" STANDARDIZATION AND EVALUATION OF RTS FROM BLENDS OF INDIAN ROSELLE (*Hibiscus sabdariffa L.*) AND ALOE VERA *(Aloe barbadensis M.)*"

M.Sc. (Hort.) Thesis

by

Gunjeshree Gond

DEPARTMENT OF VEGETABLE SCIENCE

COLLEGE OF AGRICULTURE

FACULTY OF HORTICULTURE

INDIRA GANDHI KRISHI VISHWAVIDYALAYA

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"STANDARDIZATION AND EVALUATION OF RTS FROM BLENDS OF INDIAN ROSELLE (*Hibiscus sabdariffa L.*) AND ALOE VERA (*Aloe barbadensis M.*)"

Thesis

Submitted to the

Indira Gandhi Krishi Vishwavidyalaya, Raipur

by

Gunjeshree Gond

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CERTIFICATE - I

This is to certify that the thesis entitled **"Standardization and Evaluation of RTS from blends of Indian Roselle (*Hibiscus sabdariffa L.*) and Aloe Vera (*Aloe barbadensis M.*)"** submitted in partial fulfilment of the requirements for the degree of **Master of Science in Horticulture (Vegetable Science)** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of the bonafide research work carried out by **Gunjeshree Gond** under my guidance and supervision. The subject of the thesis has been approved by the student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma or has been published/published part has been fully acknowledged. All the assistance and help received during the course of the investigation have been duly acknowledged by her.

Chairman : Dr. Annu Verma

Date: 03/10/2020

THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE

Chairman (Dr. Annu Verma)

Member (Dr. Neeraj Shukla)

Member (Dr. S.S. Porte)

Member (Dr. R.R. Saxena)

CERTIFICATE-II

This is to certify that the thesis entitled **"Standardization and Evaluation of RTS from blends of Indian Roselle (*Hibiscus sabdariffa L.*) and Aloe Vera (*Aloe barbadensis M.*)"** submitted by **Gunjeshree Gond** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur, in partial fulfillment of the requirements for the degree of **Master of Science in Horticulture (Vegetable Science)** in the Department of Vegetable Science has been approved by the external evaluator and Student's Advisory Committee after oral examination, under the chairmanship of head of the Department.

Signature of Head of the Department

(Name: Dr. Neeraj Shukla)

Date: 16/10/2020

Major Advisor 16.10.2020 Dr Anuu Verma.

Faculty Dean _____

Approved/Not approved

Director of Instructions _____

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Department of Vegatable Science

College of Agriculture,

I.G.K.V. Raipur (Chhattisgarh)

(Gunjeshree Gond)

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LIST OF ABBREVIATIONS

Abbreviations	Description
ºB	Degree Brix
ºC	Degree Celcius
%	Per cent
ha	Hectare
q	Quintal
mg	Milli gram
ml	Milli litre
mm	Milli meter
cm	Centi meter
Kg	Kilogram
g	gram
T	Treatment
hrs	Hours
CD	Critical difference
CV	Coefficient of Variation
SE±	Standard error of mean
NS	Non-significant
et al.	And co-workers/or others
/	Per
RTS	Ready-to –serve
TSS	Total soluble solids
CRD	Completely randomized design
C.G.	Chhattisgarh
S.No.	Serial number
Fig.	Figure

THESIS ABSTRACT

Title of the Thesis	:	Standardization and Evaluation of RTS from blends of Indian Roselle (*Hibiscus sabdariffa L.)* and Aloe Vera (*Aloe barbadensis M.*)
Full Name of the Student	:	Gunjeshree Gond
Major Subject:	:	Vegetable Science
Name and Address of the Major Advisor	:	Dr. Annu Verma (Professor) Department of Vegetable Science, College of Agriculture Raipur C.G.)
Degree to be awarded	:	Master of Science in Horticulture (Vegetable Science)

Signature of Major Advisor

Signature of the Student

Date : 03/10/2020

Signature of Head of the Department

ABSTRACT

This investigation has been completed at the Laboratory of Vegetable Science of IGKV, Raipur, (C.G.) during the year 2019-2020. The goal of this study is to develop blended RTS in order to maximise potential nutritional and processing benefits of roselle and aloe vera. Inspite of having lots of benefits roselle remain under utilized. High acidity and heavy sour flavour restrict its fresh consumption. Aloe vera has bitter taste and is distasteful in its raw state so the blending of these two fruit juice is the best choice for enhancing its palatibility.

Fully matured fresh calyces of roselle were obtained from Dantewada and fresh aloe vera was collected from Department of Physiology, IGKV, Raipur, (C.G.). were used for processing under this investigation. The recipe standardization of roselle and aloe vera blended RTS with 11 treatments and 3 replications were carried out under Completely Randomized Block Design. The aim of the investigation was to study the physico-chemical composition of fresh roselle calyces and aloe vera leaves, to standardize the blended RTS recipe; to determine the sensory and physico-chemical characteristics of the prepared RTS during storage under ambient conditions. During recipe standardization treatment T_3 having 80 per cent roselle juice and 20 per cent aloe vera scored highest. Throughout the storage period it was observed that TSS of T_1 having maximum TSS content decreased from (9.2% to 8.1%) and T_{11} having minimum content decreased from (5% to 4%) , pH for T_{11} noted

maximum pH content which decreased from (3.40 to 3.16) and T_1 having minimum pH decreased from (2.8 to 2.6) , acidity for T_1 was noted highest which increased from (0.18% to 0.24%) and minimum was observed in T_{11} which decrease from (0.14% to 0.18%), ascorbic acid content was recorded highest for T_{11} decreased from (4.6 mg/100g to 3.5mg/100g) and minimum was noted on T_1 which decreased from (3.0 mg/100g to 2.4mg/100g), reducing sugar for T_{11} having maximum and T_1 having minimum sugar content decreased from (2.25% to 1.75%) and (1.6% to 1.25%) respectively, T_{11} having highest non-reducing sugar content decreased from (9.11% to 7.09%) and minimum was noted on T_1 which reduced from (6.48% to 5.07%) , T_{11} recorded highest total sugar and decreased from (11.36% to 8.84%) and T_1 having minimum total sugar content decreased from (8.08% to 6.32%), T_1 having maximum anthocyanin content reduced from (1.12mg/100ml to 1.18mg/100ml) for T_1, while T_{11} recorded lowest with no anthocyanin content followed by T_{10} which decrease from (0.64 to 0.6 mg/100ml) with the advancement in storage period upto 75 days. T_2 is the most stable treatment among all having 90 per cent roselle juice and 10 per cent aloe vera juice, from this investigation it is also clear that all the physico-chemical parameters decreased except acidity with increase in storage days.

शोध सारांष

(अ) शोध शीर्षक	:	इंडियन रोजेल (हिबिस्कस सबद्रिफा एल.) और ऐलोवेरा (एलो बार्बडेंसिस एम.) के मिश्रणों से आर. टी. एस का मानकीकरण और मूल्यांकन।
(ब) छात्रा का पूरा नाम	:	गुंजेश्री गोड़
(स) प्रमुख विषय	:	सब्जी विज्ञान विभाग
(द) प्रमुख सलाहकार का नाम और पता	:	डॉ. अन्नू वर्मा (सहायक प्राध्यापक) सब्जी विज्ञान विभाग, कृषि महाविद्यालय रायपुर (छ.ग.)
(इ) उपाधि का नाम	:	एम.एस.सी. (वनस्पति विज्ञान)

प्रमुख सलाहकार के हस्ताक्षर

दिनांक : 03/10/2020

छात्रा के हस्ताक्षर

विभागाध्यक्ष के हस्ताक्षर

सारांष

सत्र 2019–2020 के दौरान, इंदिरा गांधी कृषि विश्वविद्यालय रायपुर (छ.ग.) के सब्जी विज्ञान विभाग की प्रयोगशाला में किये गए अध्ययन, जिसका प्रमुख उद्देश्य मिश्रित आर.टी.एस. विकसित करना है ताकि रोसेल और ऐलोवेरा के संभाविक पोषण और प्रसंसकरण लाभों को अधिकतम किया जा सके ।

अत्यधिक लाभ होनें के बावजूद भी रोसेल कम उपयोग में लाया जाता है । उच्च अम्लता और भारी खट्टा स्वाद इसकी ताजा खपत को प्रतिबंधित करता है । एलोवेरा का कड़वा स्वाद तथा कच्ची अवस्था में अरूचिकर लगना, इसकी ताजी खपत को कम करता है । अतः इन दो फलों के एस का सम्मिश्रण इनकी स्वाद को बढ़ाने के लिए सबसे अच्छा विकल्प है ।

जिला दंतेवाड़ा से रोसेल की पूरी तरह से परिपक्व ताजा कलियां प्राप्त की गई थीं और जांच के तहत प्रसंस्करण के लिए इंदिरा गांधी कृषि विश्वविद्यालय परिसर से ताजा एलोवेरा एकत्र किया गया था । रोसेल और ऐलोवेरा से प्राप्त नुस्खा के मानकीकरण में 11 उपचारों के साथ आर.टी.एस. को मिश्रिम किया और पूरी तरह से यादृच्छिक ब्लॉक डिजाइन के तहत 3 प्रतिकृति की गई । जॉच का उद्देश्य मिश्रित आर.टी.एस. नुस्खा को मानकीकृत करने के लिए ताजा रोसेल की कलियां और ऐलोवेरा के पत्तियों की भौतिक रासायनिक संरचना का अध्ययन करना, परिवेशी परिस्थितियों में भंडारण के दौरान तैयार आर.टी.एस. की संवदी और भौतिक रासायनिक विशेषताओं का निर्धारण करना था ।

रेसिपी मानकरीकरण उपचार के दौरान T3, जिसमें 80 प्रतिशस रोसेल रास और 20 प्रतिशस ऐलोवेरा रस था ने सर्वोत्तम अंक प्राप्त किए । जांच के दौरान यह देखा गया कि T1 की अधिकतम TSS सामग्री (9.2 %से 8.1%) तक घट गई और T11 की न्यूनतम सामग्री घटकर (5%से 4%) कम हो गई , T11 के लिए अधिकतम पी.एच सामग्री का उल्लेख किया गया जो 3.40 से 3.16 तक घट गया । और T1 में न्यूनतम पी.एच. (2.8 %से 2.6%) की कमी हुई । T1 के लिए अम्लता सबसे अधिक नोट की गई, जो (0.180 %से 0.24%) तक बढ़ गई और T11 में न्यूनतम अम्लता देखी गई, जो (0.14 %से 0. 18%) तक घट गई , एस्कांर्बिक एसिड T11 के लिए सबसे अधिक पाई गई जो (4.6 मि.ग्रा./100 ग्रा. से 3.5. मि.ग्रा./100 ग्रा.) कम हो गई । जबकि T1 पर एस्कॉर्बिक एसिड की मात्रा न्यूनतम पाई गई जो कि समय के साथ घट कर (3.0 मि.ग्रा./100 ग्रा. से 2.4 मि.ग्रा./100 ग्रा.) हो गई । रिडयूसिंग शुगर T11 के लिए सबसे अधिकतम और T1 के लिए सर्वाधिक न्यूनतम पाया गया जो कि (2.25 %से 1.75%) और (1.6 %से 1.25%) हुई । T11 में उच्चतम बॉन रिडयूसिंग शुगर सामग्री पायी गई जो घटकर (9.11 %से 7.09%) हो गई जबकि इसका न्यूनतम मान T1 पर 5.0 पाया गया जो कि 6. 48%से 5.07% तक कम गया । कुल शुगर की उच्चतम सामग्री T11 में दर्ज की गई जो (11.36 %से 8.84%) तक घट गई और T1 में न्यूनतम कुल शुगर दर्ज की गई जो (8.08 %से 6.32%) तक कम हो गई । T1 की एंथ्रोसायनिन सामग्री सबसे अधिकतम पाई गई जो (1.12 मि.ग्रा./100 मि.ली. से 1. 18 मि.ग्रा./100 मि.ली.) तक कम हो गई, जिबकी T11 में एंथ्रोसायनिन सामग्री सबसे कम दर्ज किया गया है, जिसके बाद T10 में कमी आई जो 0.64 से घटकर 0.6 हो गई ।

इस जांच से यह भी स्पष्ट है कि भंडारण के दिनों में वृद्धि के साथ अम्लता को छोड़कर सभी भौतिक रासायनिक मापदंडो में कमी आई है ।

CHAPTER-I
INTRODUCTION

Roselle, *Hibiscus sabdariffa L*. (2n=4x=72) is a shrub belonging to the family Malvaceae. It is a Hibiscus plant native to India and Malaysia that has been introduced to other parts of the world, including Central America, West Indies and even Africa (Bruke, 1975).

