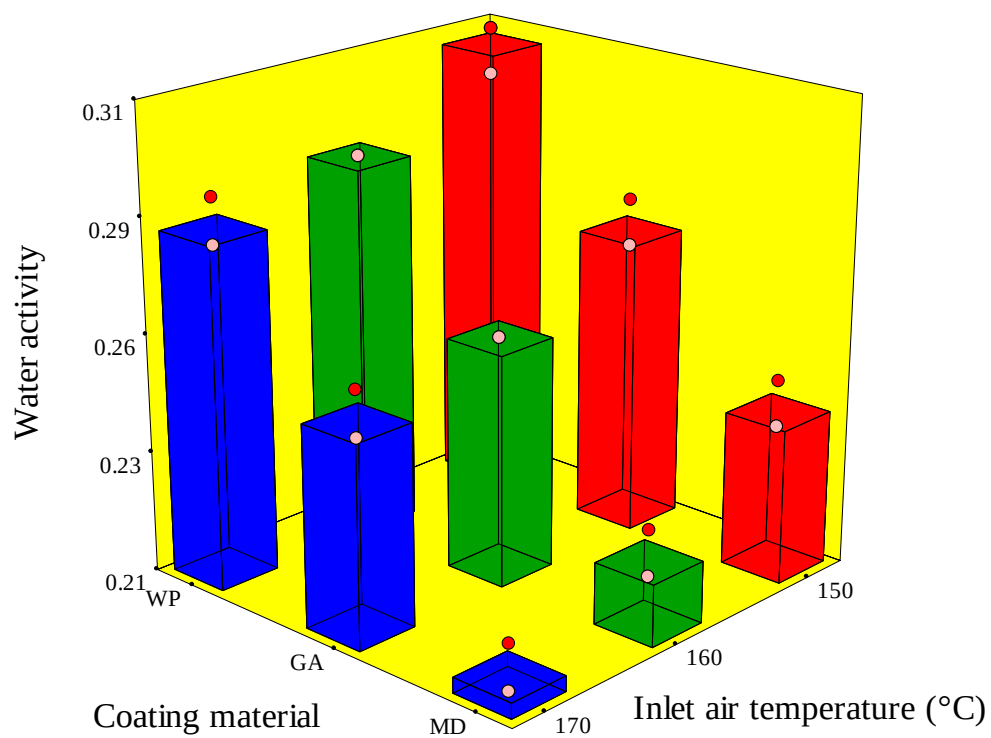


Fig. 48. Effect of different coating materials and inlet air temperatures on water activity of microencapsulated Roselle anthocyanin powder

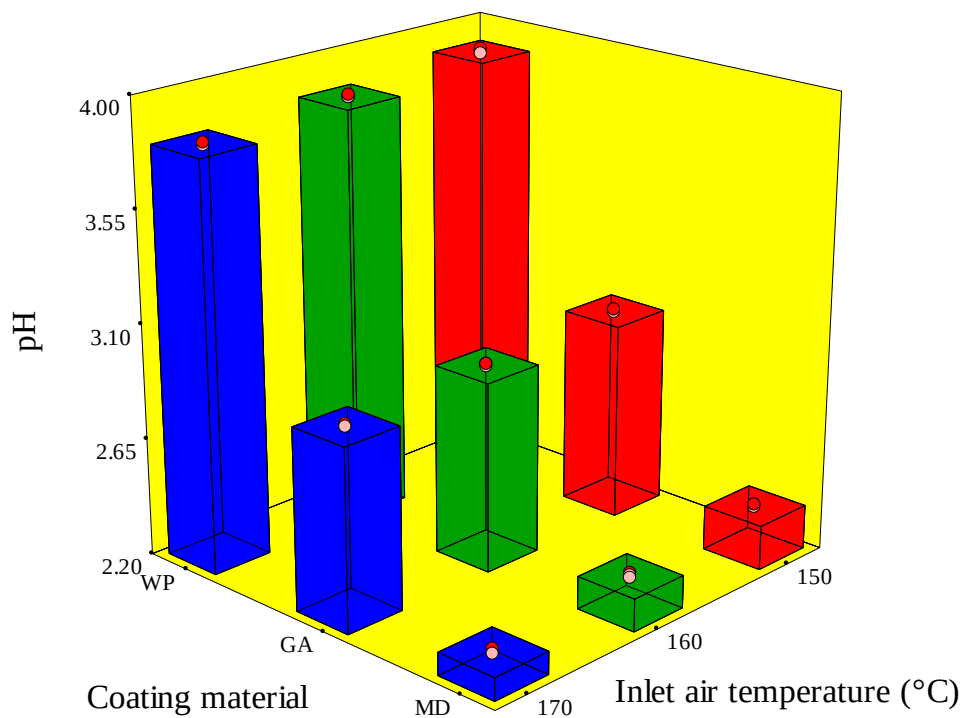


Fig. 49. Effect of different coating materials and inlet air temperatures on pH of microencapsulated Roselle anthocyanin powder

From the figure, it can be noticed that the pH was found lowest using maltodextrin (MD) as coating material followed by gum arabica (GA) and whey protein (WP). From the figure, it can also be observed that the pH of the microencapsulated Roselle anthocyanin powder decreased with an increase in inlet air temperature. Similar results were reported by Karaaslan and Dalgic (2014) for spray drying of liquorice extract, they reported that maltodextrin and high inlet air temperature resulted in decrease in pH value. The obtained result indicates that the pH value was in the range of 2.29-3.82, which means all the treatments are in high acidic range.

5.14.5 Effect of different coating materials and inlet air temperatures on titratable acidity of microencapsulated Roselle anthocyanin powder

The variations in titratable acidity of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 50. The treatment M3 recorded 25.16% higher titratable acidity compared to M7. From the figure, it can be noticed that the titratable acidity was found highest using maltodextrin (MD) as coating material followed by gum arabica (GA) and whey protein (WP). From the figure, it can also be observed that the titratable acidity of the microencapsulated Roselle anthocyanin powder decreased with an increase in inlet air temperature. Similar results were reported by Karaaslan and Dalgic (2014) for spray drying of liquorice extract, they reported that maltodextrin and high inlet air temperature resulted in higher acidity. However, Santhalakshmy *et al.* (2015) reported lower acidity at higher inlet air temperature for spray drying of jamun fruit powder.

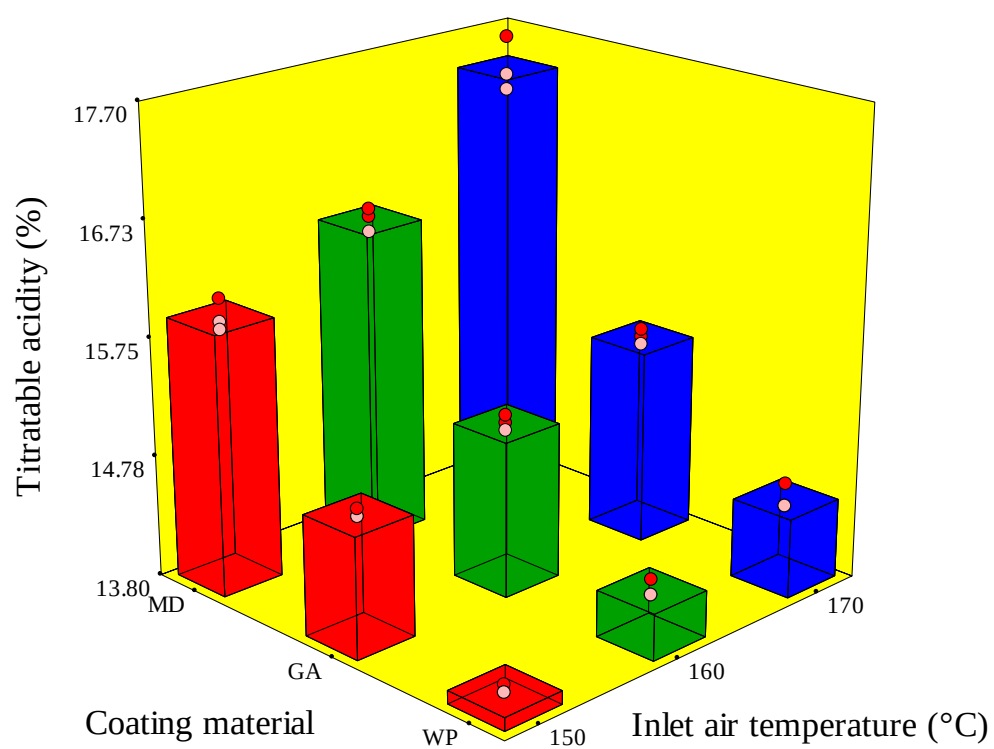


Fig. 50. Effect of different coating materials and inlet air temperatures on titratable acidity of microencapsulated Roselle anthocyanin powder

5.14.6 Effect of different coating materials and inlet air temperatures on loose and tapped bulk densities of microencapsulated Roselle anthocyanin powder

The variations in loose bulk density and tapped bulk density of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 51 and Fig. 52, respectively. The treatment M3 recorded 73.92% and 85.71% lower loose and tapped bulk density, respectively compared to M7. From the figure, it can be noticed that the loose bulk density and tapped bulk density was found lowest using maltodextrin (MD) as coating material followed by gum arabica (GA) and whey protein (WP). This effect may be attributed to the fact that maltodextrin addition minimizes thermoplastic particles from sticking. In addition, maltodextrin may cause an increase in the volume of air trapped in the particles, as maltodextrin is a skin-forming material. Generally, an increase in the volume of trapped air causes a decrease in the apparent density of the particles and this apparent density primarily determines the powder bulk density (Goula and Adamopoulos, 2010). Similar results were also reported by Vardin and Yasar (2012) who carried out tests on spray drying of pomegranate juice powder and Karsaalan and Dalgic (2014) for spray drying of liquorice.

Tonon *et al.* (2010) observed higher bulk density values for spray dried acai powder, when tapioca starch was employed as carrier agent. The authors attributed this behaviour to the highest molecular weight of tapioca starch in comparison to maltodextrin and gum arabic. The heavier the material, more easily it accommodates into the spaces between the particles, occupying less space and resulting in higher bulk density values. Similar results were also pointed out by Osorio *et al.* (2011), working with guava powder produced by spray drying. Powder encapsulated with maltodextrin exhibited a minor bulk density than that of gum arabic. Similar results were reported by Ferrari *et al.* (2012) for spray drying of blackberry powder. Chegini and Ghobadian (2005) reported that spray dried powders with higher moisture contents tend to have a higher bulking weight because of the presence of water, which is considerably denser than the dry solid. This behaviour can be associated with the results observed in the present study because Roselle anthocyanin powders produced with whey protein showed higher moisture content and higher bulk density.

From the figure, it can also be observed that the loose bulk density and tapped bulk density of the microencapsulated Roselle anthocyanin powder decreased with an increase in inlet air temperature. This could be due to the faster evaporation and more porous structure or fragmented structure of the dried powder which is implying a lower shrinkage of the droplets and giving lower density of the powder (Fazaeli *et al.*, 2012). Increase in inlet air temperature often resulted in a rapid formation of dried layer at the droplet surface and it was due to skinning over or case hardening of the droplets at the higher temperatures. This leads to the formation of vapour impermeable films on the drop surface, followed by the formation of vapour bubbles and consequently, droplet expansion (Jumah *et al.*, 2000; Martinez *et al.*, 2010; Karaaslan and Dalgic, 2014).

Goula and Adamopoulos (2010) reported that evaporation rates are faster and products dry to a more porous or fragmented structure, there was a greater tendency for the particles to be hollow. These results were in line with the findings of Kha *et al.* (2010) for spray dried Gac fruit aril powder, Chegini and Ghobadian (2005) for spray dried orange juice powder, Renata *et al.* (2010) for spray dried acai juice powder, Asun *et al.* (2012) for spray dried coconut sugar powder and Cai and Corke (2000) for spray dried *Amaranthus* betacyanin powder. Goula and Adamopoulos (2010) also observed a reduction of powders bulk density with increasing air temperatures, studying the spray drying of orange juice powders. Similar results were observed by Sarabandi *et al.* (2014) for spray drying of grape syrup and Sathyashree *et al.* (2016) for spray drying of sweet orange juice.

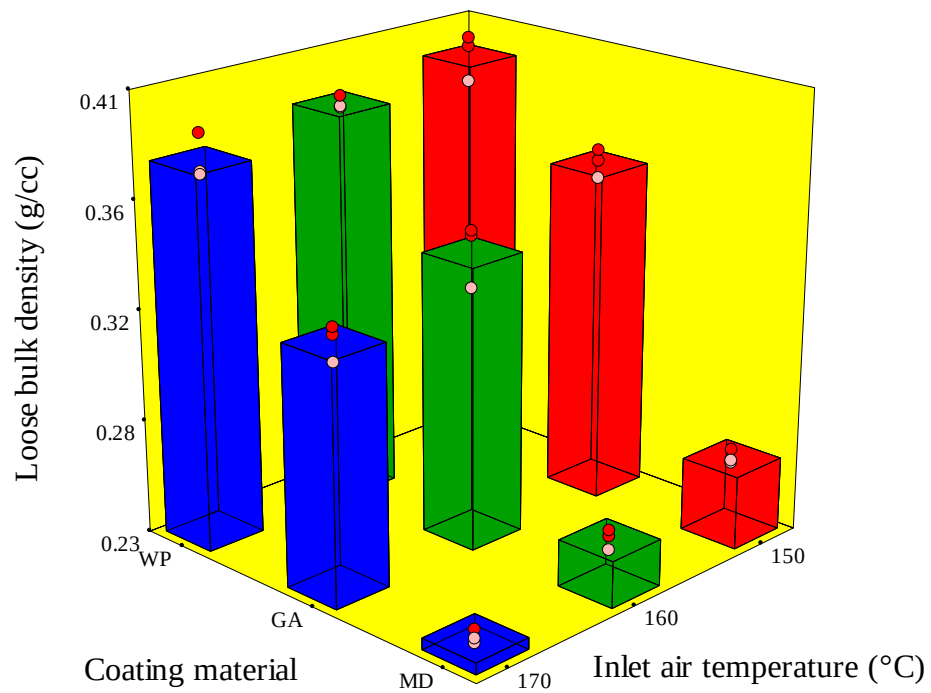


Fig. 51. Effect of different coating materials and inlet air temperatures on loose bulk density of microencapsulated Roselle anthocyanin powder

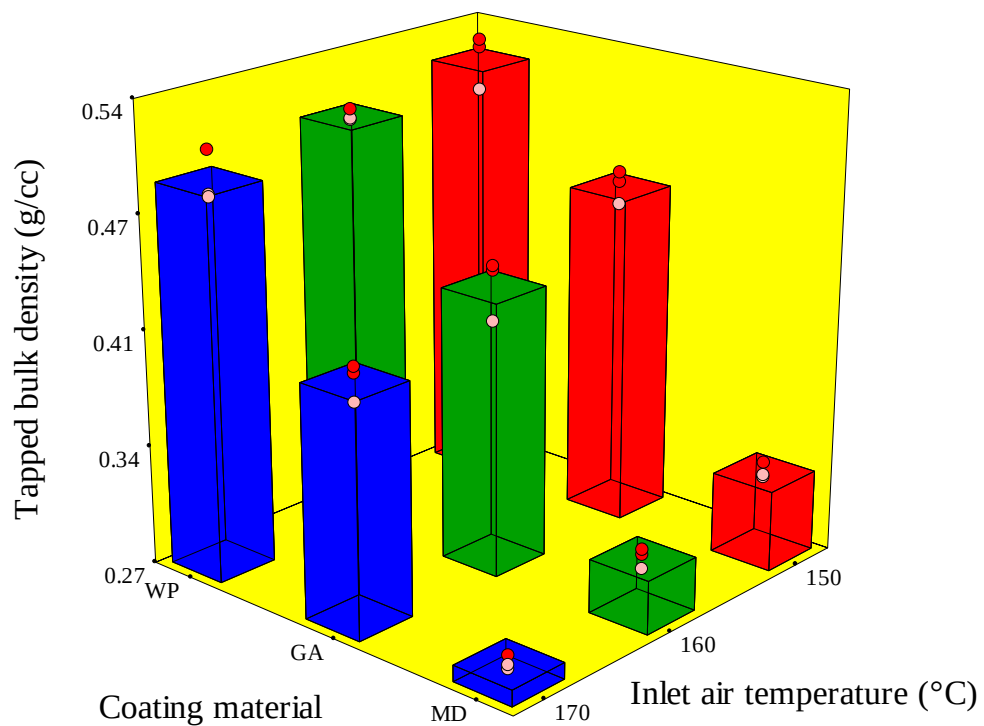


Fig. 52. Effect of different coating materials and inlet air temperatures on tapped bulk density of microencapsulated Roselle anthocyanin powder

5.14.7 Effect of different coating materials and inlet air temperatures on Carr's index of microencapsulated Roselle anthocyanin powder

Flowability is an important property for dried particles and was expressed as the Carr index (CI). The higher value of the Carr index indicates poor flowability (Santhalakshmy *et al.*, 2015). The variations in Carr's index of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 53. The treatment M3 recorded 47.79% lower Carr's index compared to M7. From the figure, it can be noticed that the Carr's index was found lowest using maltodextrin (MD) as coating material followed by gum arabica (GA) and whey protein (WP). This may be due to lower moisture content of spray dried Roselle anthocyanin powder using maltodextrin as a coating material. Moisture content had a significant effect on the flowability and cohesiveness of powder. Liquid bridges and capillary forces acting between powder particles reduced flowability and increased the cohesiveness of powder. In addition, moisture plasticizes the powder material, especially the water soluble constituents, results in deformation of the powder (Kim *et al.*, 2009; Moreira *et al.*, 2009).

Carr's index is a measure of powder bridge strength and stability (Shah *et al.*, 2008). Zhang and Youan (2010) reported that Carr's index value less than 25% indicates a fluid powder (good flow) whereas, value greater than 25% indicates a cohesive powder with poor (Gonnissen *et al.*, 2008) powder flow, which might be due to insufficient drying related to the great amount of water added into the diffusion process. In practice, poor powder flowability was correlated with high moisture content in spray dried powder shown in previous reports (Dawoodbhai and Rhodes, 1989).

From the figure, it can also be observed that increase in the inlet air temperature results in reduction of Carr's index of the encapsulated Roselle anthocyanin powder. It can be explained that high inlet air temperature provides the good driving force for moisture removal which increased the rate of heat transfer from hot air to the particle (Goula and Adamopoulos, 2008; Asun *et al.*, 2016) which intern resulted in lower Carr's index. Kalal *et al.* (2016) reported that with the increase in inlet air temperature which resulted in decrease in the Carr's index of the spray dried bitter gourd juice powder. The

result obtained is in agreement with Santhalakshmy *et al.* (2015) for spray drying of jamun fruit juice powder.