It is grown on an area of approximately 1.5 lakh hectares, and the average national crop productivity is around 10q/ha. In India, roselle is cultivated in larger parts covering areas from Karnataka to Tripura including Maharashtra, Andhra Pradesh, West Bengal, Bihar, Orissa and Meghalaya. As it is one of the minor crop it is not culivated commercially in Chhattisgarh. It is known by different names in different countries including roselle, razelle, sorrel, red sorrel, Indian sorrel, Guinea sorrel, sour-sour and Queensland jelly plant.

The plant is an erect, annual or perennial herb up to 3.4 m tall. It generally have taproot which is 18-30 cm long with few laterals roots and sometimes with adventitious roots. Stem often woody at the base with prickles or bristles, entirely green, green with red nodes, green with red patches or entirely red. The leaves are green with reddish veins and long or short petioles and are deeply 3 to 5 lobed, 8-15 cm long, arranged alternately on the stem. Flowers solitary, axillary, bisexual, pentamerous and are up to 12.5 cm wide, yellow or buff with a rose or maroon eye. The fruit is a velvety capsule, which is 1.25-2.5 cm long, green when immature, 5 valved, with 3-5 seeds in each valve.

Roselle is a multipurpose plant and is used as a folk remedy in the treatment of cancer, cough, dyspepsia, fever, heart ailments, hypertension and strangury (Duke, 1985). They are recorded to be antihypertensive, antiseptic, sedative, diuretic, digestive, purgative, emmolient, and astringent (Odigie *et al*., 2003). Calyces contain high amounts of organic acids, namely: citric acid, malic acid, tartatic acid and hibiscus protocatechuric acid (Kerharo, 1971;,Khafaga and Koch, 1980a; Tseng *et al*., 1996). It also contain antioxidants including flavonoids, gossypetine, hibiscetine and sadderetine.

Red colour of roselle is due to pigment known as “Anthocyanin”. The anthocyanin found in roselle calyces have been reported to contain delphinidin-3-sambubioside, cydine-3-sambubioside, delphinidin-3-monoglucoside and cynidin-3-monoglucoside (Hong and Wrostland,1990). Vitamins like ascorbic acid, niacin and pyridoxine were also present in appreciable amounts (Puro *et al*., 2014). Roselle calyces is used in flavouring sauces, jellies, soft drinks and marmalades, also used for culinary and confectionary purpose or as a food colouring agent due to its brilliant red colour.

The fresh calyces of roselle are rich in pectin 3.19 per cent and citric acid 3.74 per cent. The fresh calyces per 100 g, contained 49 calories, 84.5 per cent moisture, 1.9 g protein, 0.1 g fat, 12.3 g total carbohydrate, 14 mg ascorbic acid, 2.3g fibre, 1.72mg Ca, 57mg P, 2.9mg Fe (Duke and Atchley 1984). A fresh calyces of roselle contains 88.2 per cent moisture, 1.46 per cent crude fibre, 0.86 per cent ash, 0.10 per cent Ca, 0.051 per cent P, 0.021 per cent Fe along with 0.82 per cent reducing sugars and 0.24 per cent sucrose.

Aloe vera (*Aloe barbadensis M.*) is a perennial succulent plant belonging to family Liliacea. Aloe vera contains aloins, a small quantity of volatile oil, resin, gum, anthraquinone, chrysophanic acid and coumarins. The plant has rigid, fleshy, grey-green, lance shaped leaves with serrated edges, rising at the base to almost 20-50 cm long and 10 cm wide. The aloe leaf is a treasure house of vitamins, minerals, protein, amino acids, anthroquinones and sterols. Aloe juice contains 99.5 per cent moisture, 0.025 per cent reducing sugar, 0.18 per cent total sugars, 0.013 per cent proteins. Aloe vera is unpleasant in its raw state and has bitter taste so its palatability can be improved by adding some other fruit juices. The nutritious and medicinal properties make aloe vera the most commonly used and commercially available medicinal plant (Olariu, 2009). It is used in treatments of various diseases such as diabetes, arthritis, eye disease, tumor, spleen enlargement, bronchitis, asthma, jaundice and ulcers (Henery, 1979).

Roselle is one of the underexploited food crops with potential for processing by the dietry and food industries. The extract of roselle calyces have a distinctive red colour, good flavour, low sugar and high acidic content. But due to

its high acidity and strong astringent taste, it is not consumed fresh. However, roselle calyces have unlimited potential in their processed form in world trade. Aloe vera is unpleasant in its raw state and has bitter taste so its palatability can be improved by adding some other fruit juices. The present study is designed to develop blended RTS to get the potential health and processing benefits of these crops. Blended beverages are an alternative for the development of novel products which provides benefit of taste, nutrition as well as medicinal properties. Developing nutritionally value added drinks may therapeutically help in improving the health of consumer.

For this investigation attempts were made to prepare a blended RTS made from a combination of Indian roselle and aloe vera juice. Since both the crop somewhere lacks in their individual uses so the blending is the best option to get the potential benefit of these nutritional and medicinal crops. Aloe vera gel is less acidic as compared to roselle so it can neutralize the acidity of roselle, and bitter taste of aloe vera can be reduced by blending it with roselle juice. The blended RTS was analyzed for its physio-chemical, storage stability and sensory properties. The present study entitled **"Standardization and Evaluation of RTS from blends of Indian Roselle (*Hibiscus sabdariffa L.*) and Aloe Vera (*Aloe Aloe barbadensis M.)***" is being proposed with the following objectives:

1) To standardize the recipe for preparation of blended Indian roselle and aloe vera RTS.
2) Physico-chemical and organoleptic evaluation of blended RTS.
3) To study the shelf life of the prepared blended RTS.

CHAPTER-II
REVIEW OF LITERATURE

Some progresses have been made on the utilization of Indian fruits for the preparation of beverage blends to satisfy consumer's thrist for more nutritious, medicinal and flavoured drinks. The literature available pertaining to the studies entitled **"Standardization and Evaluation of RTS from blends of Indian Roselle (*Hibiscus sabdariffa L.)* and Aloe Vera (*Aloe barbadensis M*.)"** has been reviewed in this chapter under the following main headings:

The related literature has been reviewed under the following headings:

2.1 Roselle (*Hibiscus sabdariffa L.)*

Wealth of India (1959) reported that edible species of roselle are grown in warm countries especially in the Philippines, Malaya and Indonesia for their calyces. In India they are grown as monsoon crop (Kharif crop) in Punjab, Uttar

Pradesh, Bihar, Bengal, Assam, Orissa, Maharashtra, Karnataka, Andhra Pradesh and Chennai.

Bruke (1975) reported that *Hibiscus sabdariffa* is native to India but it has been introduced to other parts of the world, including Central America, West Indies and even Africa.

Morton (1975) found that the production of calyx ranged from 1.5 kg (California) to 2 kg (Puerto Rico) to 7.5 kg/plant in (Southern Florida and Hawaii). Roselle intercropped with other crops yielded 16,000 kg/ha and 1900 kg when planted alone. Dual purpose planting will yield 1700 kg of herbage in 3 cuttings and 6,300 kg of calyces in future.

Duke (1983) revealed that there are 2 main forms of the *Hibiscus sabdariffa* plant. Variety sabdariffa has inflated red or pale yellow edible calyces and fiber of poor quality, Whereas variety altissima is grown for its fibre not for its calyces as they are inedible.

Duke and Atchley (1984) find that roselle belongs to the Malvaceae family, which is possible native to ancient world tropics in Eastern India, now grown throughout the tropics.

Morton *et al.,* (1987) confirmed roselle's widespread distribution in both hemisphere's Tropics and Subtropics in many areas of the Caribbean and Central America.

2.2 Aloe vera (*Aloe barbadensis Miller.)*

2.2.1 Food value of aloe vera

Lee *et al.,* (1999) prepared aloe vera juice vinegar with the help of *Acetobacter sp*. They also made yoghurts using aloe vera powder.

Volger and Ernst (1999) reported that aloe vera is a medicinal herb that belongs to the Liliaceae family. It was traditionalty used as a folk remedy. Out of 250 species among aloe vera, *Aloe barbadensis Miller* and *Aloe aborescens* are most common ones.

Dutt (2002) reported that aloe vera has been used as a natural product with therapeutic and medicinal properties.

Wei *et al.,* (2004) prepared a health drink from fresh aloe vera leaves after washing, disinfecting, grinding, and separating it. The gel is then mixed with different amounts of Dangshen and Maidong juices and Chinese herbs.

Wolf and Danninger (2004) filed a patent for the manufacture of aloe vera gel and powder and their use in food production.

Chandegara and Varshney (2013) reported that aloe vera plant is rigid, fleshy, grey-green, lance- shaped with serrated edges rising from the central base to almost 30-50 cm long and 10 cm wide at the base.

2.2.2 Pharmaceutical value of aloe vera

Meadow (1980) reported about six catalysts present in the aloe vera gel including cellulose, amylase, catalase, bradykinase, oxidase and carboxypepdidase. An essential enzyme, carboxypeptidase inactivates and induces anti-inflammatory effects of bradykinase.

Chung *et al.,* (2006); Ro (2006) said that for its tremendous nutritional and medicinal benefit, aloe vera is now grown as an industrial crop. Aloe vera gel is found to possess immune modulatory, anti-inflammatory, anti-allergic, anti-bacterial and wound healing properties as well as excellent skin hydrating and moisturizing agent.

Hamman (2008) reported that recently aloe vera has been used as a ingredient in the cosmetics and pharmaceutical industries and has been used to produce cream, lotion, soaps, shampoos, facial cleaners and other cosmetics.

2.2.3 Extraction of aloe gel

Robert (1997) claimed that the filleting process had to be performed within 36 hours of aloe leaf, in order to prevent the decomposition of biological activity.

Lawless and Allan (2000) found that in order to keep all ingredients at maximum concentration, the aloe leaves should be clear, undamaged, free from disease and mature.

Ramachandra and Rao (2008) revealed that the gel extracted from the leaf was more stable than the gel remaining in the leaf.

Ramachandra and Rao (2008); Chandegara and Varshney (2013) found that biological operation of the leaves began to lose after harvesting and storage in ambient condition within 24 hours and refrigerated storage helped to minimize the loss.

Eshun (2003); Ramachandra and Rao (2008); Chandegara and Varshney (2013) reported that in order to avoid bad taste and loss of aloe vera gel biological activity, HTST treatment at (85-95˚C for 1-2 minutes) has been documented as an effective method.

Bohani *et al.,* (2012); Sasikumar *et al.,* (2013) and Sasikumar (2015a) carried out studies and revealed that maximum yield of gel can be obtained from thick, fully formed aloe vera leaves.

2.3 Chemical compostion of roselle and aloe vera

Mayadas (1947) stated that citric and d-malic acid, tartaric acid, hibiscus acid and ascorbic acid are the principal water soluble acids present in calyces.

Anon (1959) reported that fresh roselle calyces contain moisture of 86.5 per cent, crude protein of 1.46 per cent, carbohydrate of 5.86 per cent, ash of 0.87 per cent, calcium of 0.108 per cent, phosphorus of 0.052 per cent, iron of 0.021 per cent, reducing sugar of 0.24 per cent and sucrose of 0.24 per cent.

Main *et al.,* (1978) found roselle as an alternative to obtain natural colour from calyces.

Henry (1979) found that biologically active chemical components of aloe leaves are water and polysaccharide. Aloe gel contins 98.5 per cent water with 4.5 pH and active polysaccharide, glucomannan and acemannan. Glucomannan is widely used in beauty products because it is a good hydrating agent or moisturizer. The major carbohydrate fraction in the gel is acemannan which help in wound healing, modulates immune function and had antiviral effect.

Hussain *et al.,* (1984) performed chemical analysis of whole mature roselle seeds (*Hibiscus sabdariffa L.)* and recorded first time quantified protein (25.20 per cent) and lipids (21.20 per cent). The most prevelant fatty acid followed by palmatic and stearic acid was oleic acid.

Mamta *et al.,* (1999) reported that Anthocyanin pigment present in the Roselle calyces has been used as food colouring agents. Also observed that crude extract and pure Hibiscus isolates have contraceptive, antispermatogenic, androgenic, antimicrobial, hypotensive, antispasmodic, antioxidant, anticancer, hypoglacamic, antiarthric and diuretic properties.

Saroj *et al.,* (2004) said that aloe is one of the most common plants with superb therapeutics uses which occur naturally. A combination of glycosides called " aloin" is the active principle of aloe. Aloin's main constituents is " barbaloin" which is an "aloe emodin glucoside".

Chandegara and Varshney (2005) reported that aloe gel contains 99.8 per cent water or moisture content, 0.2 per cent fibre content, 1.91 per cent total sugar content, 0.026 per cent reducing sugar content and 0.93º brix total soluble solid content.

Ramachandra and Rao (2008), Chandegara and Varshney (2013) reported that the pH of aloe juice has been modified between 3.0 and 3.5 to boost the taste of aloe vera juice by adding citric acid.