5.14.8 Effect of different coating materials and inlet air temperatures on Hausner ratio of microencapsulated Roselle anthocyanin powder

The variations in Hausner ratio of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 54. The treatment M3 recorded 10.08% lower Hausner ratio compared to M7. From the figure, it can be noticed that the Hausner ratio was found lowest using maltodextrin (MD) as coating material followed by gum arabica (GA) and whey protein (WP). This may be due to lower moisture content of spray dried Roselle anthocyanin powder using maltodextrin as a coating material. Moisture content had a significant effect on the flowability and cohesiveness of powder. Liquid bridges and capillary forces acting between powder particles reduced flowability and increased the cohesiveness of powder. In addition, moisture content plasticizes the powder material, especially the water soluble constituents, results in deformation of the powder (Kim *et al.*, 2009; Moreira *et al.*, 2009). It can be concluded that the powders with MD were characterised by a Hausner ratio lower than that with GA and WP, which indicates their better flowability. The result obtained is in agreement with Samborska *et al.* (2015) for spray drying of honey powder.

In terms of handling properties, the spray dried Roselle anthocyanin powder had similar flow characteristics for all treatments in the present study and were considered as “possible” and “fair” powders by their Hausner ratio (HR) given in Table 57, Chapter IV as classified in Table 2, Chapter III. This was in accordance with their medium Carr’s index (CI) (Table 57, Chapter IV) which indicated that their flowability was “possible” and “fair” (Table 2, Chapter III). Teunou *et al.* (1999) also stated the presence of water in a powder could significantly affect its flowability, sticking and caking properties. In general, the greater the water content of a powder, the more cohesive the powder becomes and the more difficult to flow.

From the figure, it can also be observed that increase in the inlet air temperature results in reduction of Hausner ratio of the encapsulated Roselle anthocyanin powder.

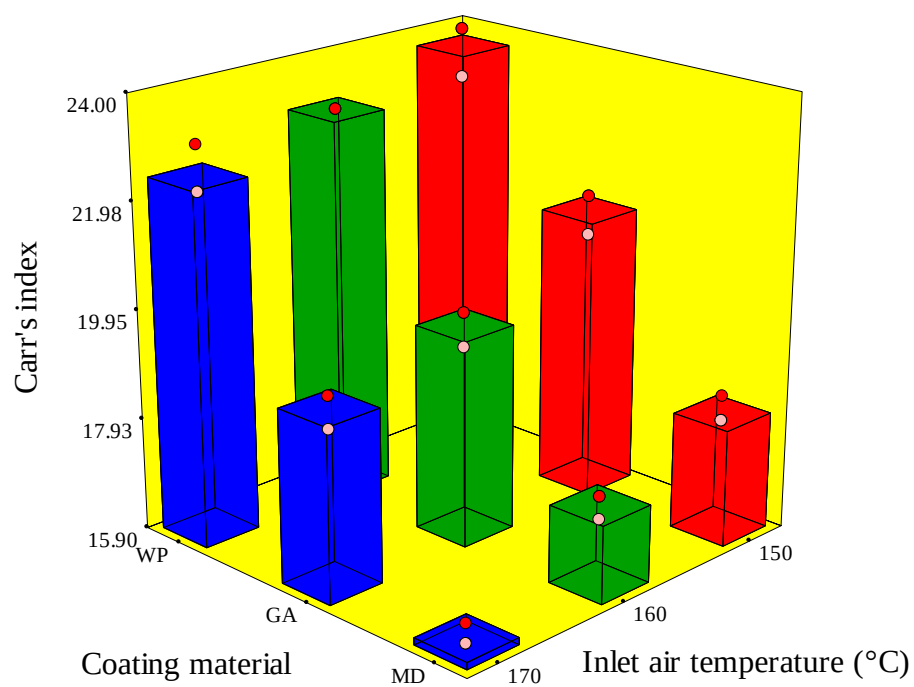


Fig. 53. Effect of different coating materials and inlet air temperatures on Carr's index of microencapsulated Roselle anthocyanin powder

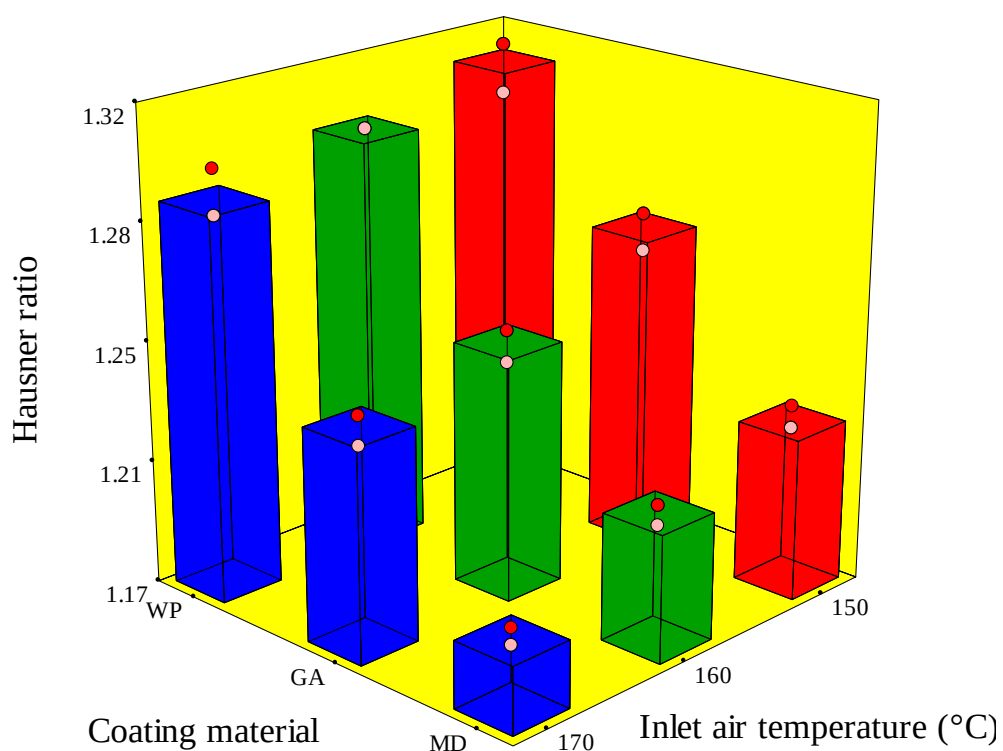


Fig. 54. Effect of different coating materials and inlet air temperatures on Hausner ratio of microencapsulated Roselle anthocyanin powder

It can be explained that high inlet air temperature provides the good driving force for moisture removal which increased the rate of heat transfer from hot air to the particle (Goula and Adamopoulos, 2008; Asun *et al.*, 2016) which intern resulted in lower Hausner ratio. Kalal *et al.* (2016) reported that with the increase in inlet air temperature which resulted in decrease in the Hausner ratio of the spray dried bitter gourd juice powder. The result obtained is in agreement with Santhalakshmy *et al.* (2015) for spray drying of jamun fruit juice powder.

5.14.9 Effect of different coating materials and inlet air temperatures on particle size of microencapsulated Roselle anthocyanin powder

The variations in particle size of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 57. The treatment M3 recorded 229.95%% lower particle size compared to M7. From the Fig. 55, it can be noticed that the particle size was found lowest using maltodextrin (MD) as coating material followed by gum arabica (GA) and whey protein (WP) (Fig. 56). Spray dried Roselle anthocyanin powder with whey protein has showed higher particle size, which may be due to whey protein causes increase in feed viscosity, resulting in formation of powder particles of bigger size. Increase in moisture content of powders was found to increase the cohesion between powder particles, resulting in higher particle size (Bhusari *et al.*, 2014). Samborska *et al.* (2015) reported that GA mixtures had higher viscosity than MD, which could lead to the production of larger droplets and particles of spray dried honey powder. The maltodextrin has the characteristic of having low viscosity at high solid concentrations which results in lower particle size (Costa *et al.*, 2015).

From the Fig. 57, it can be noticed that the particle size reduced with increase in inlet air temperature. This could be due to increase in inlet air temperature results in rapid formation of dried layer on the droplet surface. Furthermore, it can cause the particle size change beside the skinning over or casehardening on the droplets. This process leads to the formation of vapour-impermeable films on the droplet surface followed by the formation of vapour bubbles and the droplet expansion (Chegini and Ghobadian, 2005). The similar results were reported by Cai and Corke (2000) for spray drying of *Amaranthus* betacyanin powder and Suzihaque *et al.* (2015) for pineapple and banana milk powder.

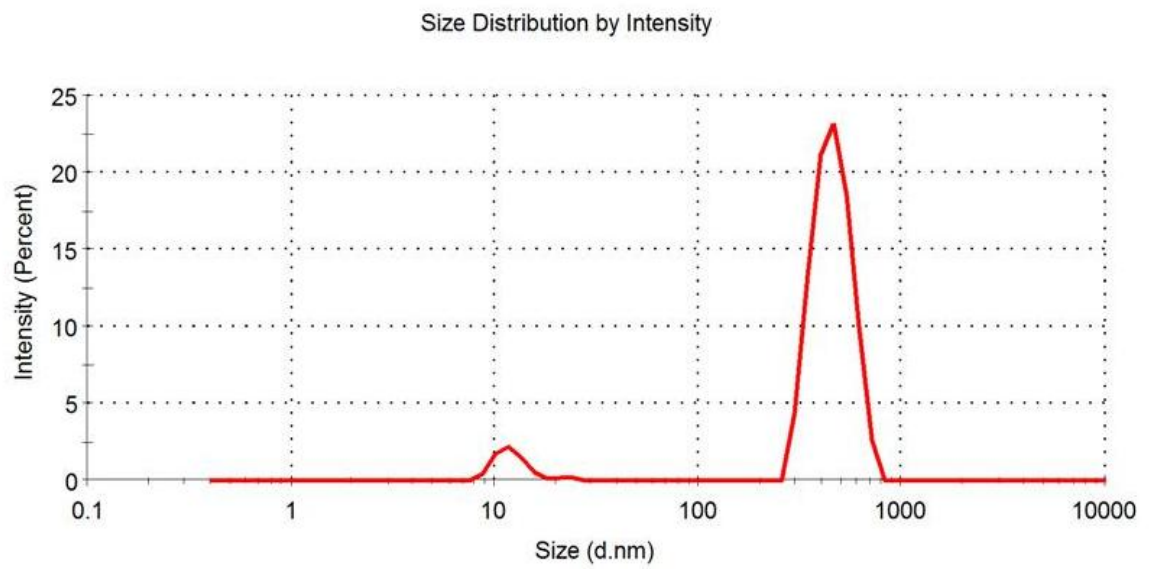


Fig. 55. Z average particle size (d.nm) of M3 treatment

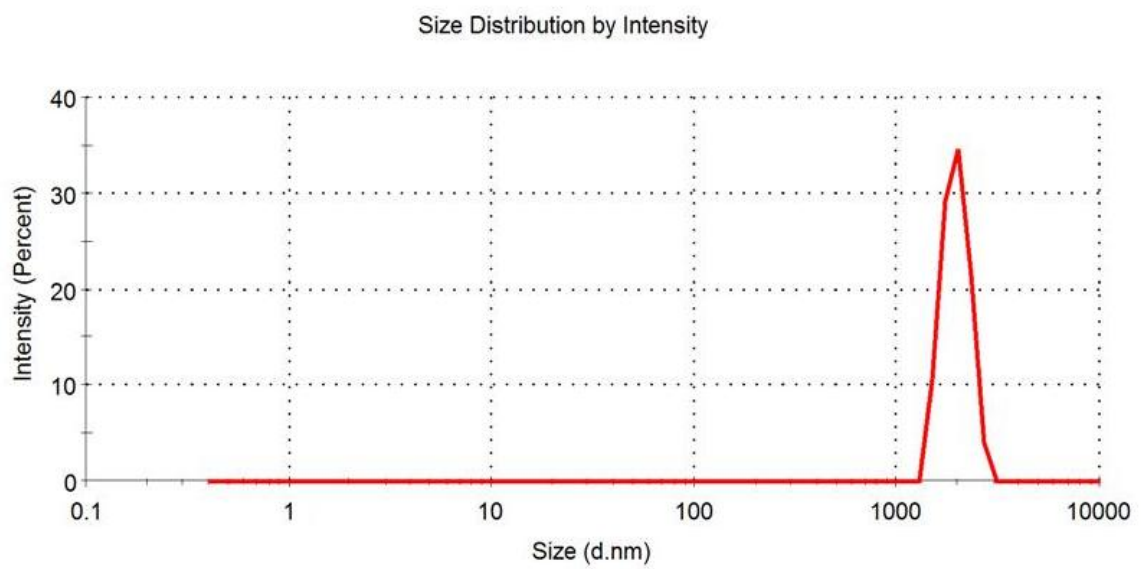


Fig. 56. Z average particle size (d.nm) of M7 treatment

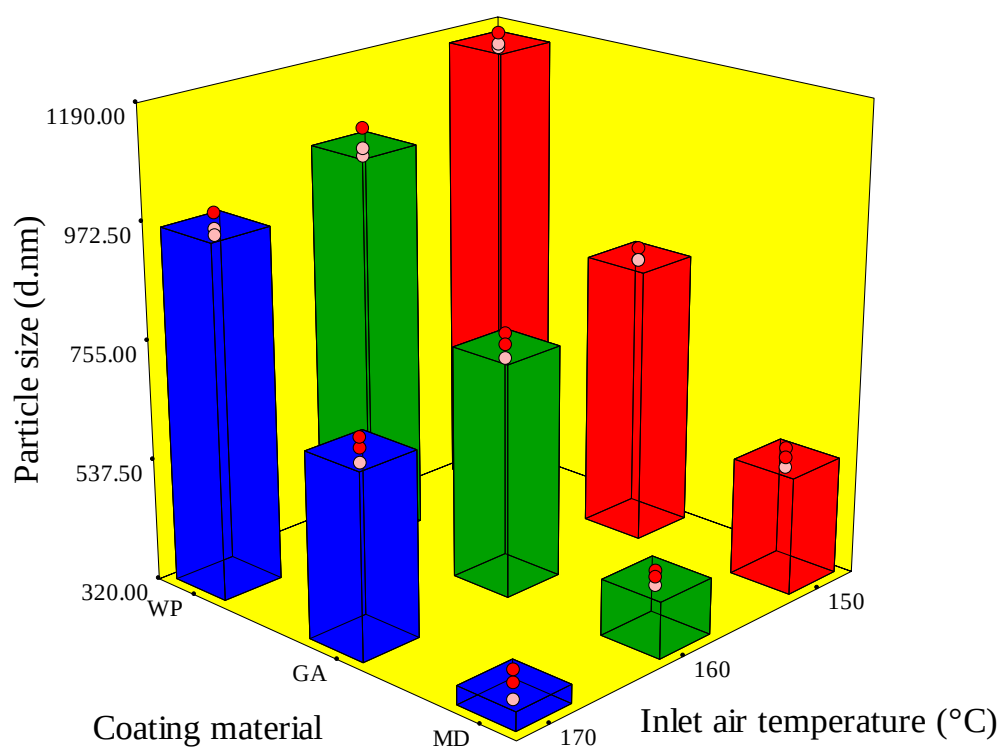


Fig. 57. Effect of different coating materials and inlet air temperatures on particle size of microencapsulated Roselle anthocyanin powder

5.14.10 Effect of different coating materials and inlet air temperatures on colour value of microencapsulated Roselle anthocyanin powder

L^* value

The variations in L^* value of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 58. The treatment M3 recorded 8.67% lower L^* value compared to M7. From the figure, it can be noticed that the L^* value was found lowest using maltodextrin (MD) as coating material followed by gum arabica (GA) and whey protein (WP). This might be due to higher a^* value in the MD samples which resulted from higher retention of anthocyanin content. Maltodextrins of different dextrose equivalent (DE) are commonly used as wall material by its high water solubility, low viscosity, low sugar content and their solutions are colourless. These properties make them useful ingredients in the food industry (Avaltroni *et al.*, 2004; Robert *et al.*, 2010).

From the figure, it can also be observed that increase in the inlet air temperature resulted in decrease in L^* value of the encapsulated Roselle anthocyanin powder. This can be explained by the fact that the hydrolysis of pyrilium ring or 3-Glycoside structure and, in consequence, chalcon compound production, are the main factors responsible for the development of brown colour, with temperature being the major determining factor, which results in lower L^* value at higher temperature (Laleh *et al.*, 2006; Yousefi *et al.*, 2011). Ahmed *et al.*, (2010) reported colour changes verified in spray-dried purple sweet potato were related to degradation reactions that promoted the formation of polymeric anthocyanins, which have a more brownish shade, and resulted in darker powders (lower lightness values). Similar results were reported by Ferrari *et al.* (2013) for spray drying of blackberry.

a^* value

The variations in a^* value of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 59. The treatment M3 recorded 308.92% higher a^* value compared to M7.

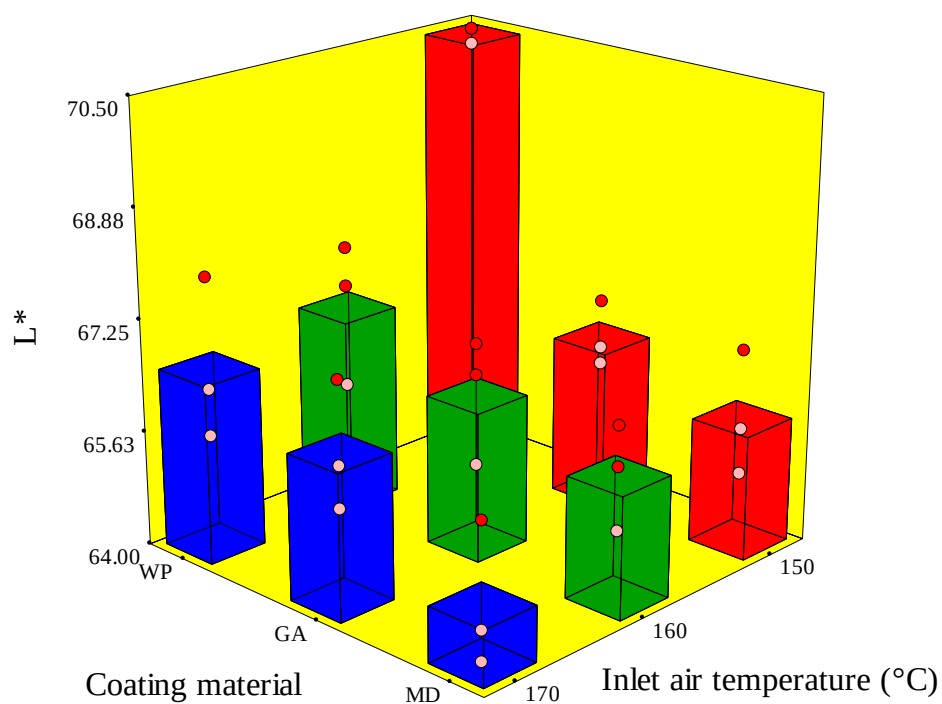


Fig. 58. Effect of different coating materials and inlet air temperatures on L^* value of microencapsulated Roselle anthocyanin powder

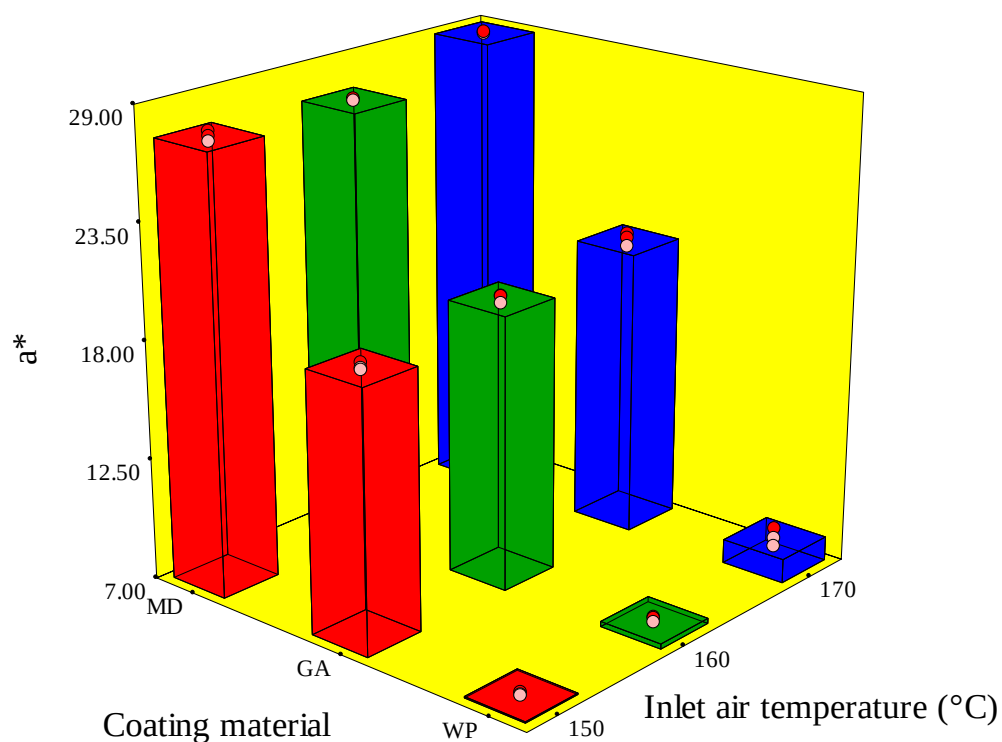


Fig. 59. Effect of different coating materials and inlet air temperatures on a^* value of microencapsulated Roselle anthocyanin powder

From the figure, it can be noticed that the a^* value was found highest using maltodextrin (MD) as coating material followed by gum arabica (GA) and whey protein (WP). The use of maltodextrin significantly increased parameter a^* as compared to GA and WP. This behaviour can be related to anthocyanin content and antioxidant activity results, because powders produced with maltodextrin showed better pigment retention and higher antioxidant activity. Similar results were reported by Ferrari *et al.* (2012) for spray drying of blackberry powder.

From the figure, it can also be observed that increase in the inlet air temperature results in increase in a^* value of the encapsulated Roselle anthocyanin powder. Ahmed *et al.* (2010) reported colour changes verified in spray-dried purple sweet potato were related to degradation reactions that promoted the formation of polymeric anthocyanins, which have a more brownish shade and resulted in darker powders. Similar results were reported by Ferrari *et al.* (2012a) for spray drying of blackberry.

b^* value

The variations in b^* value of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 60. The treatment M3 recorded 202.05% lower b^* value compared to M7. From the figure, it can be noticed that the b^* value was found lowest using maltodextrin (MD) as coating material followed by gum arabica (GA) and whey protein (WP). This might be due to higher a^* value in the MD samples which resulted from higher retention of anthocyanin content. The optical properties of the encapsulated powder were affected by the type and concentration of the carrier material- powder produced with WP showed the higher b^* value. This is because of the yellow colour of whey protein.

From the figure, it can also be observed that increase in the inlet air temperature results in decrease in b^* value of the encapsulated Roselle anthocyanin powder. This might be due to the degradation of colour of the spray dried samples at higher temperature. Similarly, Reddy *et al.* (2014) reported that b^* values of Osmanabadi goat milk powder slightly decreased with increase in inlet air temperature.

5.15 Reconstitution properties of microencapsulated Roselle anthocyanin powder

The effect of spray drying viz., coating material (MD, GA and WP) and inlet air temperature (150, 160 and 170 °C) on reconstitution properties such as solubility, wettability and dispersibility of microencapsulated Roselle anthocyanin powder are discussed hereunder.

5.15.1 Effect of different coating materials and inlet air temperatures on solubility of microencapsulated Roselle anthocyanin powder

The variations in solubility of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 61. The treatment M3 recorded 176.03% higher solubility compared to M7. From the figure, it can be noticed that the solubility was found highest using maltodextrin (MD) as coating material followed by gum arabica (GA) and whey protein (WP). This may be due to higher solubility of MD when compared to GA and WP. Maltodextrins of different dextrose equivalent (DE) are commonly used as wall material by its high water solubility, low viscosity, low sugar content and their solutions are colourless. These properties make them useful ingredients in the food industry (Avaltroni *et al.*, 2004; Robert *et al.*, 2010). Similar results were reported by Cai and Corke (2000) for spray drying of *Amaranthus* betacyanin pigments.

From the figure, it can also be observed that increase in the inlet air temperature results in increase in solubility of the encapsulated Roselle anthocyanin powder. Solubility showed an increase with an increase in inlet air temperature. This is due to the effect of inlet air temperature on residual moisture content. The lower the powder moisture content, the more soluble is the powder. In addition, increasing the drying air temperature generally produces an increase in particle size, and so a decrease in time required for the powder to dissolve (Goula and Adamopoulos, 2005a). Similar results were reported by Abadio *et al.* (2004), Tonon *et al.* (2008), Karaaslan and Dalgic (2014) and Sarabandi *et al.* (2014) for spray drying of pineapple juice, acai, liquorice and grape syrup, respectively.

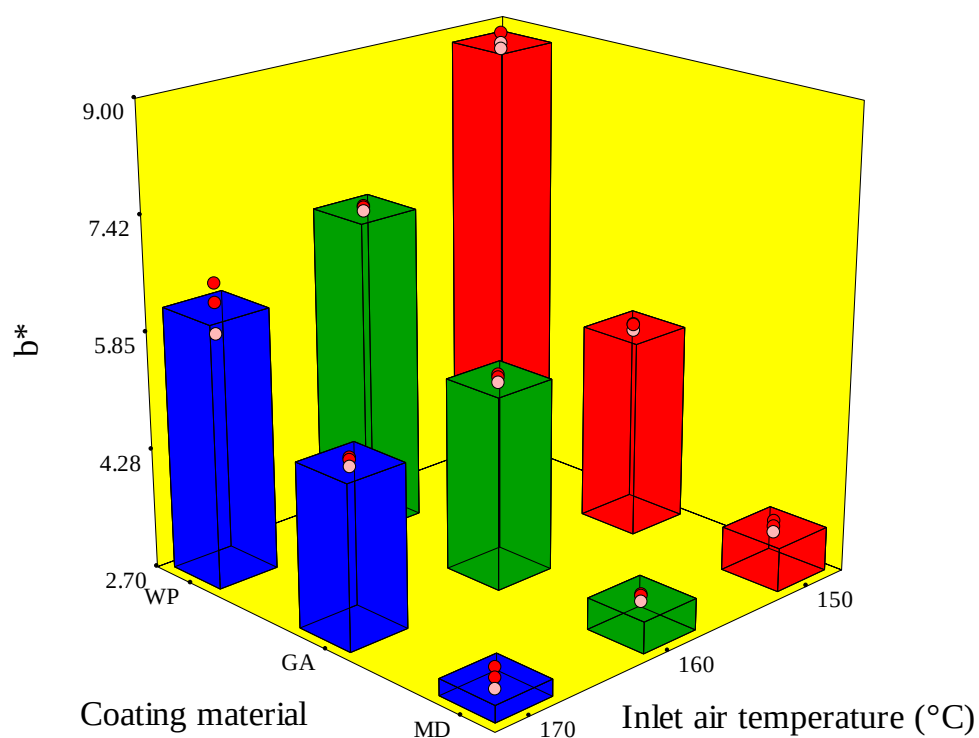


Fig. 60. Effect of different coating materials and inlet air temperatures on b^* value of microencapsulated Roselle anthocyanin powder

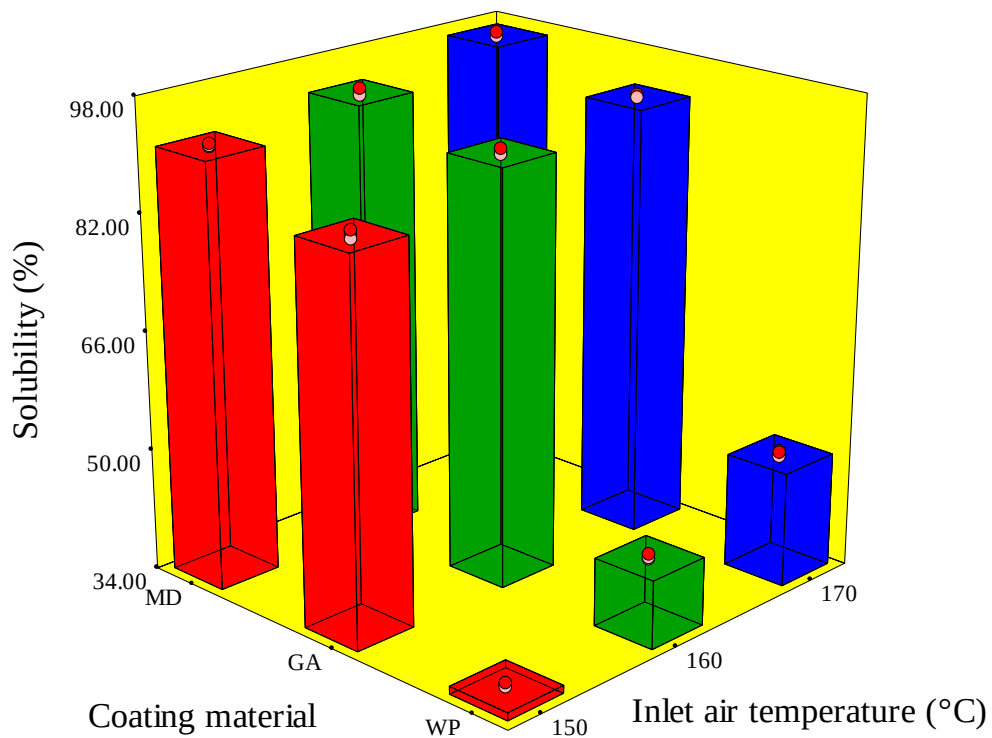


Fig. 61. Effect of different coating materials and inlet air temperatures on solubility of microencapsulated Roselle anthocyanin powder

5.15.2 Effect of different coating materials and inlet air temperatures on wettability of microencapsulated Roselle anthocyanin powder

Wettability can be defined as the ability of a powder bulk to be penetrated by a liquid because of the capillary forces (Ferrari *et al.*, 2012). The variations in wettability of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 62. The treatment M3 recorded 226.00% lower wettability compared to M4. From the figure, it can be noticed that the wettability was found lowest using maltodextrin (MD) as coating material followed by whey protein (WP) and gum arabica (GA). This may be due to higher solubility of MD makes it to wet early, when compared to WP and GA. Maltodextrins of different dextrose equivalent (DE) are commonly used as wall material by its high water solubility, low viscosity, low sugar content and their solutions are colourless. These properties make them useful ingredients in the food industry (Avaltroni *et al.*, 2004; Robert *et al.*, 2010). Similar results were reported by Samborska *et al.* (2015) for spray drying of honey and Kothakota *et al.* (2014) for spray drying of dahi.

From the figure, it can also be observed that increase in the inlet air temperature results in decrease in wettability time of the encapsulated Roselle anthocyanin powder. Vega and Roos (2006) reported that the wettability of the microencapsulated powder depends on powder particle size, density, porosity, surface area and its surface activity. With the increase in inlet air temperature wettability time was found to decrease which could be attributed to the decreased moisture content of the product that the powder absorbs water and wet the surface fast. The similar results were reported by Sariga and Prince (2016) for microencapsulation of vanilla extract. Increase in the inlet temperature decreased the wettability time. Wettability is inversely related to the particle size because larger particles show more spaces between them, being more easily penetrated by water. On the other hand, smaller particles are less porous, making it more difficult for the liquid to penetrate into the food matrix, which results in poor reconstitution properties (Cynthia *et al.*, 2015; Santhalakshmy *et al.*, 2015). It may be due to several reasons such as the decrease of residual moisture content, porous structure and particle size of the powder (Chegini and Ghobadian, 2005).

5.15.3 Effect of different coating materials and inlet air temperatures on dispersibility of microencapsulated Roselle anthocyanin powder

Dispersibility is the ability of a powder to separate into individual particles when dispersed in water with gentle mixing (Nishad *et al.*, 2017). The variations in dispersibility of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 63. The treatment M3 recorded 20.57% higher dispersibility compared to M4. From the figure, it can be noticed that the dispersibility was found highest using maltodextrin (MD) as coating material followed by whey protein (WP) and gum arabica (GA). Maltodextrin is most used in spray drying because of its physical properties, including high solubility in water (Ekpong *et al.*, 2016). Hence, the dispersibility of MD powder is more compared to WP and GA.

From the figure, it can also be observed that increase in the inlet air temperature results in increase in dispersibility of the encapsulated Roselle anthocyanin powder. Sharma *et al.* (2012) reported that the dispersible powders typically exhibit good wettability and are agglomerated, with the absence of fine particles. They are needed to determine how easily a powder goes into solution under normal home-mixing conditions. Kanha and Laokuldilok (2014) reported that the increase in inlet air temperature increases the dispersability of the spray dried black glutinous rice bran. The higher temperature gave a better porous structure after drying, generally as a result of a significantly reduced processing time. This has been shown to improve the reconstitution properties of food products (Franco *et al.*, 2015; Ekpong *et al.*, 2016).

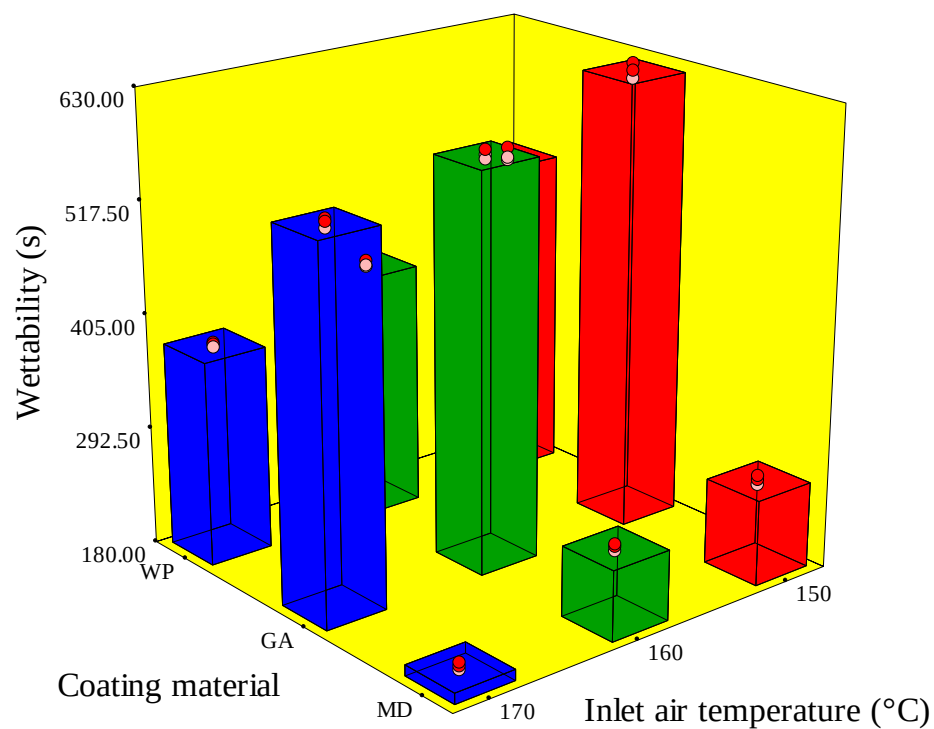


Fig. 62. Effect of different coating materials and inlet air temperatures on wettability of microencapsulated Roselle anthocyanin powder

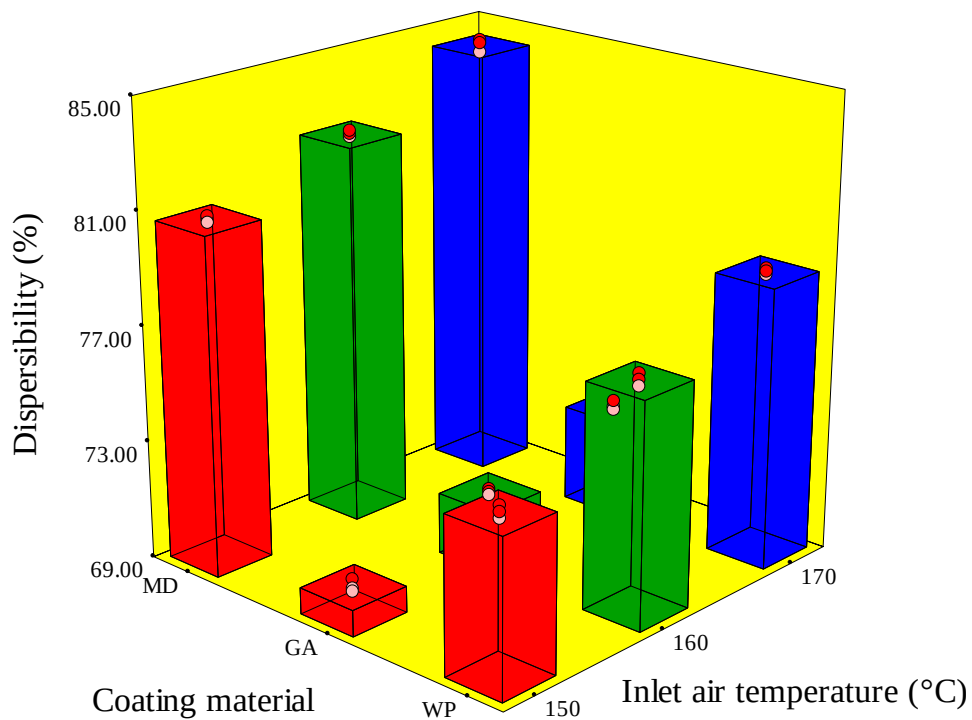


Fig. 63. Effect of different coating materials and inlet air temperatures on dispersibility of microencapsulated Roselle anthocyanin powder

5.16 Functional properties of microencapsulated Roselle anthocyanin powder

The effect of spray drying viz., coating material (MD, GA and WP) and inlet air temperature (150, 160 and 170 °C) on functional properties such as total phenolic content, antioxidant activity, total flavonoids and total anthocyanin content of microencapsulated Roselle anthocyanin powder are discussed hereunder.