2.4 Food and medicinal uses of roselle and aloe vera

Anon (1959) reported that tender roselle leaves and stalk are eaten as salad or used for curry seasoning. They are acidic in taste, and can be used to make jelly, flavouring extracts, syrup and wine.

Morton (1961) reported that a cough syrup is made by cubing and washing the aloe pulp and pressing it in a cloth to extract all the bitter juice, then boil it in water for an hour and cook it again with two parts of sugar.

Sharaf (1962) confirmed the hypotensive nature of the calyces and found that it has antipasmodic, antibacterial and anthelmintic.

Joshi (1997) claimed that the gel's polysaccharide is believed to be involved in gel clinical activity. They help to reduce inflammation and accelerate healing. The aloe gel is usually considered harmless, and even internal ingestion considered safe.

Perry (1980) studied the utility of roselle in arteriosclerosis and as an intestinal antiseptic. He also stated that the Taiwanese find seed to be diuretic, laxative and tonic, and the bitter root is used as an apertite and tonic.

Duke (1985) has claimed that *Hibiscus sabdariffa* ia a folk remedy for abscesses, cancer, cough, debility, dyspepsia, fever, hangover, heart ailments, hypertension, neurosis, scurvy and strangury.

Morton *et al.,* (1987) revealed that the aqueous extract and the calyces coloring matter are lethal to mycobacterium tuberculosis.

Hong and Wrostland (1990) found that *Hibiscus sabdariffa* contains antioxidants such as flavonoids, gossypetine, hibiscetine and sadderetine. Some of the roselle anthocyanins detected by the chromatographic method include delphinidine-3-sambubioside, cyanidings sambubioside and delphinidin-3-glucose.

Haji and Haji (1999) recorded a decreased of 11.2 per cent in systoilc blood pressure and a decrease of 10.7 per cent in diastolic pressure after 12 days in 31 patients with moderate critical hypertension taking Hibiscus tea compared with control group.

Kattimani *et al.,* (2001) reported that aloe juice is used for dyspepsia, burns, colic hepatopathy, skin disease and in painful inflammations, chronic ulcers, fever and tonic preparation as a local treatment.

2.5 Processing of roselle calyces

Clydescale *et al.,* (1979) found that seeds, leaves and calyces are different parts of the roselle and used for various uses such as vegetables, oils, cooling drinks and food preserves.

Facciola (1990) researched the possibility of making a refreshing beverage by boiling the calyces sweetening it with sugar and adding ginger.

Babalola (2000) reported that the red and dark red coloured calyces are extracted and sweetened to make a soothing drink while the green *Hibiscus sabdariffa* calyces and leaves are used to make vegetable stew.

Schippers (2000) stated that fresh succulent calyces, together with ginger, pimento and other spices, can also be used to make a kind of chutney.

Badrie (2004) reported that sorrel puree from enzymatic extracted sorrel or roselle calyces are used in sauce production and to assess customer approval of sauces with 0.3 per cent and 0.4 per cent xanthum gum. Sorrel sauces overall acceptability makes it an ideal ingredient for use in the food service industry.

Amusa *et al.,* (2005) investigated in Ibdan, Nigeria , the microbiological and nutritional consistency of freshly prepared processed and hawked zobo drink. Several bacteria harboured in the freshly distilled zobo drinks. Higher than regulation was found to be the essential protein content of the hawked refined zobo drinks. The level of vitamin C, calcium content and strength of the freshly processed zobo beverages were higher than that of the hawked zobo beverage sample.

Fasoyiro *et al.,* (2005) reported the flavouring of the roselle extract with different fruits; orange, pineapple and apple. Total solid, proximate, mineral and vitamin C content of roselle fruit drinks is determined . Roselle fruit drinks had higher acceptability in terms of taste, falvour and overall acceptability than the typical roselle (zobo) drink.

Fasoyiro *et al.,* (2005) found that drinks made from dried calyces of roselle and apple fruits, orange and pineapple were made from roselle fruit. Those were procesed under three conditions of storage. Samples in ambient storage had a shelf life of less than five days, while those stored for around a week in refrigeraton condition, and samples in freezing condition could be stored for more than two weeks.

Fasoyiro *et a.,l* (2005) reported besides being a popular homemade drink, Jarriots, a popular Mexican soft drink brand, make carbonated beverages flavoured with Jamaica. Imported Jarriots can be found readily in the U.S. Roselle is commonly sold on the street of Africa, particularly the Sahel. He conducted a study on the chemical and storability of fruit flavoured *Hibiscus sabdariffa* drink.

Nnam and Onyeke (2005) regarding nutrient and antinutrient compositions , they investigated red and yellow types of sorrel calyces and the drinks made from them. Fresh calyces were purchased of both kinds, washed and dried. The yellow variety was 9.08 per cent higher in protein and ascorbate than the red calyces. The beverages made from yellow variety.

Pascale *et al.,* (2006) produced roselle calyces wines with varying calyces puree and total soluble solids, which were found to be acceptable.

2.6 Formulation of RTS and its storage

2.6.1 Formulation of RTS beverages

Sheetal and Sruthi (2014) reported that beverages made from fruits and vegetables are essential for human diet and are an excellent medium for supplementing neutraceutical enrichment components.

Bhagwan and Awadhesh (2014) stated that the blending technology has become an important tool in the production of modern fruit beverages, and RTS beverages are popular among people of all age groups.

2.6.1.1 Physico-chemical Properties

Sunita and Ananya (2013) found that biochemical properties play an important part in the nutritional and sensory properties of RTS beverages. In addition to the aloe juice with bael juice, the TSS content of blended RTS beverages increased from 14.10 to 14.25˚Brix.

Elbandy *et al.,* (2014) found that aloe gel supplemented mango nectar showed a small increase in total soluble solids from 15.0 to 15.4 per cent compared to 25 per cent aloe gel supplementation (without aloe gel).

Elbandy *et al.,* (2014) reported that the application of 25 per cent aloe gel in mango nectar resulted in an increase in total sugar from 14.08 to 14.12 per cent and reducing sugar from 2.52 to 2.55 per cent.

Hamid *et al.,* (2014) recorded aloe gel supplemented in orange-carrot blend nectar showed increased in reducing sugar but total sugar and non- reducing sugar decreased of the blend nectar.

Shubhra *et al.,* (2014) reported that reducing sugar and total sugar content of kinnow nectar supplemented by 4 per cent blanched and unblanched aloe juice was more than a control sample of aloe free juice.

2.6.1.2 Sensory evaluation of formulated RTS

Sasikumar *et al.,* (2013) reported that with an improvement in aloe gel concentration upto 70 per cent improved appearance, colour, taste, flavour and overall acceptability of aonla ginger RTS beverages.

Sasikumar (2015b) reported that blended functional beverages scored higher for appearance than control and 60 percent aloe supplement bael juice recorded the highest score for appearance. He also reported that colour, taste, flavour and overall acceptability score increased for blended aloe and bael fruit beverages with 60 per cent supplementation recorded the highest score for colour, taste, flavour overall acceptability.

2.6.2 Storage of RTS formulation

2.6.2.1 Physico-chemical Properties

Girdharilal (1988) and Yadav *et al.,* (2010) had found that changes in chemical properties are the reason for variations in pH during RTS beverage storage.

Klimczak *et al.,* (2007) reporeted that increased antioxidant activity may be due to Maillard's reaction products.

Nagpal and Rajalakshmi (2009) reported that, due to oxidation of ascorbic acid responsive to oxygen, light, heat by both enzymatic and non-enzymatic catalysts, during storage ascorbic acid content of juice decreases.

Morales-de la pena *et al.,* (2011) stated that an increase in antioxidant activity during storage was due to polymerisation reactions of polyphenols.

Chauhan *et al.,* (2012) reported that a functional herbal RTS beverage over the course of two months of storage showed negligible change in TSS.

Chauhan et al., (2012) noticed decrease in antioxidant activity of basil and tinospora extract RTS beverages during storage.

Gao and Rupasingh (2012) reported a decrease in antioxidant activity during storage of the apple carrot juice blends.

Hridyani (2015) and Hridyani and Soni (2016) reported that TSS of traditional medicinal RTS beverage increased during storage.

Jhakar and Pathak (2012), Singh and Kumar (1995), Sasikumar (2015a), Tiwari and Deen (2015), Talib *et al.,* (2016) reported that the incremental rise in TSS during storage can be due to the conversion of polysaccharides into monosaccharides and oligosaccharides and other juice constituents into sugar.

Sasikumar *et al.,* (2013) found that rise in acidity was the reason why PH decreased affecting the organoleptic consistency of the juice.

Hamid *et al.,* (2014) recorded increases in the reducing and total sugar content of aloe gel supplemented orange-carrot nectar during storage for 3 months.

Hamid *et al.,* (2014) reported that during storage at room temperature, antioxidant activity of orange-carrot blended nectars supplemented with aloe gel and control increased.

Pawar *et al.,* (2011), Sasikumar *et al* (2015a), Sasikumar *et al* (2015b) stated that pH and acidity are inversely proportional to each other and pH of beverage decreased and acidity increased during storage.

Sasikumar *et al.,* (2013) stated that the increase in acidity was the reason for a decrease in pH that affects the organoleptic quality of juice.

Elbandy *et al.,* (2014) reported an increase in the sugar content of mango nectar during storage due to the conversion of sucrose into glucose and fructose under acidic condition of nectar.

Shubhra *et al.,* (2014) found that losses of vitamin during storage can be due to the impact of light, contact with metallic ions and prevailing high ambient temperature conditions and substantial decreases in ascorbic acid content from 2.5 to 0.5 mg per cent in both blanched and unblanched aloe juice supplemented kinnow nectar at the end of the 6 month storage period in ambient conditions.

Sasikumar *et al.,* (2015) found that the reducing sugar of functional beverage during storage time showed a substantial increase from the initial 11.21 per cent to 15.8 per cent for aloe and bael fruit blend drinks.

Sasikumar *et al.,* (2015b) observed a minimum increase of 12.32 to 17.44 per cent overall sugar in 60 per cent aloe supplemented bael fruit beverage and an increase in overall sugar due to higher metabolic juice rate and a slowdown of 60 per cent overall aloe juice blend beverage production.

Sasikumar *et al.,* (2015b) found that increased acidity during storage was caused by the introduction of citric acid to functional drinks and increased acidity in aloe juice blended with bael fruit juice compared to alone during storage.

Tiwari and Deen (2015) recorded a gradual increase in total RTS acidity from 0.25 per cent to 0.30 per cent during storage.

Tiwari and Deen (2015) studied that the increase in reducing sugar from 2.30 percent to 5.29 per cent and total sugar from 10.21 per cent to 11.21 per cent was observed during the entire 3 month storage period of bael-aloe blended RTS drinks.

Talib *et al.,* (2016) reported an increase in acidity in RTS with aloe content and bacterial spoilage.

Talib *et al.,* (2016) reported a decrease in the ascorbic acid content of aloe based RTS drinks over a period of 90 days and further losses in high aloe derived

RTS but a substitution of 20 per cent juice with aloe provides a shelf life of 60 days.

2.6.2.2 Sensory evaluation

Bohani *et al.,* (2012) recorded that storage studies of blended papaya aloe gel showed that 10 per cent aloe gel can be incorporated into blended RTS beverages and stored for a duration of 3 months without significant loss of chemical and organoleptic properties.

Bohani *et al.,* (2012) and Sasikumar *et al*., (2013) found that during storage, the overall sensory consistency profile of papaya-aloe RTS drinks and aloe and ginger RTS drinks decreased marginally during storage for 4 months but was sufficient for storage for up to 3 months.

Elbandy *et al.,* (2014) registered a decrease in mango nectar colour score when aloe gel mango nectar was stable during storage and a decrease in taste and odour scores and a stable consistency score for all storage treatments. But colour and consistency scores were best for 20 per cent and 25 per cent aloe gel supplemented mango nectar without aloe gel supplemented mango nectar and taste scores were maximum for mango nectar without aloe gel and 5 percent aloe gel supplementation while odours quality were best for mango nectar without aloe gel.

Shubhra *et al.,* (2014) recorded that overall acceptability scores of aloe juice combined with unblanched aloe kinnow nectar obtained the highest score (8.2) folowed by control (8.1) and blanched aloe juice (8.0) and decreased during storage due to loss of kinnow nectar flavour and colour.

Sasikumar *et al.,* (2015) tested the storage of aloe mixed bael fruit juice beverage recorded a decrease in the flavour, colour and organoleptic taste scores of juice mixtures as the storage time progressed. And a juice blend of 60 percent aloe juice with bael fruit juice was the highest score for colour, taste, flavour and acceptability overall compared to other blends at the end of the 6 month storage period.

CHAPTER-III
MATERIALS AND METHODS

The present work entitled **"Standardization and Evaluation of RTS from blends of Indian Roselle (*Hibiscus sabdariffa L.)* and Aloe Vera (*Aloe barbadensis M*.)"** was carried out in the Laboratory of Vegetable Science, IGKV Raipur, (C.G.) during the year 2019-2020. This chapter deals with a detailed overview of the materials and methodology used during the investigation. The various materials required and methods used in the present study are given below with suitable headings.

3.1 Geographical situation of Experimental site

Raipur is located in the central part of Chhattisgarh state and lies between latitude 21.16^{o} N and longitude 81.36 ^{o} E with an altitude of 289.56 m above mean sea level.

3.2 Climate

Raipur comes under dry, sub-humid region of Chhattisgarh plains. The average annual rainfall ranges from 1200-1400 mm, about 85 per cent of which is received from third week of June to mid of September and very little during October to February. May and December is the hottest and the coolest month. The maximum temperature in the summer is as high as 46 ^{o} C and minimum as 6 ^{o} C in the winter.

3.3 Weather condition during investigation period

The meteorological observations during the period of investigation have been presented in Appendix-I in Figure 3.1

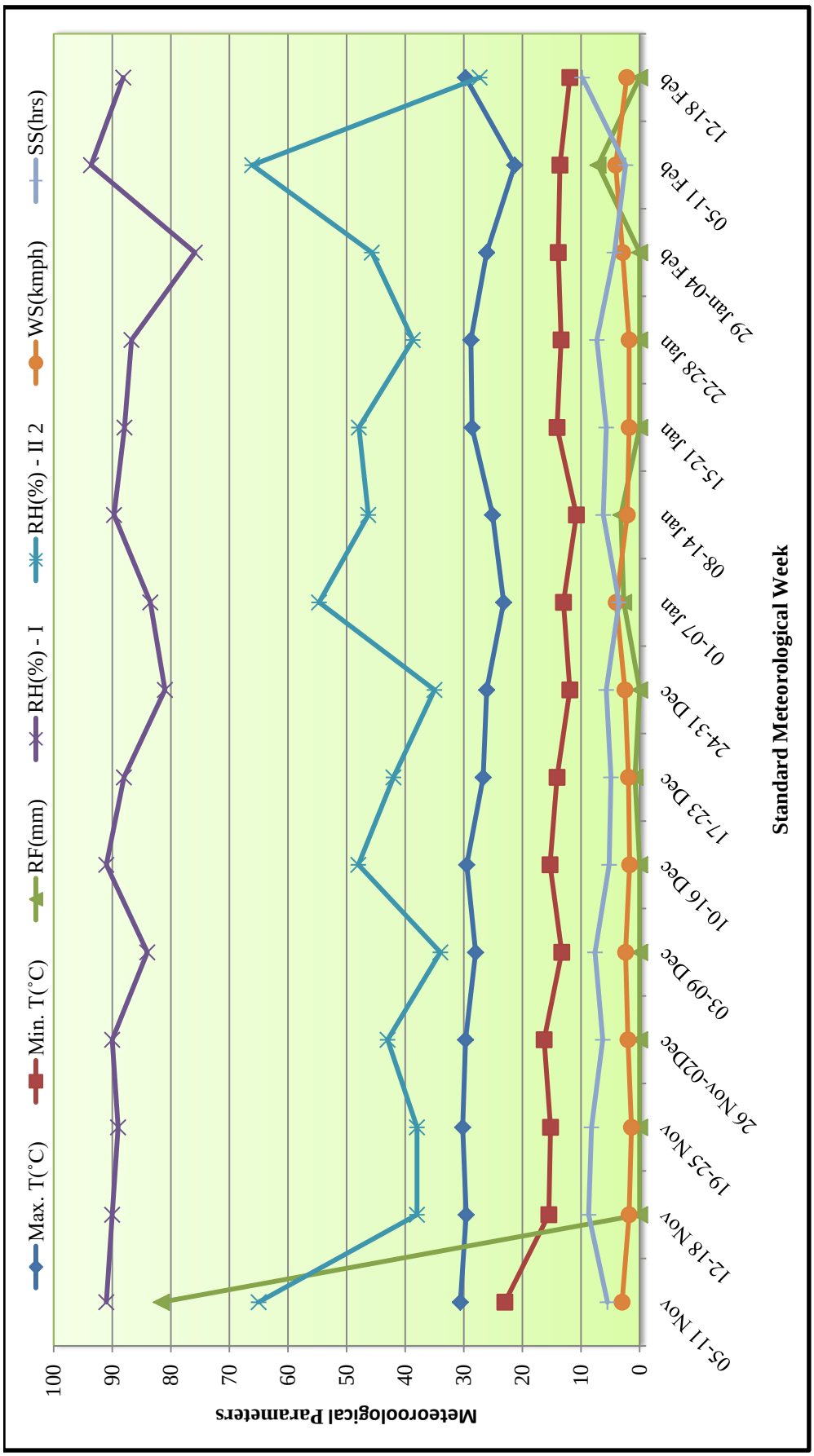

Fig 3.1 : Weekely meteorological data recorded during storage of blended roselle-aloe vera RTS (Nov 2019-Feb 2020)

3.4 Experimental Details

Crops : Roselle calyces and Aloe Vera

Processed Product : Ready -To-Serve

Design : Completely Randomized Block Design (CRBD)

Treatments : 11

Replications :3

3.4.1 Collection of roselle calyces and aloe vera

Roselle calyces were collected from the Dantewada, seeds were planted at the month of June during rainy season and was harvested in mid November onwards when the flower has dropped that time calyces were fully matured and dark pink in colour. Aloe vera was collected from Department of Physiology, IGKV Raipur.

3.4.2 Ingredients

Healthy roselle calyces, Fresh aloe vera, Good quality sugar, Citric acid, Sodium benzoate.

3.4.3 Chemicals

Chemicals like NaOH, Phenolphthalein, 2,6-dichlorophenol indophenol, Sodium bicarbonate, L-ascorbic acid, Metaphosphoric acid, Potassium oxolate, Lead acetate, Fehling solution A, Fehling solution B, Citric acid, Sodium benzoate, Methylene blue, Hydrochloric acid.

3.4.4 Packaging Materials

Juice bottles of glass were used for packing the prepared RTS.

3.4.5 Instruments

Instruments like Weighing balance, pH meter, Refractometer, Spectrophotometer.

3.5 Methodology:

The experiment is carried under controlled condition at Vegetable Science Laboratory, IGKV, Raipur (C.G.) during Rabi 2019-2020.

3.5.1 Extraction of roselle juice by Hot Water Extraction Method (HWE)

Some healthy diseased free mature calyces were selected, peeled and washed with water after that it was ready for the juice extraction. The Roselle juice was extracted by using the Hot Water Extraction (HWE) method described by (Wong *et al.*, 2003) and was performed by using 1kg dried calyces per 5 litre of distilled boiling water at (100˚C) for 20-25 minutes as described by (Aina and Odipe, 2006). The HWE method was the most effective extraction method resulting in high anthocyanin and ascorbic acid contents of 43g/l (as delphinidin-3-glucoside) and 234g/kg respectively. Fresh roselle extract were filtered with a muslin cloth.

3.5.2 Extraction of aloe vera juice

Fresh aloe leaves free from disease and pests of uniform size with no mechanical damage is selected. The conventional hand-filleting method of processing of aloe vera was created to prevent contaminating the inner fillet with the yellow sap. In this method, the lower (25 mm) portion of the leaf base and tapering point (50-100 mm) of the leaf top is removed. Spines along the leaf margin, top and bottom rind of the leaves were removed with sharp knife and leaves were blanched for 10 minutes at 60-65ºC.

3.5.3 Preparation of RTS

The extracted juice of roselle was blended with the extracted aloe juice in different ratios according to treatments. Now according to the treatments the ratio of 10 per cent roselle and aloe vera juice was blended with measured quantity of water and TSS was checked, treatment was boiled for 2-3 minutes and sugar has added to it until TSS reaches to 10 per cent and sodium benzoate 1gm/lt and citric acid 3gm/lt were added as a preservative. At last when the prepared RTS get

cooled it was filled in glass bottles and sealed properly with crown corking sealing machine and was kept at ambient temperature.

3.5.4 Treatment Details

Treatments	Proportion of juice in the Blends	
	Roselle Juice	**Aloe Vera Juice**
$\mathbf{T_1}$	100 parts	0 parts
$\mathbf{T_2}$	90 parts	10 parts
$\mathbf{T_3}$	80 parts	20 parts
$\mathbf{T_4}$	70 parts	30 parts
$\mathbf{T_5}$	60 parts	40 parts
$\mathbf{T_6}$	50 parts	50 parts
$\mathbf{T_7}$	40 parts	60 parts
$\mathbf{T_8}$	30 parts	70 parts
$\mathbf{T_9}$	20 parts	80 parts
$\mathbf{T_{10}}$	10 parts	90 parts
$\mathbf{T_{11}}$	0 parts	100 parts

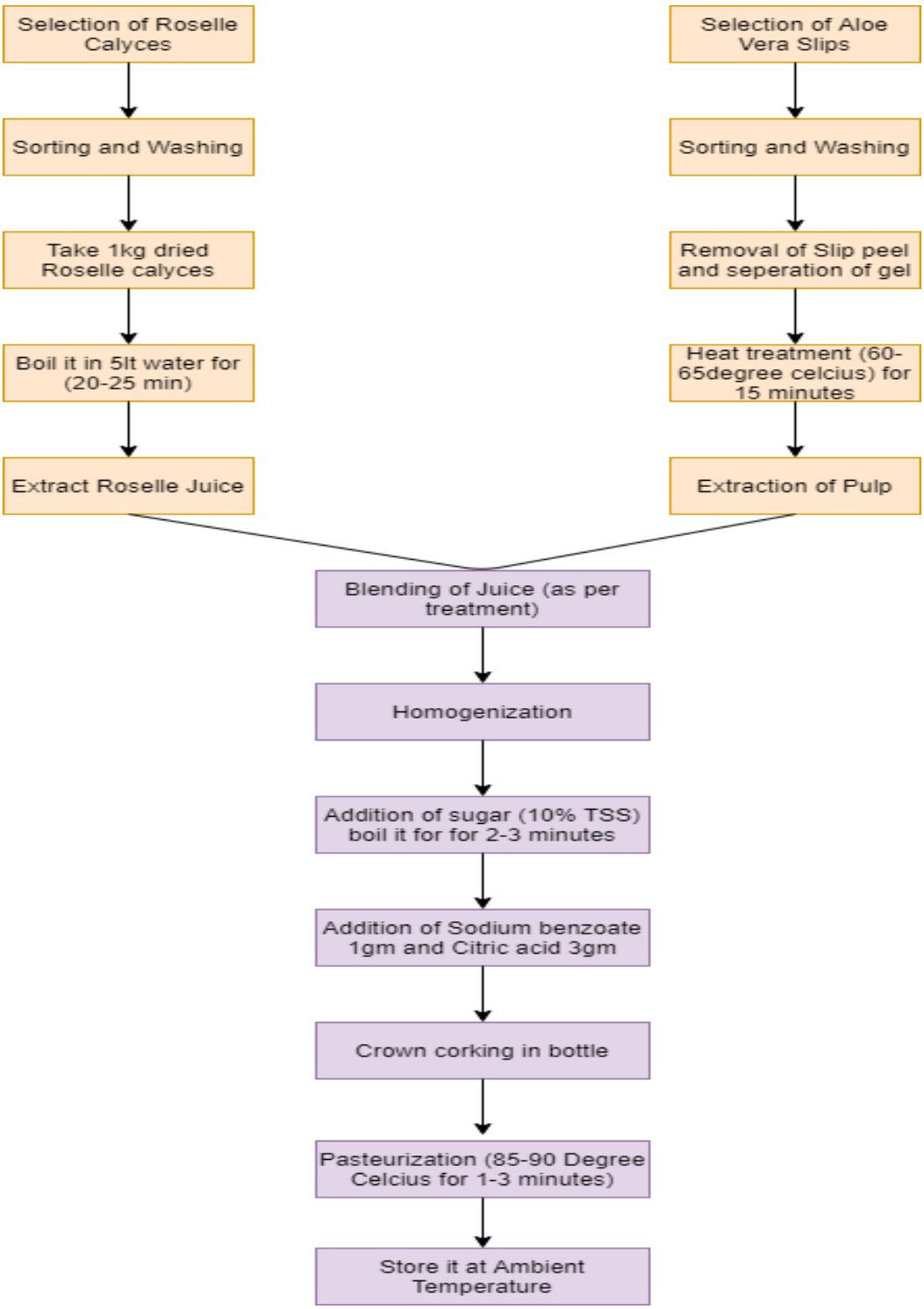

Fig. 3.2 Flow sheet for the preparation of roselle-aloe vera blended RTS during recipe standardization

(a) Boiling of roselle calyces

(b) After 10-15 minutes of boiling

(c) Straining of roselle juice

(d) Extracted roselle juice

PLATE 1:Extraction of roselle juice by HWE (Hot Water Extraction Method)

(a)Aloe vera leaves

(b) Blanching

(c) Extracted aloe vera juice

PLATE 2 : Extraction of aloe vera juice by Hand Filleting Method

(a) Replication 1

(b) Replication 2

(c) Replication 3

PLATE 3 : Prepared roselle aloe vera bleended RTS

3.6 Observations recorded

3.6.1 Physical characteristics

Ten randomly selected roselle capsules were selected from a sample lot for each observation which are required for determining the physical characteristics.