5.16.1 Effect of different coating materials and inlet air temperatures on total phenolic content of microencapsulated Roselle anthocyanin powder

The variations in total phenolic content of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 64. The treatment M3 recorded 31.06% higher total phenolic content compared to M7. From the figure, it can be noticed that the total phenolic content was found highest using maltodextrin (MD) as coating material followed by gum arabica (GA) and whey protein (WP). The increased total phenolic content might be due to interference of maltodextrin with the phenolic compounds during analysis. In addition, higher phenolic content in higher concentrations of ascorbic acid could be due to inactivation of polyphenoloxidase (Ahmed *et al.*, 2010). Similar results were reported by Barczak and Kolodziejczyk (2011) for spray drying of black current. Tonon *et al.* (2008) studied the effect of maltodextrin and reported that this drying carrier retained around 80% of the anthocyanidins present in acai juice, following spray drying, which is in agreement with results observed in the present study, in which the maltodextrin retained most of the total phenolic content. Maltodextrin is often used in the spray drying process because of its low cost, high water solubility and low viscosity in solution. Similar results were reported by Fernandes *et al.* (2014) for spray drying of extracts of guava leaves.

From the figure, it can also be observed that increase in the inlet air temperature results in increase in total phenolic content of the encapsulated Roselle anthocyanin powder. Dewanto *et al.* (2002) also found that the free phenolic content of sweet corn increased with increasing heating temperature and time. Chauhan *et al.* (2014) reported that the reason of better retention of total phenol content during spray drying process at high temperature is encapsulation with carrier agent maltodextrin, since long duration of

cooking at high temperatures had been observed as the main causes of loss of nutrients which causes disruption of cell wall and breakdown of biochemical compounds, also the single step processing of spray drying reduces the time of contact of high temperature with sample and reduces losses of essential components.

Susantikarn and Donlao (2016) reported that the highest phenolic content was found in the powder produced from 3% maltodextrin with 190 °C inlet air temperature for spray dried green tea extracts. Mishra *et al.* (2014) reported that total phenolic content of amla juice powders was reduced when inlet temperature was increased from 125 °C to 175 °C temperature, but above 175 °C there was an inverse trend. They reported that above 175 °C there is the probability of polymerization and synthesis of polyphenols at that condition which increases the total phenolic content of the amla juice powder. Bhattacharjee *et al.* (2014) reported that the heating of amla juice at 190 °C for 1 h in spray dryer might have caused either increase in some free phenolic acids (e.g., gallic acid) or polymerization of some polyphenols resulting into the increase in polyphenols content in the spray dried powder.

5.16.2 Effect of different coating materials and inlet air temperatures on antioxidant activity of microencapsulated Roselle anthocyanin powder

The variations in antioxidant activity of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 65. The treatment M3 recorded 25.37% higher antioxidant activity compared to M7. From the figure, it can be noticed that the antioxidant activity was found highest using maltodextrin (MD) as coating material followed by gum arabica (GA) and whey protein (WP). This might be due to higher amount of total phenolic content and total flavonoids content in the encapsulated Roselle powder produced from maltodextrin. Huang *et al.* (2006) and Ahmed *et al.* (2010) found that total antioxidant activity was highly correlated with total phenolic and anthocyanin content. From the figure, it can also be observed that increase in the inlet air temperature results in increase in antioxidant activity of the encapsulated Roselle anthocyanin powder. Some researchers investigated that antioxidant capacity increased in higher drying air temperature due to reactions taking place during heat treatment such as Maillard reaction.

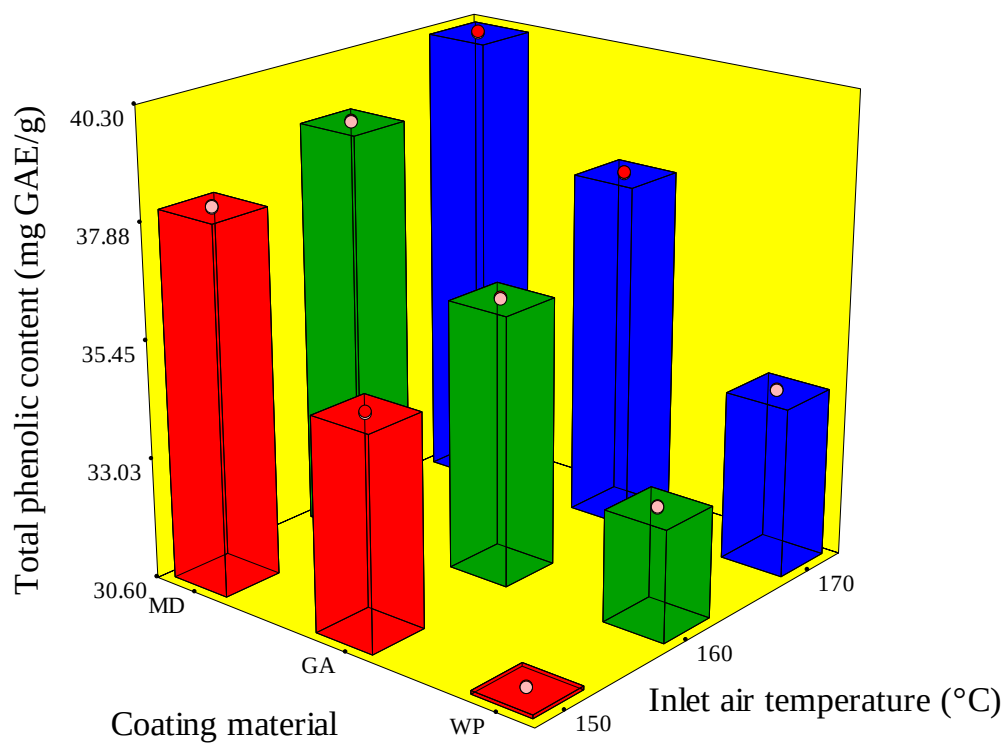


Fig. 64. Effect of different coating materials and inlet air temperatures on total phenolic content of microencapsulated Roselle anthocyanin powder

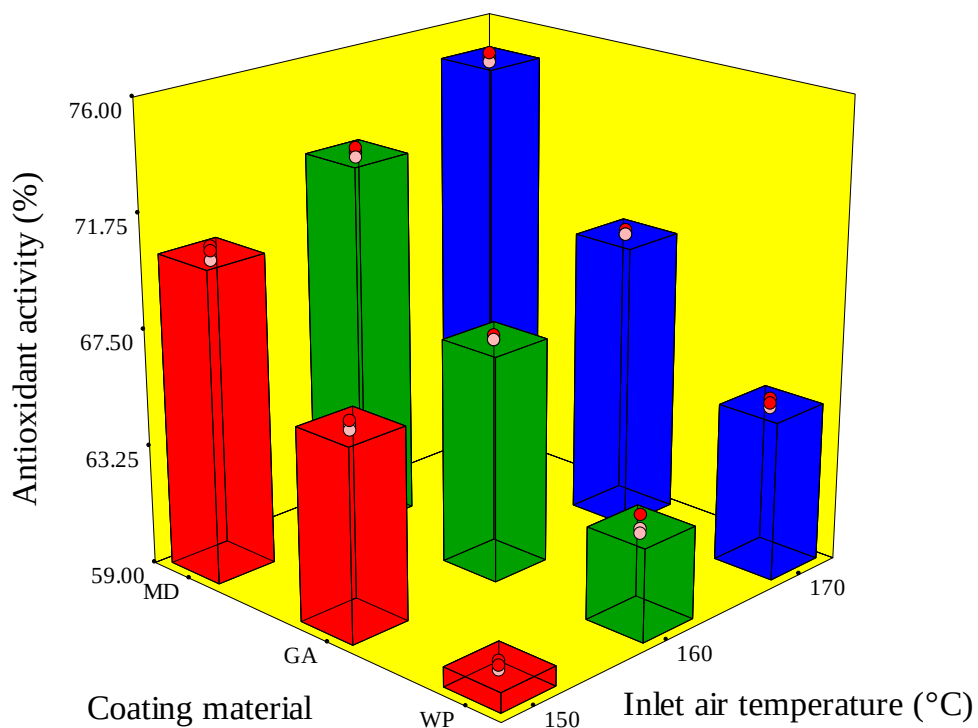


Fig. 65. Effect of different coating materials and inlet air temperatures on antioxidant activity of microencapsulated Roselle anthocyanin powder

It has been said that the products resulted from Maillard reaction have antioxidant activity (Horuz *et al.*, 2012; Djomeh *et al.*, 2016). According to a report by Ersus and Yurdagel (2007), the antioxidant activity of anthocyanin powder from black carrots had a high correlation with their anthocyanin content.

5.16.3 Effect of different coating materials and inlet air temperatures on total flavonoid content of microencapsulated Roselle anthocyanin powder

The variations in total flavonoid content of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 66. The treatment M3 recorded 59.52% higher total flavonoid content compared to M7. From the figure, it can be noticed that the total flavonoid content was found highest using maltodextrin (MD) as coating material followed by gum arabica (GA) and whey protein (WP). Tonon *et al.* (2008) studied the effect of maltodextrin and reported that this drying carrier retained around 80% of the anthocyanidins present in acai juice, following spray drying, which is in agreement with results observed in the present study, in which the maltodextrin retained most of the total flavonoid content. Peng *et al.* (2013), using maltodextrin as a carrier to spray-dry purple sweet potato flours, obtained higher retention rates of anthocyanins, flavonoids and total phenolics than those of flours dried without a carrier or with other carriers. Similar results were reported by Silva *et al.* (2014) for spray drying of jabuticaba.

From the figure, it can also be observed that increase in the inlet air temperature results in increase in total flavonoid content of the encapsulated Roselle anthocyanin powder. This trend may be explained by the occurrence of synthesis and polymerization of phenols and flavonoids which influences the total content of these compounds at higher temperature (Mishra *et al.*, 2014). Similar results were reported by Adame *et al.* (2015) for spray drying of cinnamon infusions.

5.16.4 Effect of different coating materials and inlet air temperatures on total anthocyanin content of microencapsulated Roselle anthocyanin powder

The variations in total anthocyanin content of microencapsulated Roselle anthocyanin powder obtained from spray drying of Roselle anthocyanin extract subjected

to different coating materials (maltodextrin, gum arabica and whey protein) and inlet air temperatures (150, 160 and 170 °C) are depicted in Fig. 67. The treatment M3 recorded 84.93% higher total anthocyanin content compared to M7. From the figure, it can be noticed that the total anthocyanin content was found highest using maltodextrin (MD) as coating material followed by gum arabica (GA) and whey protein (WP). This may be due to the protective effect of MD. Maltodextrin can form a thin layer around the droplets and protect anthocyanins against unfavorable conditions during the formation of the particles in the drying chamber (Yousefi *et al.*, 2011; Ferrari *et al.*, 2012; Djomeh *et al.*, 2016). Tonon *et al.* (2008) studied the effect of maltodextrin and reported that this drying carrier retained around 80% of the anthocyanidins present in acai juice, following spray drying, which is in agreement with results observed in the present study, in which the maltodextrin retained most of the total anthocyanin content.

Maltodextrin with a higher dextrose equivalent could form denser and more oxygen-impermeable wall systems to provide better retention of the anthocyanin pigments (Ferrari *et al.*, 2012). According to Shahidi and Naczsk (2004), phenolics and flavonols may form complexes with polysaccharides and the affinity of phenolics to polysaccharides depends on the water solubility, molecular size, conformational mobility and shape of polyphenol. Furthermore, the complexity formed when the flavylum cation of the anthocyanins interacted with dextrans prevented their transformation to other less stable forms (Chandra *et al.* 1993; Idham *et al.*, 2012). Selim *et al.* (2008) studied the stability of encapsulated Roselle anthocyanin pigment and found maltodextrin gave a significantly better protective effect compared with gum Arabic.

From the figure, it can also be observed that increase in the inlet air temperature results in increase in total anthocyanin content of the encapsulated Roselle anthocyanin powder. Barczak and Kolodzieczyk (2011) reported that the inlet drying temperature below 180 °C did not affect significantly the anthocyanin content in the spray dried black current powders. Additionally, smaller particles tolerate higher temperature due to the faster evaporation rate. This result is in agreement with the findings of Fazaeli *et al.* (2012) for spray dried black mulberry juice and Djomeh *et al.* (2016) for spray dried black barberry juice. Fazaeli *et al.* (2012) reported that the total anthocyanin content of final powder has high correlation with its antioxidant activity and total phenolic content.

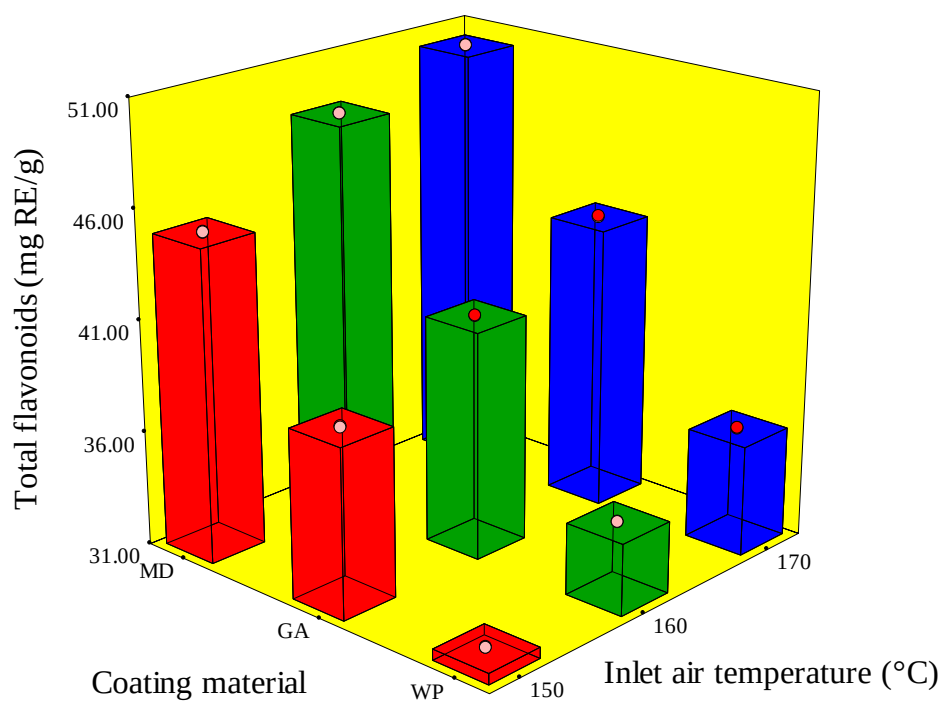


Fig. 66. Effect of different coating materials and inlet air temperatures on total flavonoid content of microencapsulated Roselle anthocyanin powder

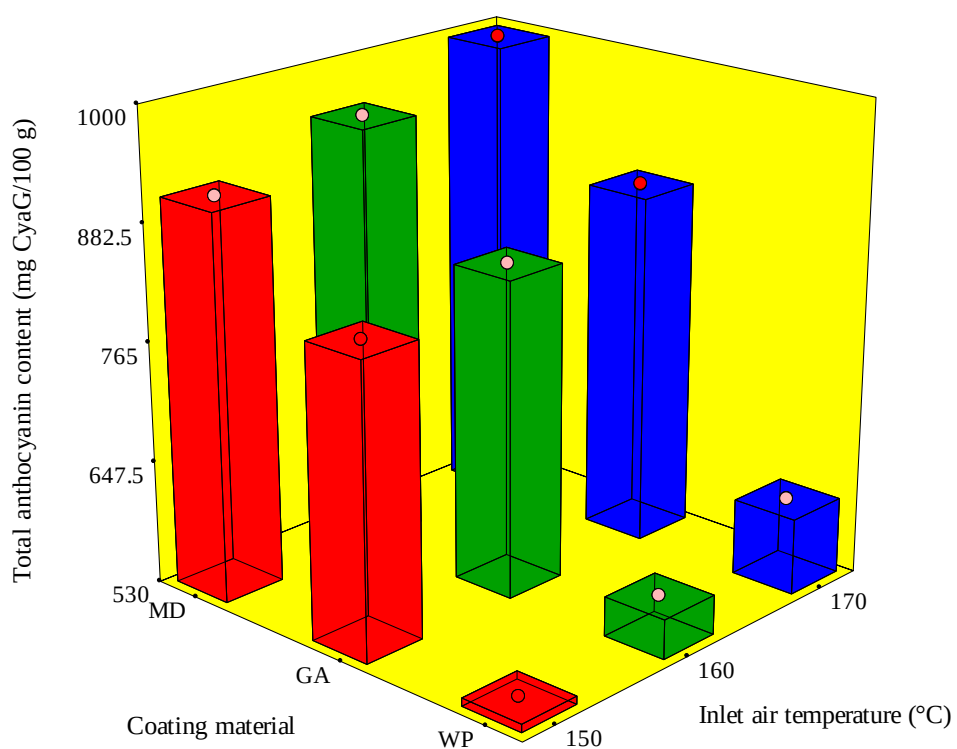


Fig. 67. Effect of different coating materials and inlet air temperatures on total anthocyanin content of microencapsulated Roselle anthocyanin powder

5.17 Optimization of process parameters for microencapsulated Roselle anthocyanin powder

Optimization of two process variables namely, coating material and inlet air temperature combinations was performed using the general factorial design in Design Expert Software 7.7.0. Numerical optimization was performed using statistical models to find the optimal process parameters of microencapsulated Roselle anthocyanin powder. In the present investigation, the independent variables were kept within the range and dependent variables were chosen as maximum and minimum.