3.6.1.1 Colour of capsule and pulp

Colour is a visual parameter, which gives an idea about its required chemical constituents for processing. Colour of capsule, calyces and pulp were determined visually.

3.6.1.2 Weight of capsule

Ten randomly selected capsules were taken from a sample lot and weighted individually on electric weighing balance. Then average weight of capsules was calculated and expressed in (g).

3.6.1.3 Weight of calyces

Ten randomly selected capsules calyces were taken and weighted individually on electric weighing balance, then average weight of calyces was calculated and expressed in (g).

3.6.1.4 Weight of waste

It includes seed coat, seed and corolla from the capsule of the ten randomly selected capsule and is expressed in (g).

3.6.1.5 Weight of seed

Ten capsules were selected from a sample lot and seed was removed and weighted, then average weight of seed was calculated and expressed in (g).

3.6.1.6 Edible index

It is a ratio of edible part of capsule, multiplied by 100 to the total weight. Separating 10 capsules and taking weight of each capsule (W_l). Edible calyces are

taken separately from the capsule and weight (W_2). It was calculated by given formula and is expressed in percentage.

$$\text{Edible index} = \frac{W_2}{W_1} \times 100$$

3.6.1.7 Waste index :

It is a ratio of waste part of capsule, multiplied by 100 to the total weight. Separating 10 capsules and taking weight of each capsule (W_1). Waste of capsule is taken separately and weight (W_2). It was calculated by given formula and is expressed in percentage.

$$\text{Waste index} = \frac{W_2}{W_1} \times 100$$

3.6.2 Chemical characteristics

The product prepared was stored at room temperature and were analyzed regularly at 15 days interval.

3.6.2.1 Determination of pH

pH refers to the negative logarithm of hydrogen ion concentration.

$$pH = -\log(H^+)$$

pH was determined by using digital pH meter. The samples were taken individually in a beaker and pH meter was dipped into it and the readings were noted carefully.

3.6.2.2 Determination of Total soluble solids (%)

Total soluble solids content of a product was determined by the index of refraction. This was measured by using a hand refractometer, and is referred to as the degrees Brix. This tests the solids concentration of a sucrose containing solution. A little quantity of sample was kept on hand refractometer and total soluble solids were recorded on the scale of the instrument and was expressed in per cent.

3.6.2.3 Determination of titrable acidity (%)

5 ml RTS sample was taken and dissolved in 50 ml of distilled water and from this 20 ml aliquot was taken out and titrated with 0.1 N NaoH using few drops of phenolphthalein as indicator. End point was judged by the appearance of pink colour. The acidity of the juice is expressed in terms of percent acidity. Acidity was calculated by finding out titre value with the help of following formula.

$$\text{Acidity (\%)} = \frac{\text{Titre value} \times \text{Normality} \times \text{Eq. Wt. of Acid} \times \text{Vloume made up} \times 100}{\text{Weight of sample taken} \times \text{Sample taken for estimation} \times 1000}$$

3.6.2.4 Determination of ascorbic Acid (mg/100g)

Reagents

1. **Metaphosphoric acid (HPO_3) solution (3%)**
   For the preparation of 3 per cent metaphosphoric solution 30 gm of metaphosphoric acid sticks was diluted in 1 litre of distilled water.
2. **Dye solution**
   50 mg of 2,6-dichlorophenol-indophenol was dissolved in about 150 ml of hot distilled water, containing 42 mg of Sodium bicarbonate and was cooled and diluted to 200 ml with distilled water, solution was stored in a brown bottle in a refrigerator at 3˚C and standardize every day.
3. **Standard ascorbic acid solution**
   100 mg of L-ascorbic acid was weighted properly and dissolved in a small amount of 3 per cent metaphosphoric acid and volume make up to 100ml with the same solution. 10 ml of this stock solution was diluted to 100 ml with 3 per cent metaphosphoric acid (0.1 mg ascorbic acid/ml).

Standardization of dye

5 ml of standard ascorbic acid solution and 3 percent metaphosphoric acid each was taken in a volumetric flask and was titrated with dye solution filled in the microburette, until pink colour persists for 10 second. Dye factor was calculated (mg of ascorbic acid/ ml of dye) as follows.

$$\text{Dye Factor} = \frac{0.5}{\text{Titre Value}}$$

Sample preparation and titration

10 ml of sample was taken and make upto 100 ml with 3 per cent metaphosphoric acid and filtered. 10 ml of filterate was pipet out into a conical flask and was titrated with standard dye till pink red end point appears.

$$\text{Ascorbic Acid (mg/100g)} = \frac{\text{Titre} \times \text{Dye factor} \times \text{Volume made up} \times 100}{\text{Volume of filterate taken} \times \text{Volume of sample taken}}$$

3.6.2.5 Sugars (%)

Sugars were determined by Lane and Eynon method as described by Ranganna (1997).

Reagents

1. Fehling's solution A : Copper sulphate 69.28g and volume made up to 1 litre.
2. Fehling's solution B : Potassium sodium tartarate 346g and Sodium hydroxide 100g and volume made up to 1 litre.
3. Methylene blue indicator : 1% Aqueous methylene blue.
4. Neutral lead acetate (45%) solution
5. Potassium oxalate (22%) solution
6. Standard invert sugar solution: AR sucrose 9.5 g and concentrated HCL 5 ml and volume made up to 100 ml . The solution was allowed to stand for further three days at 20-25˚C for inversion to take place and could be used for 8-9 month during analysis.

25 ml of invert sugar solution was taken in a flask and to it 50 ml distilled water was added, and then neutralized with 20 per cent NaOH in the presence of phenolphthalein as an indicator until solution turned into pink colour, then acidified with 1 N HCL till pink colour disappears. The volume was made up to the mark with distilled water (1 ml = mg of invert sugar).

3.6.2.5.1 Reducing sugar (%)

Reducing sugar was estimated by taking 25 ml of ready sample and diluted with 100 ml of distilled water, 1 to 2 drop of phenolphthalein was added and titrated with 1 N NaOH till pink colour appears. 2 ml of Lead acetate solution was added it was shaken and allowed to stand for 10 minutes. Thereafter, 2 ml of potassium oxalate solution was added and volume make up of 250 ml was made and the filterate was made by filtering it with the filter paper.

In a 250 ml conical flask, Fehling's solution A and B were taken. Burette was filled with the clarified sugar solution. In an open flame, the conical flask was heated. In hot condition only, 2-4 ml sugar solution was poured and 1-2 drop methylene blue indicator was added immediately. The end point appeared with brick-red colour. The reducing sugar was expressed in per cent and calculated by the following formula.

$$\text{Reducing sugar (\%)} = \frac{\text{Invert sugar (mg)} \times \text{Dilution} \times 100}{\text{Titre} \times \text{Weight or volume of sample taken} \times 1000}$$

3.6.2.5.2 Total sugar (%)

The total sugar was estimated by taking 50 ml aliquot of clarified and deleaded solution. In it 5 ml HCL was added and allowed to stand for 24 hours at room temperature. This has been neutralized with concentrated NaOH solution and volume made up to 250 ml. An aliquot was taken and total sugar were determined as invert sugars.

$$\text{Total sugar as invert sugar (\%)} = \frac{\text{Invert sugar (mg)} \times \text{Dilution} \times 100}{\text{Titre} \times \text{Weight or volume of sample taken} \times 100}$$

Sucrose (%) = (%Total invert sugar - % Reducing sugar originally present × 0.95)
Total sugar (%) = Reducing sugar (%) + Sucrose (%)

3.6.2.5.3 Non-reducing sugar

Non-reducing sugar is determined by subtracting the value of reducing sugar from the total sugar.

Non- reducing sugar (%) = Total sugar (%) – Reducing sugar (%)

3.6.2.6 Determination of anthocyanin

Reagents - 0.1 N HCL

1) Preparation of standard

Standard was prepared by taking 10 ml of sample and diluted with 50 ml of distilled water.

2) Procedure

10 ml of sample was diluted with 50 ml of 0.1 N HCL and was allowed to equilibrate in the dark for 1 hour. Absorbance was recorded at the optical density (O.D.) 510 nm in spectrophotometer. Anthocyanin is determined by the formula:

$$\text{Total O.D/100 ml} = \frac{\text{O.D} \times \text{Volume made up} \times 100}{\text{ml. of juice taken}}$$

$$\text{Total anthocyanin (mg/100ml)} = \frac{\text{Total O.D/ 100 ml}}{87.3}$$

3.6.3 Organoleptic evaluation of Processed Product

The sensory assessment of the processed product was carried out by a panel of judges which include the professors and students of the Vegetable Science Department, College of Agriculture, IGKV, Raipur, (C.G.) in order to determine the acceptability of the product.

Eleven seperate coded samples of blended RTS were presented to a panel of experienced tasters. Descriptive terms were given to different quality attributes such as appearance, colour, taste, flavour and overall acceptability (Preyam and Pilgrim, 1957). Each taster were given an assessment form which includes all these attributes. The panelist were asked to assess the RTS with respect to the attributes described using a 9-point hedonic scale with a rating of extremely like to extremely dislike. The mean score for various product was determined. The final scores were ultimately obtained by averaging the marks of judges. The product which scored 7 or more for total acceptability was considered acceptable. The score card was prepared taking into account the quality characteristics of the products produced.

Organoleptic score	Scale (rating)
9	Liked extremely
8	Liked very much
7	Liked moderately
6	Liked slightly
5	Neither liked nor disliked
4	Disliked slightly
3	Disliked moderately
2	Disliked very much
1	Disliked extremely

3.7 Statistical analysis

Data obtained from chemical analysis of different treatments were recorded during the course of the investigation and subjected to one way statistical analysis by CRBD (Completely Randomized Block Design) as per "Analysis of Variance" method. Using F table, the significance and non-significance data collected from various procedures were measured. The critical difference (C.D) at 5 per cent level of significance were worked out to compare two treatment means, whenever the treatments effects were significant. The mean scores for organoleptic evaluation of the products were determined on the basis of the scores given by the panelists.

Source of Variation	Degree of freedom	Sum of squares	Mean squares	F (Calculated)	F (Tabulated)
Treatment **Experimental Error**	(t-1) T(r-1)	TrSS ESS	TrMS=TrSS÷d.f EMS= ESS÷d.f	TrMS÷d.f	
Total	(rt-1)	TSS			

CHAPTER - IV
RESULTS AND DISCUSSIONS

In present investigation entitled **" Standardization and Evaluation of RTS from blends of Indian Roselle (*Hibiscus sabdariffa L.*) and Aloe Vera *(Aloe barbadensis M.)"*** efforts have been made to use roselle calyces and aloe vera for blending to make RTS. In addition the prepared product was tested for their physio-chemical and sensory qualities at an interval of 15 days for 75 days. The results obtained during the investigation process is presented and discussed here under the headings and subheadings below. Results pertaining to each character are interpreted in this chapter.

4.1 Physico- chemical composition of roselle calyces and aloe vera leaves.

4.2 Organoleptic evaluation of roselle-aloe vera blended RTS during recipe standardization.

4.3 Changes in physico- chemical composition of blended RTS during storage.

4.1 Physico- chemical composition of roselle calyces and aloe vera leaves

The knowledge of raw material's physical and chemical properties helps in the development of processing technology. The quality of ultimate product depends on the quality of raw materials that could be measured in terms of physical and chemical properties. To justify the suitability of roselle calyces and aloe vera leaves in preparation of RTS, it is necessary to study it's physical and chemical properties. During present investigation, the physical and chemical properties of roselle calyces and aloe vera leaves were analyzed and results are summarized as follows.

4.1.1 Physical parameters of roselle calyces

The data recorded on parameters of roselle calyces are presented on (Table 4.1) The calyces of the roselle were thick and fleshy having a diameter and length

of 2.5 cm and 4.5 cm, respectively. Colour of roselle is red wine, colour of pulp is pinkish red, weight of capsule is 4.25 g, weight of calyces is 2.42 g, weight of waste from capsule is 1.83 g, weight of seed is 0.8 g and edible index and waste index recorded are 56.94 per cent and 43.05 per cent, respectively.

4.1.2 Physical parameters of Aloe vera leaves

The data recorded on parameter of aloe vera leaves are presented on (Table 4.2). Observations such as leaf length, leaf width, weight of pulp, visual leaf and gel colour and consistency of gel. The aloe vera leaves are translucent green, having a length and width of 55 and 7 cm, respectively. The weight of individual leaves ranged from 450 g and the pulp weight ranged from 350 g. The gel looked viscous, with a slimy translucent mass of light yellow green colour.

4.1.3 Chemical composition of Roselle calyces

The data recorded on parameter of roselle calyces such as total soluble solids, ascorbic acid, acidity, pH and sugars are tabulated in (Table 4.3) The total soluble solids and pH of roselle calyces was 4.8 per cent and 2.34, respectively. While acidity and ascorbic acid recorded are 3.15 per cent and 17.68 mg/100g, respectively. Total sugar recorded was 3.28 per cent, while reducing and non-reducing sugar are 1.62 per cent and 1.66 per cent respectively.

4.1.4 Chemical composition of Aloe vera gel

The data recorded on parameter of aloe vera gel such as total soluble solids, pH, ascorbic acid, acidity and sugars are tabulated in (Table 4.4) The total soluble solids, pH, acidity, ascorbic acid, total sugar, reducing sugar, non-reducing sugar and moisture were found to be 1.2 per cent, 4.33, 0.32 per cent, 3.89 mg/100g, 1.52 per cent, 0.036 per cent, 1.484 per cent and 97.34 per cent, respectively.

Table 4.1 : Physical parameters of roselle

S.No.	Parameters	Observations
01.	Diameter of roselle capsule (cm)	2.5
02.	Length of roselle capsule (cm)	4.5
03.	Colour of capsule (Visually)	Wine red
04.	Colour of pulp (Visually)	Pinkish red
05.	Weight of capsule (g)	4.25
06.	Weight of calyces (g)	2.42
07.	Weight of waste from capsule (g) (Seed coat + Seed + Corolla)	1.83
08.	Weight of seed (g)	0.8
09.	Edible index(%)	56.94
10.	Waste index (%)	43.03

Table 4.2 : Physical parameters of aloe vera leaves

S.No.	Parameters	Observations
01.	Leaf colour (Visually)	Translucent green
02.	Leaf length (cm)	55
03.	Leaf width (cm)	7
04.	Leaf weight (g)	450
05.	Pulp weight (g)	350
06.	Gel colour	Translucent mass having light yellowish green colour
07.	Gel consistency	Viscous and slimy

Table 4.3 : Chemical composition of roselle

S.No.	Parameters	Composition
01.	Total soluble solids (%)	4.8
02.	pH	2.34
03.	Acidity (%)	3.15
04.	Ascorbic acid (mg/100g)	17.68
05.	Total sugar (%)	3.28
06.	Reducing sugar (%)	1.62
07.	Non-reducing sugar (%)	1.66

Table 4.4 : Chemical composition of aloe vera gel

S.No.	Parameters	Composition
01.	Moisture (%)	97.34
02.	Total soluble solids (%)	1.2
03.	pH	4.33
04.	Acidity (%)	0.32
05.	Ascorbic acid (mg/100g)	3.89
06.	Total sugar (%)	1.52
07.	Reducing sugar (%)	0.036
08.	Non-reducing sugar (%)	1.484

4.2 Organoleptic evaluation of roselle-aloe vera blended RTS during recipe standardization

A processed product was evaluated on the basis of organoleptic score at the time of preparation for various sensory attributes, like Colour and appearance, flavour, taste and overall acceptability and was depicted in (Table 4.5) and (Figure 4.1)

4.2.1. Colour and Appearance

The data of colour and appearance score in blended RTS at the time of preparation showed that maximum score was obtained by T_2 and T_3 which scored highest score of 8.6 which is superior over all other treatments followed by T_4 which scored 8.2. Whereas T_{10} scored minimum score of 4.2.

4.2.2. Flavour

The data on organoleptic score for flavour shows that maximum score of 8.4 was obtained by T_4 followed by T_8 which scored 8. While minimum score of 3.8 was obtained by T_{10} followed by T_{11} which scored 4.2.

4.2.3. Taste

The data on organoleptic score for taste showed that maximum score was obtained by T_3 which scored highest score of 7.8 followed by T_1 which obtained 7.6. Whereas minimum score was noted on T_{10} which scored 3.8 followed by T_9 which scored 5.

4.2.4. Overall Acceptability

It is evident from the data that maximum score for overall acceptability was 8.0 which was scored by T_3 followed by T_4 which scored 7.9 and minimum score of 3.9 was scored by T_{10} followed by T_{11} which scored 4.7.

After evaluating all the organoleptic attributes, it was found that T_3 was the best treatment and superior among all the other treatments as it scored highest and was liked very much by panels of judges. T_3 has the composition of 80 per cent

Table 4.5 : Organoleptic evaluation of roselle-aloe vera blended RTS during recipe standardization

Treatments	Colour And Appearance	Flavour	Taste	Overall Acceptability	Rating
T_1 (Roselle 100 parts + Aloe vera 0 parts)	8.0	8.0	7.6	7.8	Like Moderately
T_2 (Roselle 90 parts + Aloe vera 10 parts)	8.6	7.6	7.4	7.8	Like Moderately
T_3 (Roselle 80 parts + Aloe vera 20 parts)	8.6	7.8	7.8	8.0	Like Very Much
T_4 (Roselle 70 parts + Aloe vera 30 parts)	8.2	8.4	7.2	7.9	Like Moderately
T_5 (Roselle 60 parts + Aloe vera 40 parts)	7.4	7.8	6.8	7.3	Like Moderately
T_6 (Roselle 50 parts + Aloe vera 50 parts)	7.0	6.4	6.2	6.5	Like Slightly
T_7 (Roselle 40 parts + Aloe vera 60 parts)	6.2	6.8	6.0	6.3	Like Slightly
T_8 (Roselle 30 parts + Aloe vera 70 parts)	6.2	5.2	5.0	5.4	Neither Like nor Dislike
T_9 (Roselle 20 parts + Aloe vera 80 parts)	5.2	5.4	5.0	5.2	Neither Like nor Dislike
T_{10} (Roselle 10 parts + Aloe vera 90 parts)	4.2	3.8	3.8	3.9	Dislike Moderately
T_{11} (Roselle 0 parts + Aloe vera 100 parts)	4.6	4.2	5.4	4.7	Dislike Slightly

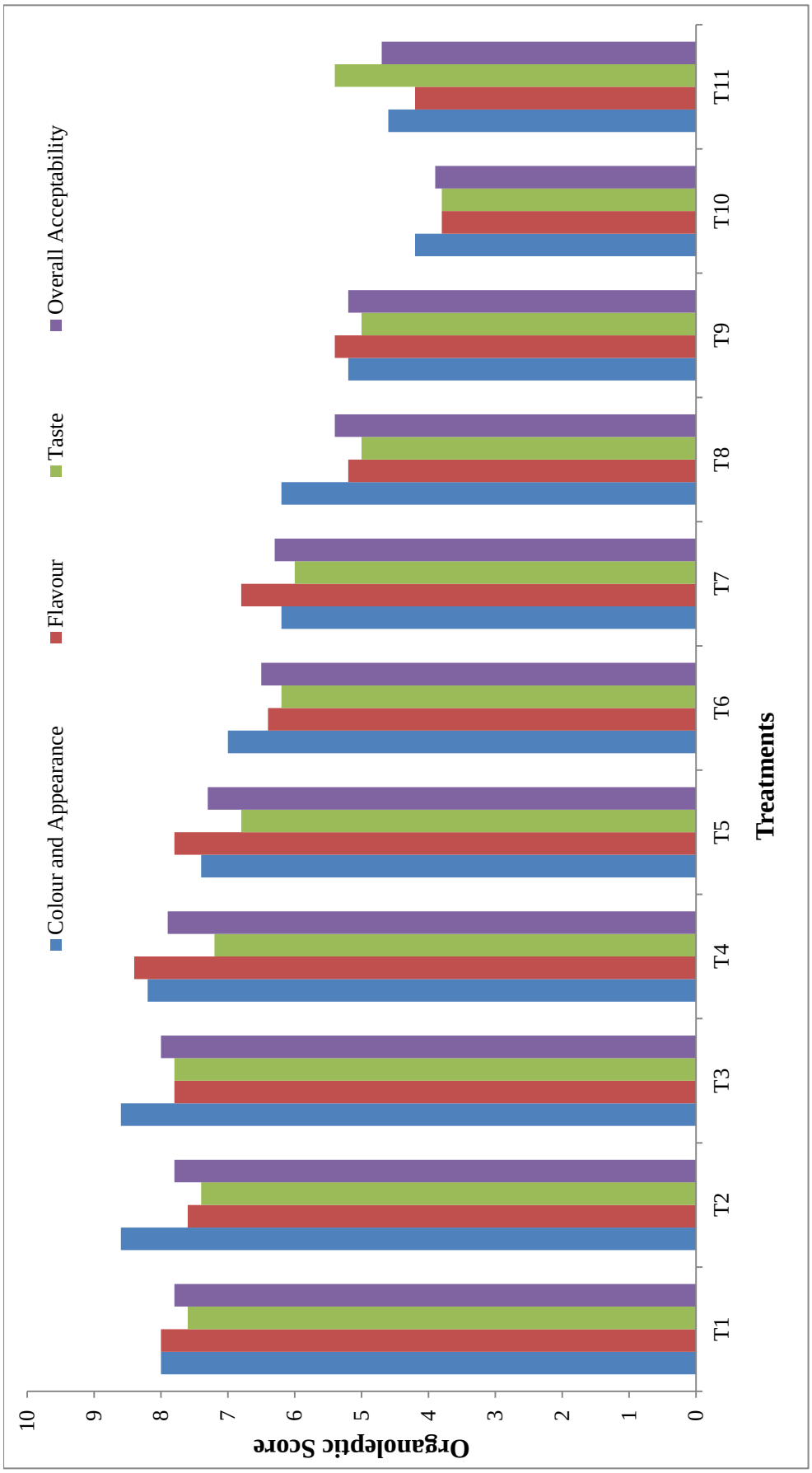

Fig 4.1 : Organoleptic evaluation of roselle-aloe vera blended RTS during recipe standardization

roselle juice and 20 per cent aloe vera juice which was found as a best combination among other treatments and was considered as a most acceptable blending combination.

4.3 Changes in physico- chemical composition of RTS during storage

Roselle-aloe vera blended RTS was prepared and analyzed for physico-chemical composition such as total soluble solids, pH, acidity, ascorbic acid, total sugar, reducing sugar, non-reducing sugar, anthocyanin. The changes in the physico-chemical composition of blended RTS on storage was analyzed and interpreted with the help of tables and graphical representations.

4.3.1 Total soluble solids (TSS)

Changes on TSS content of roselle-aloe vera blended RTS beverages having different concentrations of roselle and aloe vera at each eleven treatments are recorded at the various interval of 15 days that is 0, 15, 30, 45, 60 and 75 days of the storage period. The perusal of data pertaining to TSS of blended RTS has been presented in (Table 4.6) and graphically depicted in (Figure 4.2)

The critical evaluation of the data showed that the effect of different treatment combinations on TSS content of roselle and aloe vera blended RTS during storage period was found significant except at 0 days of storage.

At the time of preparation non-significant difference was observed in TSS per cent of blended RTS with all the treatment having TSS of 10 per cent.

The TSS value at 15 days of storage was recorded maximum for T_1 having (9.25%) followed by T_2 and T_3 having TSS (8.6%). While the minimum TSS of (5%) was noted in treatment T_{10} followed by T_{11} (6.0%).

The TSS value was recorded maximum for T_1 having TSS (8.56%) followed by T_2 and T_3 having (7.8% and 7.5%) respectively and minimum TSS was noted on T_{10} (4.2%) at 30 days of storage. The value of TSS do not show significant changes at 45^{th}, 60^{th} and 75^{th} days of storage.