The experimental sample had the optimum process conditions using maltodextrin (MD) as coating material and temperature of 170 °C with desirability of 0.97 is depicted in Fig. 68.

5.18 SEM analysis of microencapsulated Roselle anthocyanin powder

The analysis of the surface morphology and characteristics of powder particles obtained with optimized encapsulating agent and temperature was performed using SEM allowing three dimensional characteristics to be visualized. The optimized microencapsulated Roselle anthocyanin powder was obtained with the inlet air temperature of 170 °C using the maltodextrin as coating material is depicted in Fig. 69. It shows that the powder obtained with maltodextrin resulted in the formation of more homogeneous capsules. The poor capsule formation was observed with whey protein at an inlet air temperature of 150 °C is depicted in Fig. 70.

The SEM study revealed that average size of particles in the powder that was dried at higher inlet air temperature was smaller than the particles in powder dried at lower inlet air temperature. Similar findings were also observed by Cai and Corke (2000) for spray drying of *Amaranthus* betacyanin pigments. Probably, the particle size got fixed as large sized globules when there was more water in the material that was being dried. At higher inlet air temperature, due to rapid rate of drying the particles got fixed as smaller sized globules (Poonam *et al.*, 2013). Microparticles showed rough surfaces with cavities and some structural cracks. Morphological irregularities could be due to water evaporation rates during spray drying process. The higher the temperature (faster evaporation) the smoother and more defined the surfaces (Adame *et al.*, 2015).

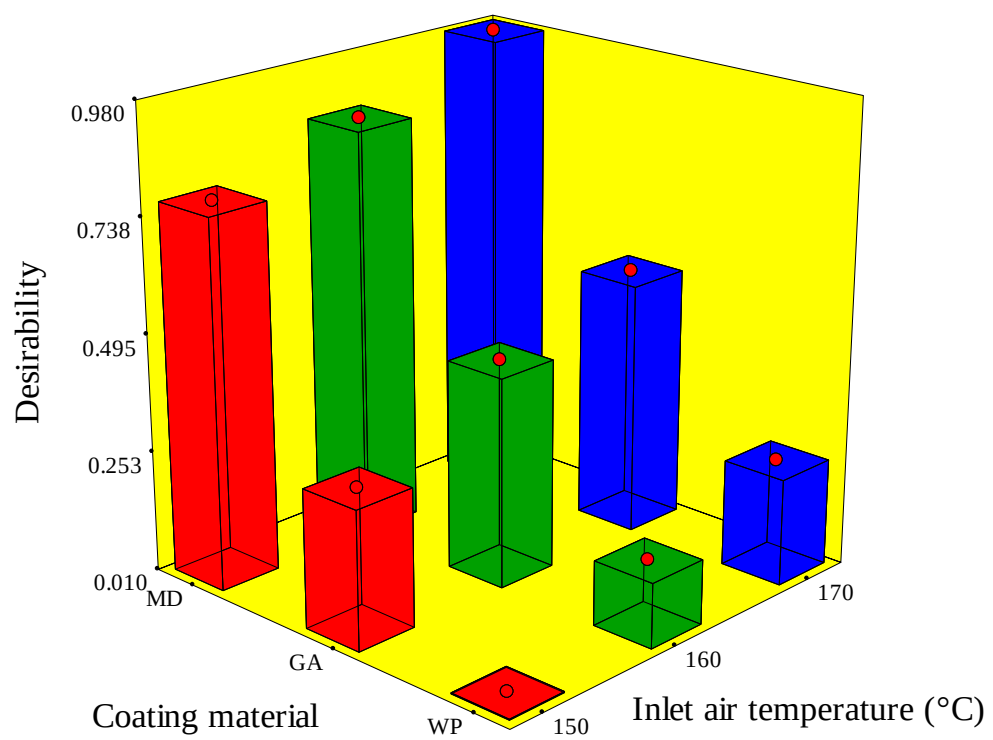


Fig. 68. Desirability graph for optimization of microencapsulation of Roselle anthocyanin powder

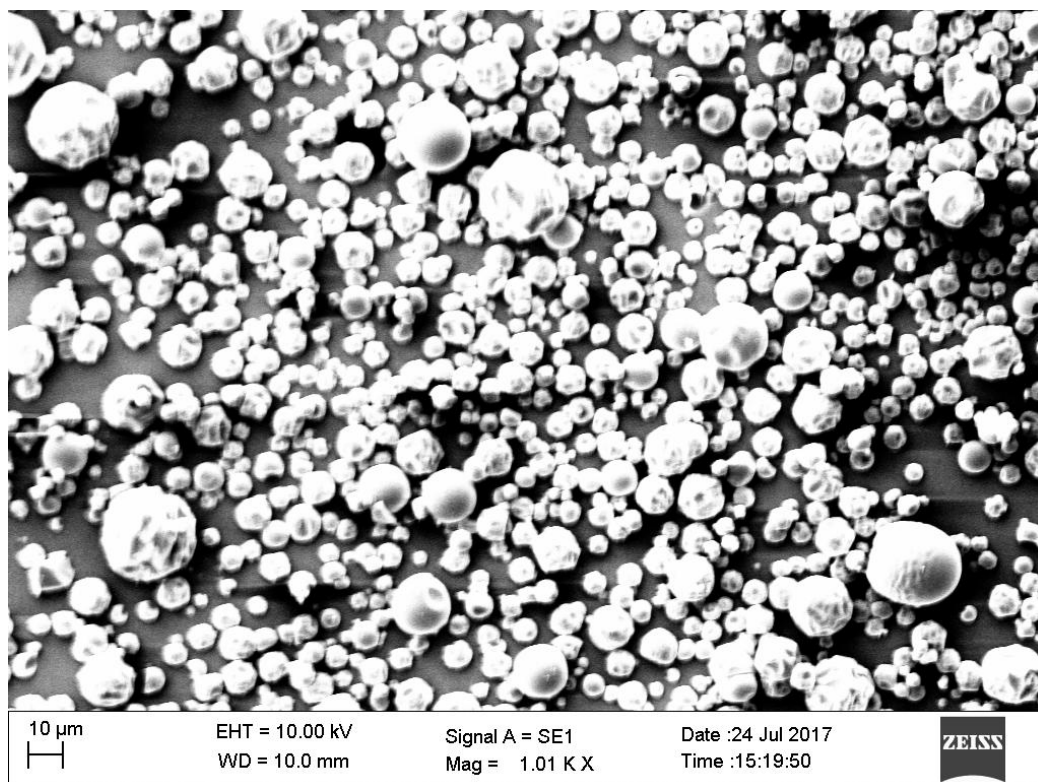


Fig. 69. Photo micrograh of microencapsulated Roselle anthocyanin powder using maltodextrin as coating material at an inlet air temperature of 170 °C.

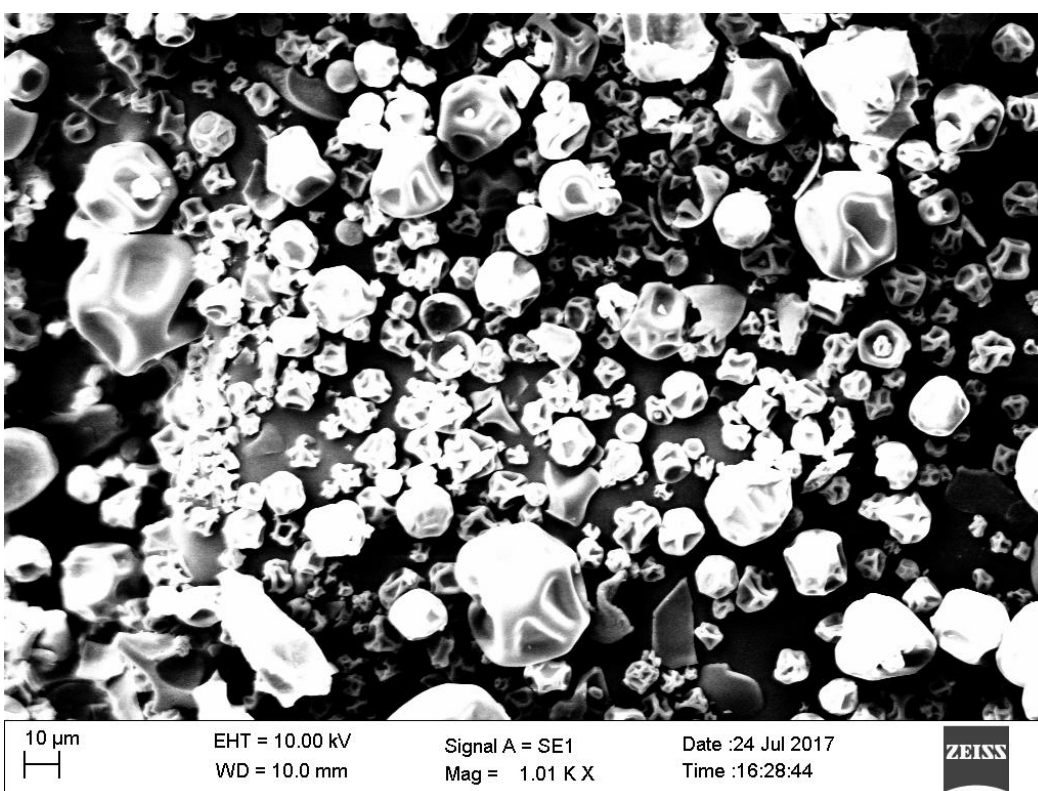


Fig. 70. Photo micrograh of microencapsulated Roselle anthocyanin powder using whey protein as coating material at an inlet air temperature of 150 °C.

This result is similar to that reported by Beltran *et al.* (2005) and Adame *et al.* (2015), who found that lower air inlet temperatures resulted in irregularly shaped microparticles with creased surfaces, while higher air inlet temperatures resulted in more rigid microparticles and porous surfaces. Shrivelling of particles increased from MD to GA to WP which may be correlated with increase in protein fraction of the carrier agents. These findings are in agreement with those of Bhusari *et al.* (2014) who concluded that intended and shrivelling surface morphology is the characteristic of protein rich spray dried powders.

5.19 Stability of microencapsulated Roselle anthocyanin

The influence of key factors, pH and temperature on the stability of microencapsulated Roselle anthocyanin is discussed hereunder.

5.19.1 Effect of pH on stability of microencapsulated Roselle anthocyanin

Effect of pH (2, 3, 4, 5, 6 and 7) on stability of microencapsulated Roselle anthocyanin are presented in Table 83, Chapter IV. The highest retention of Roselle anthocyanin was observed in case of pH 2 followed by pH 3 after 10 days of storage. Similarly, lowest retention was observed in case of pH 6 after 10 days of storage.

Several factors influencing the pigment stability have been investigated such as species, environmental and agronomic conditions; extraction and processing parameters such as pH, storage temperature, concentration, chemical structure, light, oxygen, proteins, ascorbic acid, sugars, sulfites, enzymes and metallic ions (Patras *et al.*, 2010; Cavalcanti *et al.*, 2011; Aishah *et al.*, 2013). Among these factors, pH is one of the major factors significantly influenced the pigment colour variations and stability. In general, anthocyanins are more stable in acidic media at low pH values than in alkaline solutions (Aishah *et al.*, 2013).

Cavalcanti *et al.* (2011) explained the shift of colour of anthocyanins based on their chemical structures. In aqueous solutions, anthocyanins exist basically in the form of four species in equilibrium depending on the pH: quinonoidal base (QB), flavylium cation (FC), carbinol or pseudobase (PB) and chalcone (CH). Under acidic conditions (pH<3.0), the anthocyanins exist primarily in the form of deep-red flavylium cation (FC). Increasing pH values causes rapid loss of the proton producing blue or violet quinonoidal

base forms (QB). At the same time hydration of flavylum cation (FC) occurs, generating the carbinol or pseudobase (PB) which reaches equilibrium slowly with the colourless chalcone (CH). The relative amounts of FC, QB, PB and CH forms at equilibrium condition vary according to pH (Cavalcanti *et al.*, 2011).

Similar findings were reported by Fan *et al.* (2008), where anthocyanins from sweet potato were more stable under acid conditions (pH 2.0 to 4.0) than in the subacid range (pH 5.0 to 6.0). Kirca *et al.* (2007) found that the stability of black carrot anthocyanins decreased significantly at pH above 5.0. Janna *et al.* (2007) reported that at pH 0.5-3.0 anthocyanins of all princess flowers at early development stages were stable, but at higher pH values the colour of the extracts faded. Wang *et al.* (2010) reported that anthocyanins from blueberry were stable at low pH (≤ 5.0).

5.19.2 Effect of temperature on stability of microencapsulated Roselle anthocyanin

Temperature also is another factor, which has a role in destabilising the anthocyanin molecular structure; with increase in temperature, a greater degree in anthocyanin destruction can be observed. Effect of temperature (5, 20, 35, 50, 65 and 80 °C) on stability of microencapsulated Roselle anthocyanin are presented in Table 84, Chapter IV. The highest retention of Roselle anthocyanin was observed in case of 5 °C followed by 20 °C temperature after 2 hours of storage. Similarly, lowest retention was observed in case of 80 °C temperature after 2 hours of storage. Whereas, the Roselle anthocyanin at room temperature after 2 hours of storage was found to be 913.36 mg CyaG.100 g⁻¹. The results obtained from the study of the stability of anthocyanins found in Roselle indicate that during the period of time established, a greater amount of pigment is retained at low temperatures (5 °C) and low pH (2).

Decomposition of anthocyanin frequently observed at higher temperature was probably due to non-enzymatic browning reactions. However, it must be remembered that enzymatic reactions have a role in the browning process. The enzymes that cause oxidative decolourization of anthocyanins are known as anthocyanases. Based on their functions two different groups have been identified in plants namely glycosidases and polyphenol oxidases. Glycosidases hydrolyze glycosidic bonds in anthocyanins and release sugar and glycon, a labile compound that forms colourless derivatives by itself. Polyphenol oxidases can influence anthocyanins in the presence of ortho-di-phenols.

Anthocyanins in turn, may react with each other, with amino acids or proteins and produce brown polymers (Sharifi and Hassani, 2012).

At high temperatures, the equilibrium shifts to chalcones, which means loss of colour. The return of chalcones to flavylum is slow. Anthocyanin extracts showed greater stability at lower temperatures; this was evidenced because there was less discolouration of the extracts (Vargas *et al.*, 2013). Laleh *et al.* (2006) suggested that the speedy destruction of anthocyanin in higher temperatures could be due to hydrolyzation of 3-Glycoside structure, which has a protective effect in unstable anthocyanin. The other suggestion is that the hydrolyzation of the pyrilium ring resulted in production of chalcones, which are responsible for brown colour developed in food containing anthocyanin. It is shown that anthocyanins degrade faster at 80 °C. It is supported by a finding which also said the degradation rate of anthocyanins increases during processing and storage as the temperature rises (Zaidel *et al.*, 2015).

*Summary and Conclusions*_____

VI. SUMMARY AND CONCLUSIONS

The present investigation entitled “Studies on extraction and encapsulation of anthocyanin from calyces of Roselle (*Hibiscus sabdariffa* L.)” was undertaken in the Department of Processing and Food Engineering, College of Agricultural Engineering, UAS, Raichur, Karnataka during 2015-17. The proximate composition, physico-chemical properties and functional properties of fresh calyces of Roselle were studied and summarized hereunder.

The mean values of proximate composition of fresh calyces of Roselle viz., moisture content, crude protein, crude fat, carbohydrates, crude fibre and total ash were found to be 86.08 ± 0.65 (% w.b.), $2.95 \pm 0.14\%$, $0.90 \pm 0.02\%$, $6.21 \pm 0.21\%$, $2.59 \pm 0.27\%$ and $1.09 \pm 0.02\%$, respectively.

Physico-chemical properties of fresh calyces of Roselle viz., pH, water activity, L^* value, a^* value and b^* value were found to be 2.16 ± 0.02 , 0.75 ± 0.03 , 32.44 ± 0.26 , 26.83 ± 0.05 and 9.38 ± 0.18 , respectively. The ascorbic acid was found to be 46.36 ± 0.49 mg.100g⁻¹ and titratable acidity was found to be $20.12 \pm 0.80\%$.

The average values of functional properties of fresh calyces of Roselle viz., total phenolic content was found to be 20.67 ± 0.08 mg GAE.g⁻¹, antioxidant activity was found to be $92.23 \pm 0.77\%$, total flavonoid content was found to be 38.34 ± 0.11 mg RE.g⁻¹ and total anthocyanin content was found to be 718.72 ± 1.19 mg CyaG.100g⁻¹, respectively.

Optimization of drying and milling methods of calyces of Roselle

The drying methods (solar tunnel drying, shade drying and dehumidified air drying) and milling methods (normal pulverizer, water cooled pulverizer and liquid nitrogen cooled pulverizer) had a major role on production of calyces of Roselle powder. The quality of the powder mainly depends on the type of drying, type of milling and temperature at which the operations take place.

The drying time required for solar tunnel drying, shade drying and dehumidified air drying were found to be 19 h, 24 h and 14 h, respectively for moisture content reduction from 618.444% (d.b.) to safe storage moisture content of 10% (d.b.). The drying rate of fresh calyces of Roselle dried in solar tunnel drying ranged from 0.0173 to 0.0001 g.g⁻¹ dm. min⁻¹. In case of shade drying, drying rate ranged from 0.0110 to

0.0001 g.g⁻¹ dm. min⁻¹. The drying rate of fresh calyces of Roselle dried under dehumidified air dryer at 40±1 °C and 15±1% RH ranged from 0.0287 to 0.0008 g.g⁻¹ dm. min⁻¹.

The moisture ratio was determined in order to obtain the best drying model. The different drying models namely, Newton, Page, Modified Page, Henderson-Pabis, Modified Henderson-Pabis and Logarithmic models were selected and fitted to the drying data based on their ability to model the drying phenomenon of fresh calyces of Roselle. Among the different drying models, the Page model was best fitted with the samples dried under dehumidified air dryer with the highest R² value of 0.9983, shade drying with the R² value of 0.9980 and solar tunnel drying with the R² value of 0.9952.

The proximate composition of calyces of Roselle powder viz., moisture content (9.38% d.b.) and carbohydrates (62.01%) were found to be high in solar tunnel drying with normal pulverizer. The maximum of crude protein (9.46%), crude fat (2.98%), crude fibre (11.24%) and total ash (10.57%) were found in calyces of Roselle powder obtained from dehumidified air dryer and LN₂ cooled pulverizer.