Table 4.6 : Changes in Total soluble solids (%) of roselle-aloe vera blended RTS during storage under ambient condition

Treatments	Total soluble solids (TSS) %					
	Storage periods (in days)					
	0	**15**	**30**	**45**	**60**	**75**
T_1 (100 parts Roselle juice + 0 parts Aloe vera juice)	10	9.2	8.56	8.4	8.16	8.13
T_2 (90 parts Roselle juice + 10 parts Aloe vera juice)	10	8.6	7.8	7.5	7.3	7.16
T_3 (80 parts Roselle juice + 20 parts Aloe vera juice)	10	8.6	7.5	7.5	7.2	7.16
T_4 (70 parts Roselle juice + 30 parts Aloe vera juice)	10	8.4	7.4	7.3	7.0	6.60
T_5 (60 parts Roselle juice + 40 parts Aloe vera juice)	10	8.3	7.16	7.2	7.0	6.46
T_6 (50 parts Roselle juice + 50 parts Aloe vera juice)	10	7.8	7.0	7.0	7.0	6.40
T_7 (40 parts Roselle juice + 60 parts Aloe vera juice)	10	7.5	6.7	6.6	6.2	6.20
T_8 (30 parts Roselle juice + 70 parts Aloe vera juice)	10	7.1	6.5	6.4	6.13	6.13
T_9 (20 parts Roselle juice + 80 parts Aloe vera juice)	10	7.0	6.2	6.13	6.0	5.8
T_{10} (10 parts Roselle juice + 90parts Aloe vera juice)	10	5.0	4.2	4.0	4.0	4.0
T_{11} (0 parts Roselle juice + 100parts Aloe vera juice)	10	6.0	5.6	5.4	5.4	5.0
Mean	**10**	**7.5**	**6.7**	**6.6**	**6.49**	**6.2**
SE (m)±	**_**	**0.03**	**0.033**	**0.032**	**0.032**	**0.036**
CV (%)	**_**	**0.687**	**0.849**	**0.82**	**0.847**	**0.998**
CD at 5%	**_**	**0.089**	**0.098**	**0.094**	**0.094**	**0.107**

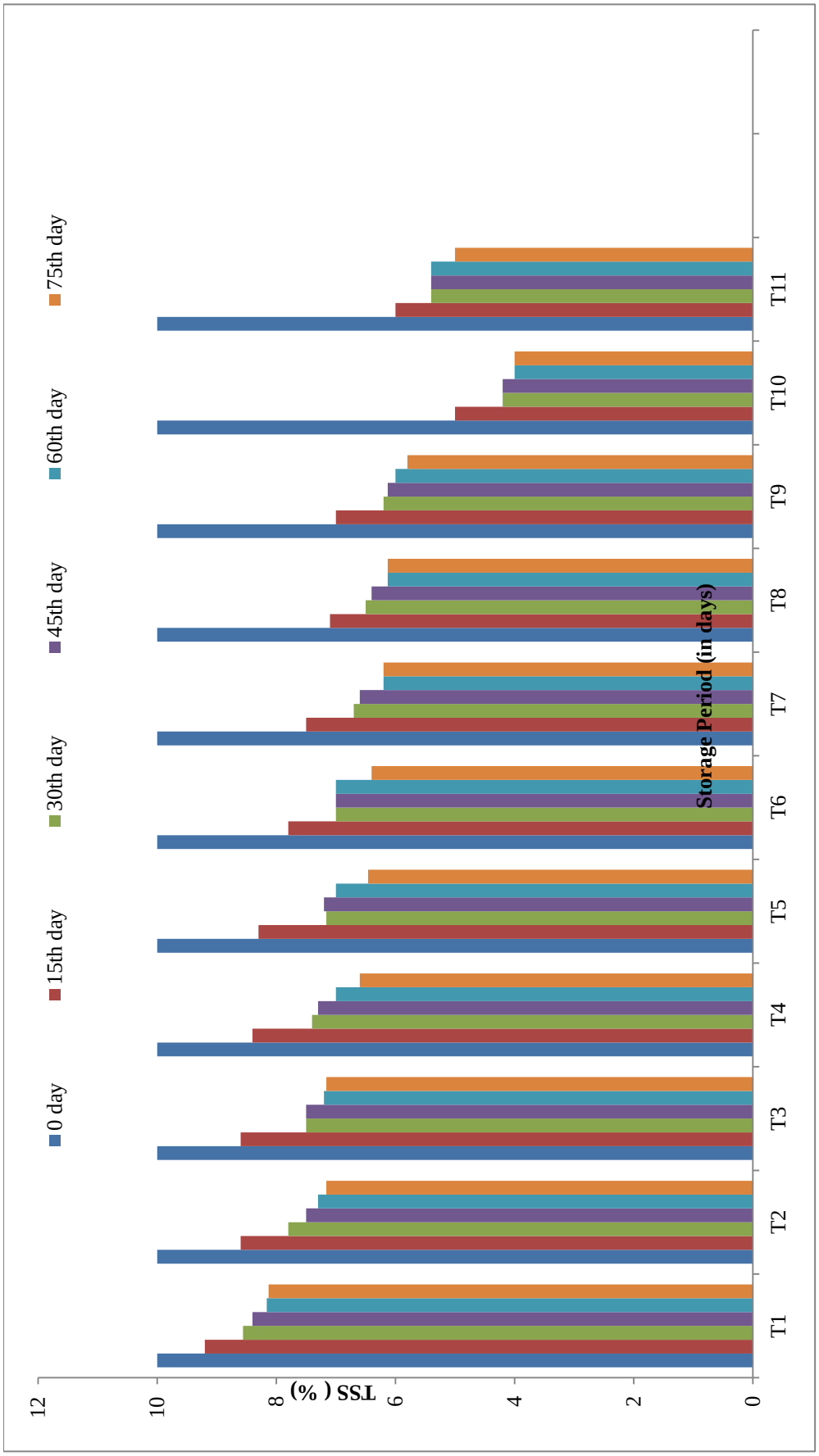

Fig 4.2: Changes in Total soluble solids (%) of roselle-aloe vera blended RTS during storage under ambient condition

From the above observation it was found that the TSS content of the blended RTS decreased significantly from 15 to 30th days of storage period. While a non-significant changes were observed at 45th, 60th and 75th days of storage. The TSS content of T_1 remains maximum during all the storage days and decreased from (9.2% to 8.13%) from 15th to 75th days and minimum TSS was recorded for T_{10} which decreased from (5% to 4%) from 15th to 75th days.

It is vivid from the above observation that minimum change in TSS was observed for T_1 which implies that it is the most stable treatment among all the other treatment having treatment combination of 100 per cent roselle juice, While T_{10} shows maximum change during storage.

A similar finding was observed by Singh and Gaikwad (2012) who found the decrease in TSS concentration of blended lemon and bitter gourd RTS during storage period of 60 days, which may be due to hydrolysis of polysaccharides into monosaccharide and oligosaccharides.

4.3.2 pH

It is apparent from the present findings (Table 4.7 and Fig 4.3) that no significant changes were observed in pH from 0 to 15th day of storage. From 30th to 75th day there is slight decrease in pH content, this slight change may be due to increased acidity during storage period. However microbial development may also contribute to a decrease in pH.

The maximum pH content (3.40) was observed in treatment T_{11} having 100 per cent aloe vera juice and minimum pH content (2.8) was observed in treatment T_1 having 100 per cent roselle juice at 0 and 15th days. It was found that pH content increased with decrease in concentration of roselle.

On 30th day maximum pH content (3.4) was found in treatment T_{11} which is same as 0 and 15th day of storage but minimum pH content decreased from 2.8 to 2.76 from 0 and 15th day to 30th day of storage.

No significant changes were observed at 45th, 60th and 75th days of storage period.

Table 4.7: Changes in pH of roselle-aloe vera blended RTS during storage under ambient condition

Treatments	pH					
	Storage period (in days)					
	0	**15**	**30**	**45**	**60**	**75**
T_1 (100 parts Roselle juice + 0% partsAloe vera juice)	2.8	2.8	2.7	2.6	2.6	2.6
T_2 (90 parts Roselle juice + 10 partsAloe vera juice)	2.8	2.8	2.7	2.7	2.6	2.6
T_3 (80 partsRoselle juice + 20 parts Aloe vera juice)	2.9	2.9	2.9	2.8	2.6	2.6
T_4 (70 parts Roselle juice + 30parts Aloe vera juice)	2.9	2.9	2.9	2.8	2.7	2.6
T_5 (60 parts Roselle juice + 40 parts Aloe vera juice)	3.0	3.0	3.0	2.8	2.7	2.7
T_6 (50 parts Roselle juice + 50 parts Aloe vera juice)	3.0	3.0	3.0	2.8	2.7	2.7
T_7 (40 parts Roselle juice + 60 parts Aloe vera juice)	3.0	3.0	3.0	2.8	2.8	2.7
T_8 (30 parts Roselle juice + 70 parts Aloe vera juice)	3.1	3.1	3.1	2.9	2.8	2.7
T_9 (20 parts Roselle juice + 80 parts Aloe vera juice)	3.2	3.2	3.1	3.1	3.0	2.9
T_{10} (10 parts Roselle juice + 90parts Aloe vera juice)	3.3	3.3	3.3	3.2	3.1	2.9
T_{11} (0 parts Roselle juice + 100 parts Aloe vera juice)	3.4	3.4	3.4	3.4	3.1	3.1
Mean	**3.04**	**3.04**	**3.03**	**2.92**	**2.81**	**2.76**
SE (m)±	**0.010**	**0.010**	**0.020**	**0.025**	**0.025**	**0.025**
CV (%)	**0.572**	**0.572**	**1.148**	**1.460**	**1.511**	**1.541**
CD at 5%	**0.030**	**0.030**	**0.059**	**0.073**	**0.073**	**0.073**

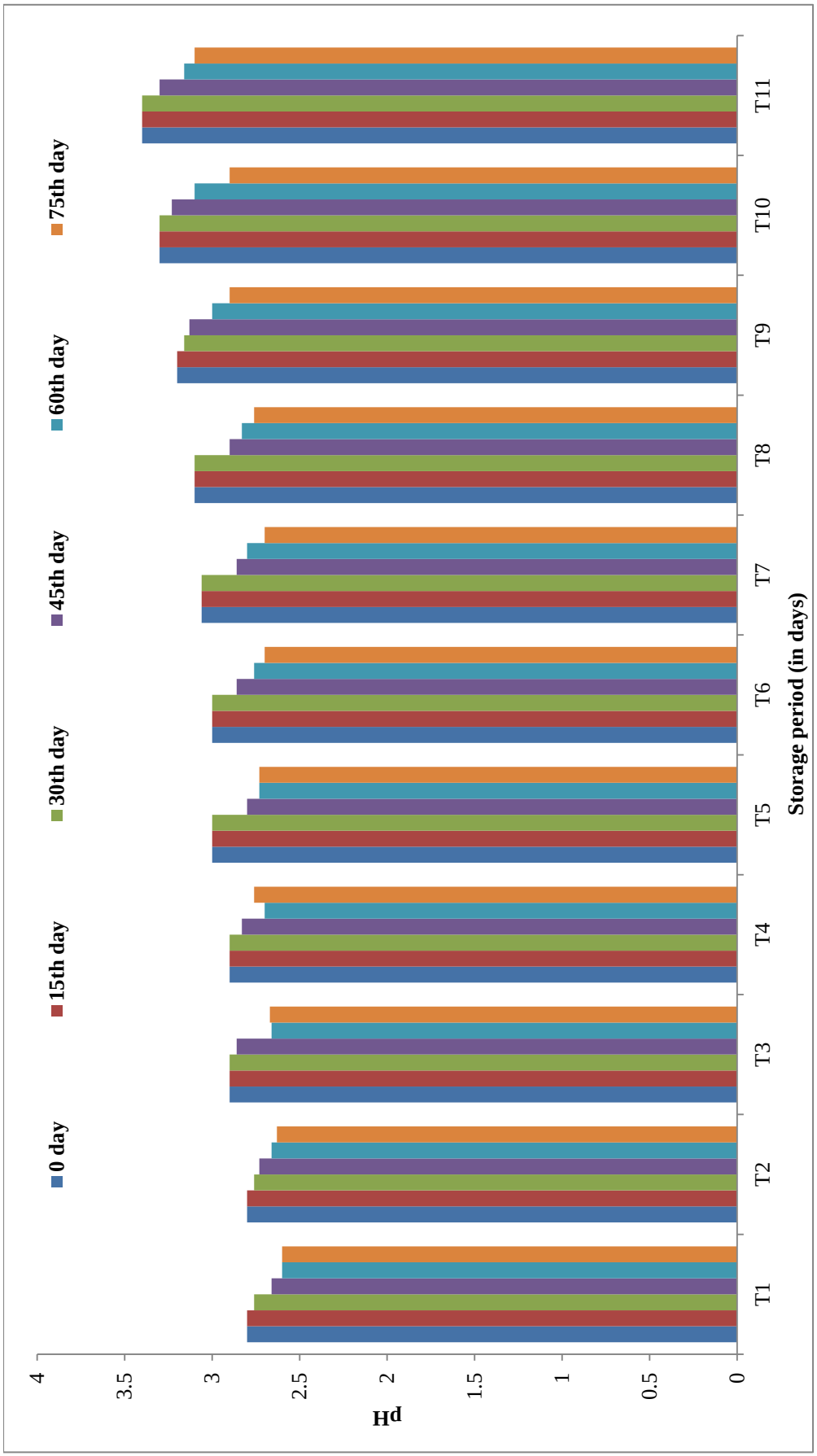

Fig 4.3 : Changes in pH of roselle-aloe vera blended RTS during storage under ambient condition

From the above result it is clear that pH content of treatment T_{11} is maximum and treatment T_1 is minimum during all the days of storage. The pH content decreased non-significantly from 30th to 75th days by showing very slight changes in pH. No significant changes were observed during 0 to 15th day of storage. Initially the pH was 2.8 for the T_1 which is reduced to 2.6 at 75th day of storage, similarly the pH was 3.40 initially for the T_{11} which was reduced to 3.16 at 75th day of storage period. The pH content increased from T_1 to T_{11} with the decreased concentration of roselle.