In case of physico-chemical properties viz., *a*^{*} value of 8.63, titratable acidity of 21.40%, ascorbic acid content of 40.42 mg.100g⁻¹ and solubility of 0.26 g.g⁻¹ were found to be highest for the calyces of Roselle powder dried under dehumidified air dryer and milling under LN₂ cooled pulverizer. The colour *L*^{*} value (46.50) and *b*^{*} value (11.21) was found to be maximum in solar tunnel drying with normal pulverixer. Similarly with respect to other physico-chemical properties viz., loose bulk density (0.47 g.cc⁻¹), tapped bulk density (0.55 g.cc⁻¹), Carr's index (14.08), Hausner ratio (1.16), pH (2.77), water activity (0.35) and particle size (2519.33 d.nm) were found to be lower in powder produced from dehumidified air drying and LN₂ cooled pulverizer.

With respect to the functional properties viz., total phenolic content (20.66 mg GAE.g⁻¹), antioxidant activity (48.58%), total flavonoids (27.85 mg RE.g⁻¹) and total anthocyanin content (593.86 mg CyaG.100g⁻¹), the dehumidified air drying and LN₂ cooled pulverizer was the best as compared to other methods used for production of calyces of Roselle powder.

The powder obtained from shade drying gave on par value with the dehumidified air drying due to low temperature which retains most of the functional components. The

water cooled pulverizing method also gave better results compared to normal pulverizer milling methods, but it was found to be lesser than LN₂ cooled pulverizer method. Solar tunnel drying and normal pulverizer milling gave comparatively less significant results. These methods adversely affect the quality of calyces of Roselle powder. The results showed that, the temperature during drying and milling plays a major role in the quality of calyces of Roselle powder.

The production cost for 100 kg of dried calyces of Roselle obtained using different drying methods viz., solar tunnel drying, shade drying and dehumidified air drying was computed as ₹ 16,200, 16,500 and 48,300 with benefit cost ratio of 1.51, 1.50 and 1.45, respectively. Similarly, the production cost of 100 kg of calyces of Roselle powder obtained using different milling methods viz., normal pulverizer, water cooled pulverizer and LN₂ cooled pulverizer was computed as ₹ 49,900, 52,600 and 59,400 with benefit cost ratio of 1.50, 1.52 and 1.68, respectively.

The optimization of process parameters was carried out using the general factorial method in Design Expert 7.7.0 software on the basis of goal of response. The optimized drying method was found to be dehumidified air dryer and milling method was found to be LN₂ cooled pulverizer. The desirability at optimum conditions was found to be 0.93.

Optimization solvent extraction of anthocyanin from calyces of Roselle

The optimized calyces of Roselle powder was subjected to solvent extraction of anthocyanin using three different solid to solvent ratio (1:5, 1:10 and 1:15) and % citric acid (1, 2 and 3%) and were studied for physico-chemical and functional properties. The detailed results are summarized hereunder.

The physico-chemical properties of solvent extracted anthocyanin from calyces of Roselle viz., extraction yield (97.33%), TSS (45.60 °Brix) and colour a^* value (0.75) were found to be higher in Roselle anthocyanin extracted using 1:10 solid to solvent ratio and 2% citric acid. Titratable acidity (23.94%) was found to be more in Roselle anthocyanin extracted using 1:10 solid to solvent ratio and 3% citric acid. Similarly, pH (1.19) was found to be less in Roselle anthocyanin extracted using 1:10 solid to solvent ratio and 3% citric acid. Higher L^* value (3.24) was found in Roselle anthocyanin extracted using 1:5 solid to solvent ratio and 1% citric acid and higher b^* value (0.22) was found in Roselle anthocyanin extracted using 1:15 solid to solvent ratio and 1% citric acid.

The functional properties viz., total phenolic content (43.19 mg GAE.g⁻¹), antioxidant activity (87.29%), total flavonoids (54.28 mg RE.g⁻¹) and total anthocyanin content (1072.27 mg CyaG.100g⁻¹) were found to be higher in Roselle anthocyanin extracted using 1:10 solid to solvent ratio and 3% citric acid as compared to other combinations used for solvent extraction of anthocyanin from calyces of Roselle.

As anthocyanins are soluble in polar solvents, extraction of anthocyanins from plant materials is commonly done using ethanol with an addition of small amounts of organic acid. The acid lowers the pH of solution and prevents the degradation of the non-acylated anthocyanin pigments. Hence the solvent extraction of anthocyanin from calyces of Roselle resulted in good functional properties and could be adopted for commercial production of Roselle anthocyanin extract.

The production cost for 100 kg of solvent extracted anthocyanin from calyces of Roselle using ethanol and citric acid (1:10 solid to solvent ratio and 2% citric acid) was found to be ₹ 1,83,600 of extract with benefit cost ratio of 2.72.

The optimization of process parameters was carried out using the general factorial method in Design Expert 7.7.0 software on the basis of goal of response. The optimized solid to solvent ratio was found to be 1:10 and concentration of citric acid (%) was found to be 2%. The desirability at optimum conditions was found to be 0.92.

Optimization of microencapsulation of Roselle anthocyanin

The optimized solvent extracted anthocyanin from calyces of Roselle was subjected to microencapsulation to produce microencapsulated Roselle anthocyanin powder. Spray drying was used for microencapsulation of Roselle anthocyanin and the effect of different process conditions viz., coating material (maltodextrin, gum arabica and whey protein) and inlet air temperature (150, 160 and 170 °C) on physico-chemical and functional properties of microencapsulated Roselle anthocyanin powder were evaluated and summarized hereunder.

The physico-chemical properties of microencapsulated Roselle anthocyanin powder viz., powder yield (92.04%) and titratable acidity (17.41%) were found to be higher in maltodextrin as a coating material and at 170 °C inlet air temperature. Similarly, moisture content (2.16%), water activity (0.21), pH (2.29), loose bulk density

(0.23 g.cc⁻¹), tapped bulk density (0.28 g.cc⁻¹), Carr's index (16.03), Hausner ratio (1.19) and particle size (354.70 d.nm) were found to be minimum in maltodextrin as a coating material and at 170 °C inlet air temperature.

The colour L^* (70.43) and b^* value (8.85) was found to be higher in case of microencapsulated Roselle anthocyanin powder obtained using whey protein as a coating material and at 150 °C inlet air temperature. The colour a^* value (28.87) was found to be higher in maltodextrin as a coating material and at 170 °C inlet air temperature.

The reconstitution properties of the microencapsulated Roselle anthocyanin powder namely, solubility (96.75%), wettability (190.80 s) and dispersability (84.30%) were found to be best using maltodextrin as a coating material and at 170 °C inlet air temperature.

The functional properties viz., total phenolic content (40.21 mg GAE.g⁻¹), antioxidant activity (74.87%), total flavonoids (50.25 mg RE.g⁻¹) and total anthocyanin content (994.99 mg CyaG.100g⁻¹) were found to be higher in microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material and at 170 °C inlet air temperature as compared to other combinations of coating material and inlet air temperature.

The production cost for 100 kg of microencapsulated Roselle anthocyanin powder using maltodextrin as a coating material and at inlet air temperature 170 °C was found to be ₹ 1,84,200 kg⁻¹ with benefit cost ratio of 3.53.

The optimization of process parameters was carried out using the general factorial method in Design Expert 7.7.0 software on the basis of goal of response. The optimized condition was obtained using maltodextrin as coating material and at an inlet air temperature of 170 °C. The desirability value at optimum conditions was found to be 0.97.

Stability studies of microencapsulated Roselle anthocyanin

The influence of key factors, pH and temperature on the stability of microencapsulated Roselle anthocyanin were studied and the results are summarized hereunder.

Effect of pH (2, 3, 4, 5, 6 and 7) on stability of microencapsulated Roselle anthocyanin represents the highest retention of Roselle anthocyanin was observed in case of pH 2 (968.69 mg CyaG.100 g⁻¹) after 10 days of storage. Similarly, lowest retention was observed in case of pH 6 (364.46 mg CyaG.100 g⁻¹) after 10 days of storage.

Effect of temperature (5, 20, 35, 50, 65 and 80 °C) on stability of microencapsulated Roselle anthocyanin represents the highest retention of Roselle anthocyanin was observed in case of 5 °C (992.96 mg CyaG.100 g⁻¹) temperature after 2 hours of storage. Similarly, lowest retention was observed in case of 80 °C (199.46 mg CyaG.100 g⁻¹) temperature after 2 hours of storage.

The results obtained from the stability study of microencapsulated Roselle anthocyanin powder indicates that during the period of time established, a greater amount of pigment is retained at low temperatures (5 °C) and low pH (2).

Important conclusions drawn from the present investigation are given below:

- ❖ The calyces of Roselle dried under dehumidified air drying took less time (14 h) as compared to solar tunnel drying (19 h) and shade drying (24 h).
- ❖ The proximate composition (moisture content of 8.98%, crude protein of 9.46%, crude fat of 2.98%, crude fibre of 11.24%, carbohydrates of 56.52% and total ash of 10.57%) and ascorbic acid (40.42 mg.100g⁻¹) of calyces of Roselle powder retained well using dehumidified air dryer and LN₂ cooled pulverizer.
- ❖ The functional properties viz., total phenolic content (20.66 mg GAE.g⁻¹), antioxidant activity (48.58%), total flavonoids (27.85 mg RE.g⁻¹) and total anthocyanin content (593.86 mg CyaG.100g⁻¹) retained well in dehumidified air drying and LN₂ cooled pulverizer.
- ❖ The optimized drying and milling method for calyces of Roselle powder was found to be dehumidified air dryer and LN₂ cooled pulverizer with desirability of 0.93.
- ❖ The highest net returns was obtained for dried calyces of Roselle produced using dehumidified air dryer and calyces of Roselle powder for LN₂ cooled pulverizer.

- ❖ The maximum extraction yield (97.33%) and a^* value (0.75) was obtained using 1:10 solid to solvent ratio and 2% citric acid.
- ❖ The highest total phenolic content (43.19 mg GAE.g⁻¹), antioxidant activity (87.29%), total flavonoids (54.28 mg RE.g⁻¹) and total anthocyanin content (1072.27 mg CyaG.100g⁻¹) of Roselle anthocyanin extract were found in 1:10 solid to solvent ratio and 2% citric acid.
- ❖ The optimized condition for solvent extracted Roselle anthocyanin was found to be 1:10 solid to solvent ratio and 2% citric acid with desirability of 0.92.
- ❖ The benefit cost ratio of the solvent extracted Roselle anthocyanin obtained using 1:10 solid to solvent ratio and 2% citric acid was found to be 2.72.
- ❖ The highest powder yield (92.04%) and a^* value (28.87) of microencapsulated Roselle anthocyanin powder was obtained using maltodextrin as a coating material and at 170 °C inlet air temperature.
- ❖ The best handling *i.e.*, flowability and reconstitution properties (Carr's index (16.03), Hausner ratio (1.19), solubility (96.75%), wettability (190.80 s) and dispersability (84.30%)) of the microencapsulated Roselle anthocyanin powder can be obtained using maltodextrin as a coating material and at 170 °C inlet air temperature.
- ❖ The functional properties *viz.*, total phenolic content (40.21 mg GAE.g⁻¹), antioxidant activity (74.87%), total flavonoids (50.25 mg RE.g⁻¹) and total anthocyanin content (994.99 mg CyaG.100g⁻¹) retained more in microencapsulated Roselle anthocyanin powder obtained using maltodextrin as a coating material and at 170 °C inlet air temperature.
- ❖ The optimized coating material and inlet air temperature for microencapsulation of Roselle anthocyanin was found to be maltodextrin and at 170 °C inlet air temperature, respectively.
- ❖ The benefit cost ratio of the microencapsulated Roselle anthocyanin powder was found to be 3.53.
- ❖ Greater amount of pigment is retained at 5 °C temperature and 2.0 pH.

FUTURE LINE OF WORK

On the basis of the investigation, the following suggestions were made for future study:

1. Application of calyces of Roselle powder and microencapsulated Roselle anthocyanin powder can be carried out for different food items for its colour stability study.
2. Nanoencapsulation of Roselle anthocyanin can be carried out using different process parameters such as coating materials, inlet and outlet temperatures, feed flow rate and atomizer speed.
3. Effect of light, oxygen, proteins, ascorbic acid, sugars, sulfites, enzymes and metallic ions on stability of Roselle anthocyanin pigment can be studied.

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*Appendices*_____

APPENDIX I

General specifications of various instruments/equipments used in the present investigation

A1. Specifications of dehumidified air dryer

Sl. No.	Parameter	Specification
1	Model	FSD-600
2	Bed drive motor	0.34 hp
3	Tray material	Aluminium
4	Desiccant Rotor	1 No.
5	Process fans motor	1.5 hp
6	Reactor fan motor	0.5 hp
7	Power requirement	3 phase, 415 V, 50 Hz
8	Make	Bry-Air

A2. Technical specifications of low temperature grinder with LN₂

I Specifications of low temperature grinder

Sl. No.	Particulars	Specification
1	Make	Crompton Gruve Ltd.
2	Model	NDA 2 TOP
3	Power supply	230 V -50 Hz
4	RPM	2820
5	Chamber size (D×W)	27×15 cm
6	Capacity	10 kg/h
7	Motor	1.0 hp

II Specifications of LN₂ container (Dewar)

Sl. No.	Particulars	Specifications
1	Make	Chengdu golden phoenix LN2 container Co. Ltd.
2	Model	YDS-10
3	Geometric volume (m ³)	10
4	Caliber (mm)	50
5	Outside diameter (mm)	303
6	Height (mm)	530
7	Liquid level (cm)	27.5

A3. Technical specifications of Kjelttec distillation unit

I. Performance data

Sl. No.	Particulars	Specification
1.	Distilling time	4-5 min
2.	Distillation capacity	10-12 samples per hour
3.	Measuring range	1-160 mg
4.	Reproducibility	±1% relative
5.	Recovery	99.5-100.5%

II. General Specifications

Sl. No.	Particulars	Specification
1.	Power supply	200-240 V 50-60 Hz
2.	Power consumption	1900 W
3.	Water supply	1 lit/min tap water at 20 °C
4.	Dimensions L × D × H	30 × 28 × 80 cm
5.	Weight	25 kg
6.	Water pressure (max)	5 bar

III. Technical specifications

Sl. No.	Particulars	Specification
1.	Altitude	2000 m
2.	Temperature	5 °C to 40 °C
3.	Maximum relative humidity	80% for temperatures up to 31 °C decreasing linearly to 50% relative humidity at 40 °C
4.	Mains supply voltage	Fluctuations not exceeding ±10% of the rated voltage

A4. Technical specifications of soxhlet apparatus

Sl. No.	Particulars	Specification
1.	Dimension (W×L)	47.25 × 53.5 cm
2.	Power	220 V
3.	Watt	360
4.	Capacity	3 × 100 ml
5.	Shipping weight	18.8kg

A5. Technical specifications of muffle furnace

Sl. No.	Parameter	Specification
1.	Capacity	323 cu in
2.	Temperature control accuracy	± 1 °C
3.	Control type	Basic
4.	Rise time	1100°C 25 minutes
5.	Chamber size	6"W $\times$ 6" H $\times$ 9" D
6.	Watts	1800
7.	Power	Hz 50/60 Hz
		V AC 120 V AC
8.	Dimensions	17"W $\times$ 21" H $\times$ 21"D
9.	Uniformity	± 5 °C
10.	Temperature range	100 1100 °C

A6. Technical specifications of Hunter lab colourimeter

Sl. No.	Particulars	Specification
1.	Port size	31.8 mm (45°/0°) 14.3 mm (diffuse/8°)
2.	Reproducibility	0.15 (Avg. DE* on BCRA II tile set)
3.	Spectral range	400–700 nm
4.	Spectral resolution	10 nm
5.	Spectral band pass	< 12 nm
6.	Photometric	0–150%
7.	Lamp life	> 500,000 flashes
8.	Display (cm)	6.4 $\times$ 6.4 LCD
9.	Colour spaces	CIE XYZ CIE L^* a^* b^* , CIE L^* , c^* , h^* Hunter L , a , b ΔE^* , ΔE , ΔE_{cmc}
10.	Indices	Whiteness, Yellowness, Meta merism, Tint, Brightness, ISO Greyscale, Gray Stain
11.	Illuminants	A, C, D65, TL84, F2 and 10 Degree observer
12.	Approximate size	36 cm (depth) $\times$ 13 cm (width) $\times$ 16 cm (height)
13.	Weight	4.5 Kg

A7. Technical specifications of rotronic water activity meter

Sl. No.	Particulars	Operating ranges
1.	Temperature measurement	5 to 50 °C
2.	Humidity measurement	0 to 100% RH
Reproducibility		
3.	Humidity measurement	<0.5% RH
4.	Temperature measurement	<0.2 °C
5.	Long term stability	<1% RH/ year
6.	Supply voltage	6 to 15 DC
7.	Current consumption	12 mA
Output signals		
8.	Digital	DIO
9.	Analogue	ROV: 0...1V=0..100% RH, 5...50 °C
10.	Load per analogue output	>1000 Ohm
Adjustment points		
11.	Humidity	35%, 80%, 10%, 5%, or 0% RH
12.	Temperature	Tmin, Tmax

A8. Technical specification of zetasizer nano series

Sl . No.	Size measurement	
1	Maximum particle size range (diameter):	0.3nm - 10µm** 0.3nm - 5µm††
2	Minimum sample volume:	12µL 20µL
3	Minimum sample concentration:	0.1mg/mL Lysozyme 0.1ppm polystyrene latex (60nm)
4	Maximum sample concentration:	40%w/v*
5	Size measurement in continuous flow for use as SEC chromatography detector :	Flow rate maximum 1mL/min
Zeta potential measurement		
1	Particle size range (diameter):	3.8nm - 100µm†
2	Zeta potential range:	No practical limits exist
3	Electrophoretic mobility range:	Minimum Zero No practical upper limit exists
4	Conductivity range:	0 to 200mS/cm
5	Sample concentration range:	Up to 40%w/v†

6	Minimum sample volume:	750µL (Folded capillary cell) 750µL (Dip cell) 150µL (High concentration cell)
Molecular weight measurement		
1	Absolute molecular weight range using Debye plot:	980Da - 2x10 ⁷ Da† 9,800Da - 2x10 ⁷ Da†
2	Molecular weight range estimated from Hydrodynamic diameter:	342Da - 2x10 ⁷ Da†
3	Minimum sample volume:	12µL 20µL
4	Minimum volume for automated measurement:	4mL
* Peak mode range (diameter), 0.6nm - 8.9 microns, sample dependent		
Note	** Temperature accuracy, 0.1°C at 25°C, 0.2°C at 0°C and 0.5°C at 90°C, 1°C at 120°C	
	† Sample dependent	

A9. Technical specifications of spectrophotometer

Sl. No.	Parameter	Specifications
1	Measurement modes	% REL and % TRN
2	Measurement principle	DUAL BEAM
3	Sphere dimension	152 mm diameter
4	Light source	Pulsed xenon
5	Wavelength range	360 nm to 740 nm
6	Wavelength interval	10 nm
7	Photometric range	0-200%
8	Optical configuration	D/8 or D/0 viewing geometry
9	Wavelength accuracy	0.1 nm (Average)
10	Repeatability	Less than 0.1 DE CIELAB (white tile)
11	Inter instrument agreement (LAV)	0.15DE (Maximum)
12	Measurement time	2 s
13	Large area view (LAV)	25.4 mm diameter
14	Medium area view (MAV)	5.0 mm×10.0 mm
15	Small area view (SAV)	12.0 mm diameter
16	Very small area view (VSAV)	3 mm diameter
17	UV filter	IN/OUT
18	Visible cut-off filter	420 nm/465 nm/560 nm
19	Specular component	Included/excluded

20	Operating temperature	150-320 °C
21	Relative humidity	<90% non-condensing
22	Electrical requirements	110-120V AC/50-60 Hz 230-240V AC/50-60 Hz
23	Weight	21 kg approx.
24	Size	54 m×29 cm×23 cm

A10. Technical specifications of rotary vacuum flash evaporator

Sl. No.	Particulars	Specifications
1.	Heating bath dimensions	Ø 230×130 mm
2.	Volume of heating bath	4 l Approx.
3.	Bath heater capacity	1.2 kW
3.	Heating bath controller	PID with display for set and actual temperature
4.	Bath temperature range	Ambient to 120 °C
5.	Temperature accuracy	± 2 °C
6.	Heating bath material	SS 304
7.	Flask size	1000 ml
8.	Environmental conditions (Temperature)	10 to 40 °C
8.	Power supply	230 V, AC, 50 Hz
9.	Rotational speed	0 to 90 rpm
10.	Lift	Manual
11.	Height adjustment	240 mm Approx.
11.	Flask ejector	Yes
12.	Condenser support	Yes
13.	Threaded connectors for utilities	Yes
14.	Feed stopcock and tube made of	PTFE

A11. Technical specifications of SMST pilot scale spray dryer

Sl. No.	Features	Specifications
1	Make	SM SCIENTECH
2	Suitability	Aqueous/Aqueous + Solvents/ Solvent feeds
3	Evaporation rate	Up to 1000 ml of water evaporation/ h
4	Drying temperature	Ambient to 350 ±1 °C
5	Diameter of inside drying chamber	0.367 m

6	Length of the drying chamber	0.91 m
7	Air heater capacity	2 kW
8	Aspirator (Blower capacity)	0-4000 rpm
9	Blower motor	180 W×12 V DC
10	Feed pump capacity	0-30 rpm with 3 mm ID tube– controlled through microprocessor
11	Feed pump	80 W×12 V DC
12	Nozzle type	Two fluid spray nozzle; Co-current
13	Nozzle diameter	0.84 mm
14	Power supply	3- phase, 415 V, AC
15	Power consumption	6 kW
16	Compressed air for 2 fluid nozzle	2-4 bar

A12. Technical specifications of scanning electron microscopy

Sl . No.	Technology Parameter	
1	Resolution:	1.9 nm, 2 nm, 3 nm @ 30 kV SE with HD, LaB6, W
2	Acceleration Voltage:	0.2 to 30 kV
3	Probe Current	0.5 pA to 5 µA
4	Model No:	ZEISS EVO LS10
5	Magnification:	< 7 – 1,000,000x
6	X-ray Analysis:	8.5 mm AWD and 35° take-off angle
7	Field of View	6 mm at Analytical Working Distance (AWD)
8	Pressure Range	10 – 3000 Pa (LS configuration)
9	10 – 3000 Pa (LS configuration)	35-175/5-80
10	Time Range (min)	5-60

APPENDIX-II

Economics of dried, milled, extracted and encapsulated calyces of Roselle

I. Economics for dried Roselle calyces

Assumptions:

$$1. \text{ Depreciation} = \frac{C - S}{L \times H}$$

where,

C= Capital investment of machine

S= Junk value or salvage value (10% initial investment of machine)

L= Life span of machine (10 years)

$$2. \text{ Interest} = \frac{C + S}{2} \times \frac{i}{H}$$

where,

i = Rate of interest at 11.5% of initial investment of machine

3. Repair at 10% of initial investment of machine

4. Housing at 1% of initial investment of machine

5. Labour charge per day per person = ₹ 300/day

6. Prevailing cost of electricity ₹ 8/kWh

7. Number of working days per year = 275 days per annum

8. Recovery = 17% of fresh Roselle

A. Fixed cost of dehumidified air dryer

$$\text{I. Depreciation} = \frac{C - S}{L \times H}$$

$$\begin{aligned} \text{Depreciation} &= \left(\frac{C - S}{L \times H} \right) = \frac{(4,00,000) - \left(\frac{4,00,000}{10} \right)}{10 \times 275 \times 8} \\ &= ₹ 16.36/h \end{aligned}$$

$$\text{II. Interest} = \frac{C + S}{2} \times \frac{i}{H}$$

$$\text{Interest} = \frac{(4,00,000) + \left(\frac{4,00,000}{10} \right)}{2 \times 275 \times 8} \times \left(\frac{11.5}{100} \right) = 11.50/h$$

Total fixed cost of Dehumidified air dryer (₹) = I + II

$$= 16.36 + 11.50 = ₹ 27.86/h$$

B. variable cost of dehumidified air dryer

I. Repair

$$\begin{aligned} 10\% \text{ of initial investment of machine (₹)} &= \left(\frac{4,00,000}{275 \times 8} \right) \times \left(\frac{10}{100} \right) \\ &= ₹ 18.18/\text{h} \end{aligned}$$

II. Housing at 1% of initial investment of dehumidified air dryer (₹) =

$$\begin{aligned} &= \left(\frac{4,00,000}{275 \times 8} \right) \times \left(\frac{1}{100} \right) \\ &= ₹ 1.81/\text{h} \end{aligned}$$

III. Electricity consumed by the dehumidified air dryer = 10.10 kWh/h

Prevailing cost of electricity ₹ 8/kWh

$$\begin{aligned} \text{Hence the total electricity charges per hour} &= 10.10 \times 8 \\ &= ₹ 80.80/\text{h} \end{aligned}$$

IV. Labour charges = ₹ 300 for 8 h

No. of labours required = 2 No.

$$\begin{aligned} &= \frac{600}{8} \\ &= ₹ 75/\text{h} \end{aligned}$$

$$\begin{aligned} \text{Total variable cost of dehumidified air dryer (₹)} &= 18.18 + 1.81 + 80.80 + 75 \\ &= ₹ 176/\text{h} \end{aligned}$$

Total operating cost of dehumidified air dryer (₹) = A + B

$$= 27.86 + 176 = ₹ 204/\text{h} \quad \dots (1)$$

C. Fixed cost of solar tunnel dryer

$$\begin{aligned} \text{I. Depreciation} &= \left(\frac{C - S}{L \times H} \right) = \frac{(1,50,000) - \left(\frac{1,50,000}{10} \right)}{10 \times 275 \times 8} \\ &= 6.13/\text{day} \end{aligned}$$

$$\begin{aligned} \text{II. Interest} &= \frac{C + S}{2} \times \frac{i}{H} \\ &= \frac{(1,50,000) + \left(\frac{1,50,000}{10} \right)}{2 \times 275 \times 8} \times \left(\frac{11.5}{100} \right) \\ &= 4.31/\text{day} \end{aligned}$$

Total fixed cost of solar tunnel dryer (₹) = I + II

$$= 6.13 + 4.31 = 10.44/\text{day}$$

$$= 25.00/\text{batch}$$

D. Variable cost of solar tunnel dryer

I. Repair

$$\begin{aligned} \text{5\% of initial investment of machine (₹)} &= \left(\frac{1,50,000}{275} \right) \times \left(\frac{5}{100} \right) \\ &= 27/\text{batch} \end{aligned}$$

II. Housing at 1% of initial investment of STD (₹) =

$$\begin{aligned} &= \left(\frac{1,50,000}{275} \right) \times \left(\frac{1}{100} \right) \\ &= 5.45/\text{batch} \end{aligned}$$

III. Labour charges = ₹ 300 for 8 h

$$\begin{aligned} \text{No. of labours required} &= 2 \text{ No.} \\ &= 1425/\text{batch} \end{aligned}$$

IV. Electricity consumed by the solar tunnel dryer = 0.4 kWh/h

Prevailing cost of electricity ₹ 8/kWh

$$\begin{aligned} \text{Hence the total electricity charges per day (₹)} &= 0.4 \times 8 \times 8 \\ &= ₹ 25.60/\text{day} \\ &= ₹ 61/\text{batch} \end{aligned}$$

$$\begin{aligned} \text{Total variable cost of solar tunnel dryer (₹)} &= \text{I} + \text{II} + \text{III} + \text{IV} \\ &= 27 + 5.45 + 1425 + 61 \\ &= ₹ 1518/\text{batch} \end{aligned}$$

$$\begin{aligned} \text{Total operating cost of solar tunnel dryer (₹)} &= \text{C} + \text{D} \\ &= 25 + 1518 = ₹ 1,543/\text{batch} \quad \dots (2) \end{aligned}$$

Considering 12 kg per tray in solar tunnel dryer, there are 50 trays in the STD.

$$\text{Total raw material hold in one batch} = 12 \times 50 = 600 \text{ kg}$$

$$\text{For 7,200 kg raw material, } = \frac{7,200}{600} = 12 \text{ batches need to be carried out}$$

$$\begin{aligned} \text{Therefore, cost incurred for drying Roselle for 12 batches} &= 12 \times 1543 \\ &= ₹ 18,516/12 \text{ batches} \end{aligned}$$

1. Estimation of cost of production of dried Roselle from 7200 kg of fresh Roselle from dehumidified air dryer

I. Total operating cost of Dehumidified air dryer

From equation (1)

Operating cost of Dehumidified air dryer (₹) = ₹ 204.00/h

In DD the time required to dry the sample in 14 h

Hence, the total operating cost of DD (₹) = 204×14

$$= ₹ 2,856.00/\text{batch}$$

For drying 7200 kg of Roselle, 144 batches are to be carried out

$$= 2856 \times 144 = ₹ 4,11,264$$

II. Raw material cost

Consider price of 1 kg of fresh Roselle (₹) = 25/-

Total cost of raw material for 7200 kg of fresh Roselle (₹) = 7200×25

$$= ₹ 1,80,000$$

Total cost of Roselle dried (7200 kg batch) (₹) = I + II

$$= 4,11,264 + 1,80,000$$

$$= ₹ 5,91,264/-$$

Considering average recovery of dried Roselle = 17.00% of fresh Roselle

From 7200 kg (₹) = $7200 \times 0.17 = 1,224.00$ kg of dried Roselle

Hence, total cost of 1 kg of dried Roselle (₹)

$$= \frac{5,91,264}{1,224}$$

$$= ₹ 483/\text{kg of dried Roselle}$$

Dehumidified dried Roselle had premium quality hence, premium selling price of ₹ 700/kg was considered for computation.

Selling price of dehumidified air dried Roselle (₹) = ₹ 700/kg

Now, Gross return = $700 \times 1224 = ₹ 8,56,800/-$

For production of 100 kg of dried Roselle:

Production cost = $483 \times 100 = 48,300$

Gross return = $700 \times 100 = 70,000$

Therefore, Net returns = $70,000 - 48,300 = 21,700$

Hence, Benefit cost ratio = $\frac{\text{Gross returns}}{\text{Production cost}} = \frac{70,000}{48,300}$

B:C ratio = 1.45

2. Estimation of cost of production of dried Roselle from 7200 kg of fresh Roselle using solar tunnel drying

I. Total operating cost of solar tunnel dryer

From equation (2)

Operating cost of solar tunnel dryer (₹) = ₹ 122/h

In STD the time required to dry the sample in 2 days

Hence, the total operating cost of STD (₹) = ₹ 1543/7200 kg

II. Raw material cost

Consider price of 1 kg of Roselle (₹) = 25/-

Total cost of raw material for 500 kg of fresh Roselle (₹) = 7200×25

= ₹ 1,80,000/7200 kg

Total cost of Roselle dried (7200 kg) (₹) = I + II

= 18,516 + 1,80,000

= ₹ 1,98,516/-

Considering average recovery of dried Roselle = 17.00% of fresh Roselle

From 7200 kg (₹) = $7200 \times 0.17 = 1224$ kg of dried Roselle

Hence, total cost of 1 kg of dried Roselle (₹)

$$= \frac{1,98,514}{1,224}$$

= ₹ 162/kg of dried Roselle

Selling price of dried Roselle (₹) = ₹ 280/kg

= 280×1224

Now, Gross return = ₹ 3,42,720/-

For production of 100 kg of dried Roselle:

Production cost = $162 \times 100 = 16,200$

Gross return = $280 \times 100 = 28,000$

Therefore, Net returns = $28,000 - 16,200 = 11,800$

Hence, Benefit cost ratio = $\frac{\text{Gross returns}}{\text{Production cost}} = \frac{28,000}{16,200}$

B:C ratio = 1.73

3. Estimation of cost of production of dried Roselle from 7200 kg of fresh Roselle in shade drying

I. Raw material cost

Consider price of 1 kg of fresh Roselle (₹) = ₹ 25/-

$$\begin{aligned}\text{Total cost of fresh Roselle for 7200 kg (₹)} &= 7200 \times 25 \\ &= ₹ 1,80,000\end{aligned}$$

II. Labour charge

Number of labours required = 2 No. for handling 600 kg/batch

$$\text{Number of batches} = \frac{7,200}{600} = 12 \text{ batches}$$

Total number of labours required = $12 \times 2 = 24$ No.

Labour charge per day per person = ₹ 300/-

Total cost of labour charge per batch in 3 days = $2 \times 300 \times 3 = ₹ 1,800/\text{batch}$

Therefore, labour charges for drying 7200 kg Roselle = $1800 \times 12 = ₹ 21,600$

$$\begin{aligned}\text{Total cost of Roselle dried (7200 kg) (₹)} &= \text{I} + \text{II} \\ &= 1,80,000 + 21,600 \\ &= ₹ 2,01,600/-\end{aligned}$$

Considering average recovery of dried Roselle = 17.00% of fresh Roselle

From 7200 kg (₹) = $7200 \times 0.17 = 1224$ kg of dried Roselle

Hence, total cost of 1 kg of dried Roselle (₹)

$$= \frac{2,01,600}{1,224}$$

$$= ₹ 165/\text{kg of dried Roselle}$$

Assuming the market selling price of 1 kg of dried Roselle is ₹ 250/kg

$$\begin{aligned}\text{Selling price of dried Roselle (₹)} &= ₹ 250/\text{kg} \\ &= 250 \times 1224\end{aligned}$$

Now, Gross returns = 3,06,000/-

For production of 100 kg of dried Roselle:

$$\text{Production cost} = 165 \times 100 = 16,500$$

$$\text{Gross return} = 250 \times 100 = 25,000$$

$$\text{Therefore, Net returns} = 25,000 - 16,500 = 8,500$$

$$\text{Hence, Benefit cost ratio} = \frac{\text{Gross returns}}{\text{Production cost}} = \frac{25,000}{16,500}$$

$$\text{B:C ratio} = 1.51$$

II. Economics for milled Roselle

1. Estimation of cost of production and B:C ratio of Roselle powder using normal pulverizer

Cost of grinder (normal pulverizer) (₹)	=	1,00,000/-
Cost of the electronic balance (₹)	=	15,000/-
Life span of the unit (n)	=	10 years
Annual usage	=	275 days
Interest rate (i)	=	11.5% per annum

I. Fixed cost per year

Fixed cost of the Equipments (E) (pulverizer, and electronic balance)	=	$\frac{i(i+1)^n}{(i+1)^n - 1} \times E$
	=	$\frac{0.115(0.115+1)^{10}}{(0.115+1)^{10} - 1} \times 1,15,000$
	=	₹ 19,949/-
Housing, Insurance and Taxes (pulverizer)	=	4% of initial cost of grinder
	=	$1,00,000 \times \frac{4}{100}$
	=	₹ 4,000/-
Total fixed cost/year (₹)	=	19,949 + 4,000
	=	₹ 23,949/- per year

II. Variable cost per year

a) Repair and maintenance charge of equipments

i) Repair and maintenance charge of grinder (₹)	=	5% of initial cost of the grinder
	=	$1,00,000 \times \frac{5}{100}$
Total repair and maintenance charges	=	₹ 5,000/-

b) Cost of energy

Energy requirement for grinder	=	30 kWh/8 h
Electricity charges	=	8.00/kWh
Electricity consumption charges (₹)	=	$(30 \times 275) \times 8$
	=	₹ 66,000/-
Total cost of energy per year (₹)	=	₹ 66,000/-

c) Labour charges

No. of labours	=	3
Labour charge per day (₹)	=	300/-
Cost of labour per year (₹)	=	900×275
	=	₹ 2,47,500/-

d) Cost of raw materials

Cost of dried Roselle	=	483/kg
Total quantity of Roselle powder required per batch	=	80 kg/batch/day
Total quantity of Roselle powder production per year	=	$80 \times 275 = 22,000$ kg
Cost of Roselle powder per year	=	$483 \times 80 \times 275$
	=	₹ 1,06,26,000/-

Total variable cost/year (₹)	=	$a + b + c + d$
	=	$5,000 + 66,000 + 2,47,500 + 1,06,26,000$
	=	₹ 1,09,44,500/- per year

Total cost of the production Roselle powder (₹)	=	Total fixed cost + total variable cost
	=	$23,949 + 1,09,44,500$
	=	₹ 36,20,449/-

Cost of production of one kg Roselle powder (₹)	=	$\frac{\text{Total cost of operation}}{\text{Total quantity of Roselle powder}}$
	=	$\frac{1,09,68,442}{22,000}$

Cost of production of one kg of Roselle powder	=	₹ 499/kg
Selling price of Roselle powder	=	₹ 750/kg
	=	$22,000 \times 750 = 1,65,00,000$
Gross return	=	₹ 1,65,00,000

Hence, for production of 100 kg milled Roselle:

Production cost	=	$499 \times 100 = ₹ 49,900$
Gross returns	=	$750 \times 100 = ₹ 75,000$
Therefore, Net returns	=	$75,000 - 49,900 = ₹ 25,100$
Hence, Benefit cost ratio	=	$\frac{\text{Gross returns}}{\text{Production cost}} = \frac{75,000}{49,900}$

B:C ratio	=	1.50

2. Estimation of cost of production and B:C ratio of Roselle powder using water cooled pulverizer

Cost of water cooled pulverizer (₹)	=	40,000/-
Cost of the electronic balance (₹)	=	15,000/-
Life span of the unit (n)	=	10 years
Annual usage	=	275 days
Interest rate (i)	=	11.5% per annum

I. Fixed cost per year

Fixed cost of the Equipments (E) (Water cooled pulverizer and electronic balance)	=	$\frac{i(i+1)^n}{(i+1)^n - 1} \times E$
	=	$\frac{0.115(0.115+1)^{10}}{(0.115+1)^{10} - 1} \times 55,000$
	=	₹ 9,584/-
Housing, Insurance and Taxes (water cooled pulverizer)	=	4% of initial cost of water cooled pulverizer
	=	$40,000 \times \frac{4}{100}$
	=	₹ 1,600/-
Total fixed cost/year (₹)	=	9,584 + 1,600
	=	₹ 11,184/- per year

II. Variable cost per year

a) Repair and maintenance charge of equipments

i) Repair and maintenance charge of water cooled pulverizer (₹)	=	5% of initial cost of the water cooled Pulverizer
	=	$40,000 \times \frac{5}{100}$
	=	₹ 2,000/-

Total repair and maintenance charges (₹)	=	2,000/-

b) Cost of (water+ water tank + pipe and fittings + electricity)

Total quantity of water required per batch	=	25 litre
Total Cost of (water + tank + pipe and	=	$30 \times 25 \times 3 \times 275$

fittings + electricity) per year (₹)

$$= ₹ 6,18,750/-$$

c) Cost of energy

Energy requirement for water cooled pulverizer = 30 kWh/8 h

Electricity charges = 8.00/kWh

Electricity consumption charges (₹) = $30 \times 275 \times 8$

$$= ₹ 66,000/-$$

Total cost of energy (₹) = 66,000/-

d) Labour charges

No. of labours = 3

Labour charge per day = 300/-

Cost of labour per year (₹) = 900×275

$$= ₹ 2,47,500/-$$

e) Cost of raw materials

Cost of dried Roselle = 483/kg

Total quantity of Roselle powder prepared per day = 80 kg/day

Cost of Roselle powder per year (₹) = $483 \times 80 \times 275$

$$= ₹ 1,06,26,000/-$$

Total cost of raw materials (₹) = ₹ 1,06,26,000/-

Total variable cost/year (₹) = $a + b + c + d + e$

$$= 2,000 + 6,18,750 + 66,000 + 2,47,500 + 1,06,26,000/-$$

$$= ₹ 1,15,60,250/- \text{ per year}$$

Total cost of the production of Roselle powder (₹) = Total fixed cost + total variable cost

$$= 11,184 + 1,15,60,250$$

$$= ₹ 1,15,71,434/-$$

Total production of Roselle powder/year (₹) = $80 \times 275 = 22,000 \text{ kg/year}$

Cost of production of one kg of Roselle powder (₹) = $\frac{\text{Total cost of operation}}{\text{Total quantity of Roselle powder}}$

$$= \frac{1,15,71,434}{22,000}$$

Cost of production of one kg of Roselle powder	=	₹ 526/kg
Selling price of Roselle powder	=	₹ 800/kg
Gross return	=	22,000 × 800 = ₹ 1,76,00,000
Hence, for production of 100 kg of milled Roselle:		
Production cost	=	526 × 100 = ₹ 52,600
Gross returns	=	800 × 100 = ₹ 80,000
Therefore, Net returns	=	80,000 - 52,600 = ₹ 27,400
Hence, Benefit cost ratio	=	$\frac{\text{Gross returns}}{\text{Production cost}} = \frac{80,000}{52,600}$
B:C ratio	=	1.52

3. Estimation of cost of production and B:C ratio of Roselle powder using LN₂ cooled pulverizer

Cost of LN ₂ cooled pulverizer (₹)	=	2,50,000/-
Cost of the electronic balance (₹)	=	15,000/-
Life span of the unit (n)	=	10 years
Annual usage	=	275 days
Interest rate (i)	=	11.5% per annum

I. Fixed cost per year

Fixed cost of the Equipments (E) (LN ₂ cooled pulverizer and electronic balance) (₹)	=	$\frac{i(i+1)^n}{(i+1)^n - 1} \times E$
	=	$\frac{0.115(0.115+1)^{10}}{(0.115+1)^{10} - 1} \times 2,65,000$
	=	₹ 45,969/-
Housing, Insurance and Taxes (LN ₂ cooled pulverizer) (₹)	=	4% of initial cost of grinder
	=	$2,50,000 \times \frac{4}{100}$
	=	10,000/-
Total fixed cost/year (₹)	=	45,969+10,000
	=	₹ 55,969/- per year

II. Variable cost per year

a) Repair and maintenance charge of equipments

i) Repair and maintenance charge of LN₂ cooled pulverizer (₹) = 5% of initial cost of the pulverizer

$$= 2,50,000 \times \frac{5}{100}$$

$$= ₹ 12,500/-$$

Total repair and maintenance charges (₹) = 12,500/-

b) Cost of LN₂

Total quantity of LN₂ required per batch = 50 kg

Cost of 1 kg LN₂ (₹) = 150/-

Total cost of LN₂ required per year (₹) = 150 × 50 × 275

$$= ₹ 20,62,500/-$$

c) Cost of energy

Energy requirement for LN₂ cooled pulverizer = 30 kWh/8 h

Electricity charges = 8.00/kWh

Electricity consumption charges (₹) = 30 × 275 × 8

$$= ₹ 66,000/-$$

Total cost of energy (₹) = 66,000/-

d) Labour charges

No. of labours = 3

Labour charge per day = 900/-

Cost of labour per year (₹) = 900 × 275

$$= ₹ 2,47,500/-$$

e) Cost of raw materials

Cost of dried Roselle (₹) = ₹ 493/kg

Total quantity of Roselle powder required per day = 80 kg/day

Total cost of raw materials (₹) per year = ₹ 1,06,26,000/-

$$\begin{aligned}
\text{Total variable cost/year (₹)} &= a + b + c + d + e \\
&= 12,500 + 20,62,500 + 66,000 + 2,47,500 + 1,06,26,000 \\
&= ₹ 1,30,14,500 \text{ /- per year} \\
\text{Total cost of the production of Roselle powder (₹)} &= \text{Total fixed cost} + \text{total variable cost} \\
&= 55,969 + 1,30,14,500 \\
&= ₹ 1,30,70,469\text{-} \\
\text{Total production of Roselle powder/year (₹)} &= ₹ 22,000 \text{ kg/year} \\
\text{Cost of production of one kg of Roselle powder(₹)} &= \frac{\text{Total cost of operation}}{\text{Total quantity of Roselle powder}} \\
&= \frac{1,06,81,969}{22,000} \\
\text{Cost of production of one kg of Roselle powder} &= ₹ 594/\text{kg} \\
\text{Selling price of Roselle powder} &= ₹ 900\text{-} \\
\text{Gross return} &= 22,000 \times 900 = 1,98,00,000\text{-} \\
\text{Hence, for production of 100 kg of milled Roselle:} & \\
\text{Production cost} &= 594 \times 100 = 59,400 \\
\text{Gross returns} &= 1000 \times 100 = 1,00,000 \\
\text{Therefore, Net returns} &= 1,00,000 - 59,400 = 40,600 \\
\text{Hence, Benefit cost ratio} &= \frac{\text{Gross returns}}{\text{Production cost}} = \frac{1,00,000}{59,400} \\
\text{B:C ratio} &= 1.68
\end{aligned}$$

III. Economics for solvent extracted Roselle anthocyanin extract

Estimation of cost of production of Roselle extract/year from solvent (ethanol) extraction

$$\begin{aligned}
\text{Cost of the rotary vacuum evaporator (₹)} &= 6,30,000\text{-} \\
\text{Cost of the electric balance (₹)} &= 1,00,000\text{-} \\
\text{Life span of the unit (n)} &= 10 \text{ years} \\
\text{Annual usage} &= 275 \text{ days} \\
\text{Interest rate (i)} &= 11.50 \% \text{ per annum}
\end{aligned}$$

I. Fixed cost per year

Fixed cost of the Equipments (E) (rotary vacuum flash evaporator and electric balance)

$$= \frac{i(i+1)^n}{(i+1)^n + 1} \times E$$
$$= \frac{0.115(0.115+1)^{10}}{(0.115+1)^{10} + 1} \times 6,30,000$$

$$\text{Total fixed cost/year (₹)} = ₹ 54,090 /-$$

II. Variable cost per year

a) Repair and maintenance charge of equipments

i) Repair and maintenance of rotary vacuum evaporator (₹) = 5 % of initial cost of the rotary vacuum evaporator

$$= 6,30,000 \times \frac{5}{100}$$

$$= ₹ 31,500/-$$

ii) Repairs and maintenance of electric balance (₹) = 2 % of initial cost of the electric balance

$$= 1,00,000 \times \frac{2}{100}$$

$$= ₹ 2,000/-$$

Total Repair and maintenance charge (₹)

$$= \text{i + ii}$$
$$= 31,500 + 2,000$$

$$= ₹ 33,500/-$$

b) Cost of energy

Total energy requirement for evaporator = 50 kW/8h

Electricity charges = 8.00/kWh

Electricity consumption charges (₹) = No. of days × Energy per day × Cost of electricity

$$= 275 \times 50 \times 8.0$$

$$= ₹ 1,10,000/-$$

c) Labour charges

Man labour (1) @ 180/day = 3 × 300

Total labour charge/day = 300/day

$$\begin{aligned}\text{Cost of labour per year (₹)} &= 900 \times 275 \\ &= ₹ 2,47,500/-\end{aligned}$$

d) Cost of raw materials

$$\text{Cost of Roselle powder} = 594/\text{kg}$$

$$\begin{aligned}\text{Roselle powder required per day} \\ &= 25 \text{ kg}\end{aligned}$$

$$\text{Total Roselle powder required per year (kg) (₹)} = 25 \times 275 = 6875 \text{ kg}$$

$$\begin{aligned}\text{Total Cost of Roselle powder per year} \\ (\text{₹}) &= 25 \times 275 \times 594 = ₹ 40,83,750\end{aligned}$$

$$\text{Cost of ethanol for 275 days (₹)} = ₹ 68,75,000/-$$

$$\text{Citric acid required} = 700 \text{ kg}$$

$$\text{Cost of citric acid} = 700 \times 1,200 = ₹ 8,40,000$$

$$\text{Total cost of raw material (₹)} = ₹ 40,83,750$$

$$\begin{aligned}\text{Total cost} \\ &= 40,83,750 + 68,75,000 + 8,40,000 \\ &= 1,17,98,750\end{aligned}$$

$$\begin{aligned}\text{Total variable cost (₹)} &= a + b + c + d \\ &= 33,500 + 1,10,000 + 2,47,500 + \\ &\quad 1,17,98,750 \\ &= ₹ 1,21,89,750/-\end{aligned}$$

$$\begin{aligned}\text{Total cost of operation/ year (₹)} &= \text{Total fixed cost} + \text{Total variable cost} \\ &= 54,090 + 1,21,89,750 \\ &= ₹ 1,22,43,840/-\end{aligned}$$

$$\text{Considering 97\% recovery} = 6875 \times 0.97 = 6668$$

$$\text{Total Production of Roselle extract/ year} = 6,668$$

$$\begin{aligned}\text{Cost of production of one kg of Roselle extract} \\ (\text{₹}) &= 1836/\text{kg}\end{aligned}$$

Production of commercial Roselle extract

$$\text{The quantity production of Roselle extract/year} = ₹ 6,668 \text{ kg}$$

$$\begin{aligned}\text{Selling price of Roselle extract in the} \\ \text{commercial market per kg (₹)} &= ₹ 5,000/-\end{aligned}$$

$$\text{Benefit obtained/ Gross returned (₹)} = 6,668 \times 5000$$

$$= ₹ 3,33,40,000/-$$

Hence, for production of 100 kg of Roselle extract:

$$\text{Production cost} = 1836 \times 100 = ₹ 1,83,600$$

$$\text{Gross returns} = 5000 \times 100 = ₹ 5,00,000$$

$$\text{Therefore, Net returns} = 5,00,000 - 1,83,600$$

$$= 3,16,400$$

$$\text{Hence, Benefit cost ratio} = \frac{\text{Gross returns}}{\text{Production cost}} = \frac{5,00,000}{1,83,600}$$

$$\text{B:C ratio} = 2.72$$

IV. Economics for microencapsulated Roselle powder

Estimation of cost of production of microencapsulated Roselle powder/year from spray dryer equipment using maltodextrin as coating material

$$\text{Cost of the spray dryer} = 25,00,000/-$$

$$\text{Cost of the electric balance} = 1,00,000/-$$

$$\text{Life span of the unit (n)} = 10 \text{ years}$$

$$\text{Annual usage} = 275 \text{ days}$$

$$\text{Interest rate (i)} = 11.5 \% \text{ per annum}$$

I. Fixed cost per year

$$\begin{aligned} \text{Fixed cost of the Equipments (E) (Spray dryer and electric balance)} &= \frac{i(i+1)^n}{(i+1)^n + 1} \times E \\ &= \frac{0.115(0.115+1)^{10}}{(0.115+1)^{10} + 1} \times 25,00,000 \end{aligned}$$

$$\text{Total fixed cost/year (₹)} = ₹ 2,14,646 /-$$

II. Variable cost per year

a) Repair and maintenance charge of equipments

$$\begin{aligned} \text{i) Repair and maintenance of spray dryer} &= 5 \% \text{ of initial cost of spray dryer} \\ \text{(₹)} & \end{aligned}$$

$$= 25,00,000 \times \frac{5}{100}$$

$$= ₹ 1,25,000/-$$

ii) Repairs and maintenance of electric balance (₹) = 5 % of initial cost of the electric balance

$$= 1,00,000 \times \frac{5}{100}$$

$$= ₹ 5,000/-$$

i + ii

Total Repair and maintenance charge (₹) = 1,25,000 + 5,000

$$= ₹ 1,30,000/-$$

b) Cost of energy

Total energy requirement spray dryer = 75 kWh/8 h

Electricity charges = 8.00/kWh

Electricity consumption charges = No. of days × Energy per day × Cost of electricity

$$= 275 \times 75 \times 8$$

$$= ₹ 1,65,000/-$$

c) Labour charges

Men labour (1) @ 300/day = 6 × 300

Total labour charge/day = 1,800/day

Cost of labour per year = 1,800 × 275

$$= ₹ 4,95,000/-$$

d) Cost of raw materials

Cost of 1 kg of Roselle extract (₹) = ₹ 1,836/-

Roselle extract required per batch = 25 kg (3kg per hour)

Total no. of batches per day = 1 × 8.11 = 8.11

Total Cost of Roselle extract per year (₹) = ₹ 1,26,22,500

Cost of maltodextrin (₹) = ₹ 47,91,472/-

Total cost of raw material (₹) = 1,26,22,500 + 47,91,472 = 1,74,13,972

Total variable cost = a + b + c + d

$$= 1,30,000 + 1,65,000 + 4,95,000 + 1,74,13,972$$

$$= ₹ 1,82,03,972$$

$$\begin{aligned}
 \text{Total cost of operation/ year} &= \text{Total fixed cost} + \text{Total variable cost} \\
 &= 2,14,646 + 1,82,03,972 \\
 &= ₹ 1,84,18,618 /- \\
 \text{Total Production of encapsulated Roselle powder/year} &= 10,000 \text{ kg} \\
 \text{Cost of production of encapsulated powder} &= \frac{1,84,18,618}{10,000} = ₹ 1842/\text{kg}
 \end{aligned}$$

Production of commercial microencapsulated Roselle anthocyanin powder

$$\begin{aligned}
 \text{Total production of encapsulated Roselle powder} &= 10,000 \text{ kg}
 \end{aligned}$$

$$\begin{aligned}
 \text{Selling price of encapsulated Roselle powder in the commercial market per kg} &= ₹ 6,500/-
 \end{aligned}$$

$$\begin{aligned}
 \text{Benefit obtained/Gross returned} &= 10,000 \times 6,500 \\
 &= ₹ 6,50,00,000/-
 \end{aligned}$$

Hence, for production of 100 kg of encapsulated Roselle powder:

$$\begin{aligned}
 \text{Production cost} &= 1842 \times 100 = ₹ 1,84,200 \\
 \text{Gross returns} &= 6500 \times 100 = ₹ 6,50,000 \\
 \text{Therefore, Net returns} &= 6,50,000 - 1,84,200 = ₹ 4,65,800 \\
 \text{Hence, Benefit cost ratio} &= \frac{\text{Gross returns}}{\text{Production cost}} = \frac{6,50,000}{1,84,200} \\
 \text{B:C ratio} &= 3.53
 \end{aligned}$$

**STUDIES ON EXTRACTION AND ENCAPSULATION OF ANTHOCYANIN
FROM CALYCES OF ROSELLE (*Hibiscus sabdariffa* L.)**

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ABSTRACT

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The aim of this investigation was to standardize the process technologies for drying and milling, extraction and encapsulation of anthocyanin from calyces of Roselle (*Hibiscus sabdariffa* L.) and their effects on quality characteristics of the products. The anthocyanin is the main colouring component present in calyces of Roselle which was found to be 718.72 ± 1.19 mg CyaG.100g⁻¹. The optimized drying and milling method for calyces of Roselle powder was found to be dehumidified air dryer and LN₂ cooled pulverizer with total anthocyanin content of 593.86 mg CyaG.100g⁻¹. The maximum extraction yield (97.33%), and total anthocyanin content (1072.27 mg CyaG.100g⁻¹) were obtained using 1:10 solid to solvent ratio and 2% citric acid. The highest powder yield (92.04%) and total anthocyanin content (994.99 mg CyaG.100g⁻¹) of microencapsulated Roselle anthocyanin powder was obtained using maltodextrin as a coating material and at 170 °C inlet air temperature. Similarly, the particle size, morphology and reconstitution properties were found superior in microencapsulated Roselle anthocyanin powder at the optimized process parameters. The results obtained from the stability study of microencapsulated Roselle anthocyanin powder indicates that during the period of time established, a greater amount of pigment was retained at low temperatures (5 °C) and low pH (2).