From the above result it is clear that treatment T_2 is the most stable having treatment combination 90 per cent roselle and 10 per cent aloe vera as it shows minimum changes during storage and T_7 is the least stable.

The pH of blended RTS beverages could be associated inversally with the acidity of RTS beverages and decreased with an increase in storage period as stated by Girdharilal (1988). During storage changes in pH can be due to changes in chemical properties which are affected by storage conditions. Fruit juices have a low pH , since organic acids are comparatively abundant Tasnim *et al*., (2010). The decrease in pH may be associated with increased acidity due to the conversion of reducing sugar to acids resulting in lower pH of the product due to microbial action. A similar result was reported by Adegoke *et al.,* (1995) in a fermented beverage sekete who recorded a lower pH due to the high acid level produced. Singh and Gaikwad (2012) found decrease in pH and rise in acidity during storage period of 60 days, which might be due to the shortage of carbohydrates present in bitter gourd lemon drinks due to the activity of microorganisms that produces acids in the drink.

4.3.3 Acidity

The data presented in (Table 4.8 and Figure 4.4) revealed that there was a slight increase in titrable acidity from 0 to 75th days of storage under ambient condition as the stoage duration advanced.

The maximum acidity at 0 day was noted on treatment T_1 (0.18%) and minimum acidity was noted on treatment T_{11} (0.14%).

At 15th day maximum acidity remain same (0.18%) for treatment T_1 as observed in 0 day, while minimum acidity was noted on T_{11} which changes from 0.14 percent to 0.15 per cent. The acidity value at 30th to 75th days do not show much changes.

.From the above observation it was found that maximum titrable acidity was found on T_1 during all the days of storage, which increase from (0.18% to 0.24%), while minimum acidity was noted on T_{11} during all the storage period, which increases from (0.14% to 0.18%). It was noted that there was a significant increase in acidity per cent from 0 to 75th day of observation.

A maximum changes was noted on treatment T_{11}. While minimum changes were observed on treatments T_7, T_{10} and T_9 respectively and are said to be stable.

Acidity decreased from T_1 to T_{11} due to decrease in concentration of roselle in each treatment. T_1 having 100 per cent roselle shows maximum acidity percentage, and minimum was noted on T_{11} having 100 per cent aloe vera and no roselle. pH is another factor, acidity increases due to decrease in pH. The acidity of blended RTS beverages could be associated inversally with the pH of RTS beverages and increased with an increase in storage period. The increase in acidity of RTS may be due the decomposition of fermentable sugars added. Changes in the acid content of the drinks have influenced by storage temperature, storage containers and also storage periods. The rise in acidity during storage may be due to the production of organic acid by the degradation of ascorbic acid.

Fasoyiro *et al.,* (2005) found similar trend of increasing acidity while studying the storage stability of roselle drinks and Adegoke *et al*., (1995) while studying sekete (fermented drinks). Chauhan *et al.,* (1997) also reported the similar findings that acidity of health drink increased over a six month storage period which may be due to degradation of ascorbic acid or pectin hydrolysis. Karanjalker *et al.,* (2013) reported similar findings. A similar trend was reported by Lohar *et al.,* (2010) revealed that the acid content of the RTS carbonated beverage prepared from karonda fruits showed an increased in acidity during storage, regardless of storage temperature. Chitra (2000) observed a steady rise in acidity in the banana squashes prepared from clarified Rasthali, Poovan juices and combination of both

Table 4.8 : Changes in acidity (%) of roselle-aloe vera blended RTS during storage under ambient condition

Treatments	Acidity (%)					
	Storage period (in days)					
	0	15th	30th	45th	60th	75th
T_1 (100 parts Roselle juice + 0 parts Aloe vera juice)	0.180	0.180	0.200	0.200	0.220	0.240
T_2 (90 parts Roselle juice + 10 parts Aloe vera juice)	0.180	0.180	0.193	0.193	0.200	0.233
T_3 (80 parts Roselle juice + 20 partsAloe vera juice)	0.170	0.177	0.180	0.190	0.200	0.220
T_4 (70 parts Roselle juice + 30parts Aloe vera juice)	0.170	0.173	0.180	0.190	0.190	0.220
T_5 (60 parts Roselle juice + 40 parts Aloe vera juice)	0.160	0.170	0.177	0.187	0.190	0.200
T_6 (50 parts Roselle juice + 50 parts Aloe vera juice)	0.160	0.167	0.177	0.187	0.187	0.200
T_7 (40 parts Roselle juice + 60 parts Aloe vera juice)	0.157	0.160	0.173	0.180	0.187	0.190
T_8 (30 parts Roselle juice + 70 partsAloe vera juice)	0.153	0.160	0.170	0.177	0.180	0.187
T_9 (20 parts Roselle juice + 80 parts Aloe vera juice)	0.150	0.157	0.167	0.170	0.180	0.183
T_{10} (10 parts Roselle juice + 90 parts Aloe vera juice)	0.147	0.153	0.163	0.173	0.177	0.180
T_{11} (0 parts Roselle juice + 100 parts Aloe vera juice)	0.140	0.150	0.160	0.167	0.177	0.180
Mean	0.160	0.166	0.176	0.183	0.189	0.203
SE (m)±	0.002	0.002	0.003	0.003	0.002	0.002
CV (%)	1.877	2.344	2.961	2.853	1.835	2.100
CD at 5%	0.005	0.007	0.009	0.009	0.006	0.007

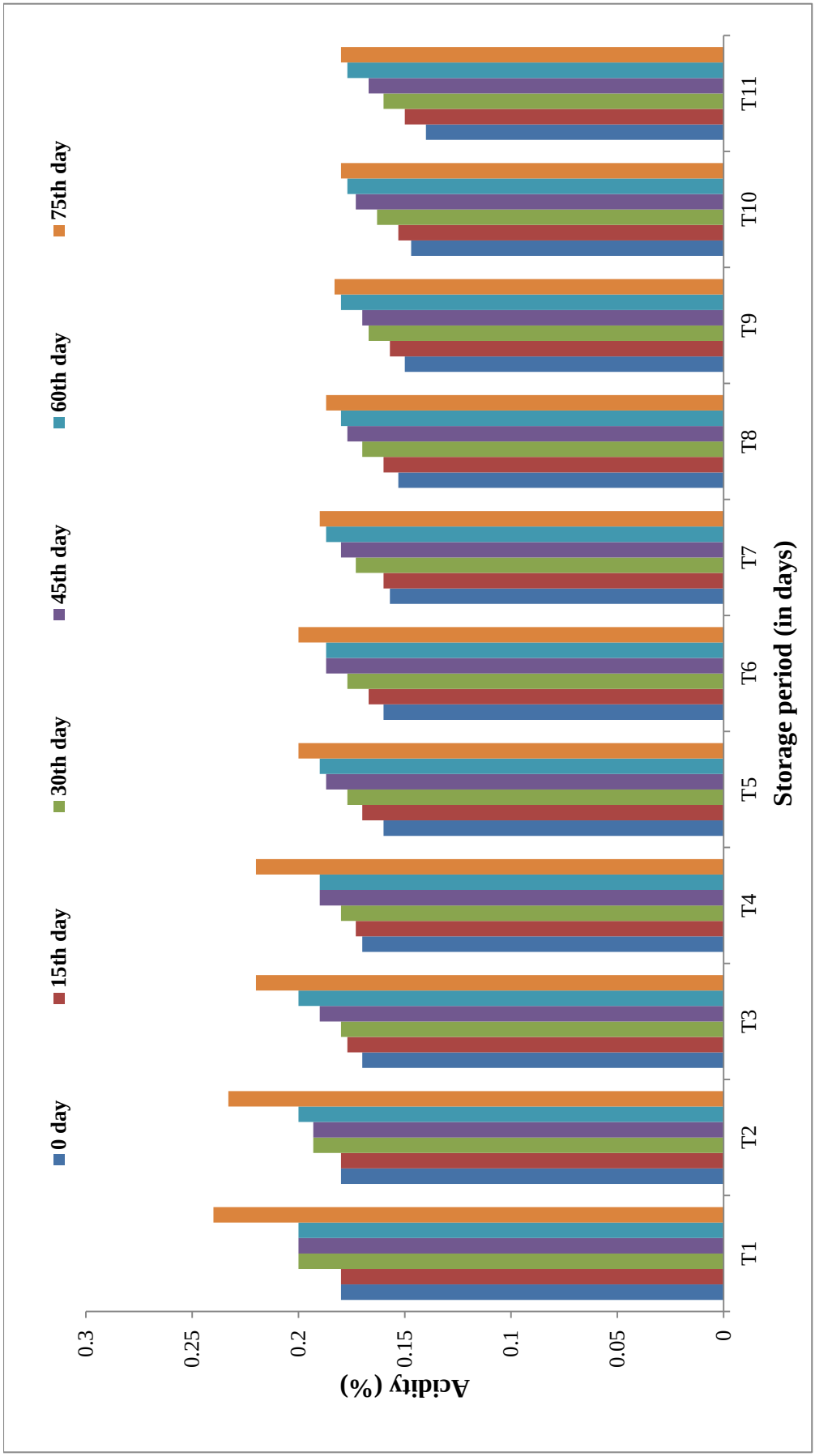

Fig 4.4 Changes in acidity (%) of roselle-aloe vera blended RTS during storage under ambient condition

packed in glass bottles and kept in room and refrigerated condition during the storage period of 300 days.

Acidity decreased from T_1 to T_{11} due to decrease in concentration of roselle in each treatment. T_1 having 100 per cent roselle shows maximum acidity percentage, and minimum was noted on T_{11} having 100 per cent aloe vera and no roselle. pH is another factor, acidity increases due to decrease in pH. The acidity of blended RTS beverages could be associated inversally with the pH of RTS beverages and increased with an increase in storage period. The increase in acidity of RTS may be due the decomposition of fermentable sugars added. Changes in the acid content of the drinks have influenced by storage temperature, storage containers and also storage periods. The rise in acidity during storage may be due to the production of organic acid by the degradation of ascorbic acid.

Fasoyiro *et al.,* (2005) found similar trend of increasing acidity while studying the storage stability of roselle drinks and Adegoke *et al*., (1995) while studying sekete (fermented drinks). Chauhan *et al.*, (1997) also reported the similar findings that acidity of health drink increased over a six month storage period which may be due to degradation of ascorbic acid or pectin hydrolysis. Karanjalker *et al.,* (2013) reported similar findings. A similar trend was reported by Lohar *et al*., (2010) revealed that the acid content of the RTS carbonated beverage prepared from karonda fruits showed an increased in acidity during storage, regardless of storage temperature. Chitra (2000) observed a steady rise in acidity in the banana squashes prepared from clarified Rasthali, Poovan juices and combination of both packed in glass bottles and kept in room and refrigerated condition during the storage period of 300 days.

4.3.4 Ascorbic acid

It is apparent from the results presented in (Table 4.9 and Figure 4.5) that the ascorbic acid content showed an decreasing trend throughout the storage period.

The maximum ascorbic acid at 0 day was recorded in T_{11} (4.6 mg/100g) and minimum ascorbic acid was recorded in T_1 (3.0 mg/100g). At 15^{th} days

maximum and minimum ascorbic acid was noted in T_{11} and T_1 are (4.2 mg/100g) and (2.7 mg/100g) respectively.

The value of ascorbic acid at 30th and 45th days do not show much changes and were found similar .

No significant changes were observed at 60th to 75th in ascorbic acid content.

It is vivid From the above observation that ascorbic acid content was found maximum for T_{11} during entire period of observation, which shows a decreasing trend and decreased significantly from (4.6 mg/100 to 3.5 mg/100g) from 0 to 75th days of storage period. Similarly minimum ascorbic acid content was noted on T_1 during entire period of observation, which also decreased from (3.0 mg/100g to 2.4 mg/100g) from 0 to 75th day. It was also observed that treatment T_2 is most stable treatment among all and treatment T_{11} is least stable.

During processing and subsequent oxidation in storage, the decline in ascorbic acid concentration may be due to thermal degradation because it is very sensitive to heat and pressure treatment, Oxidation and light. It may be due to ascorbic acid conversion to dehydroxy ascorbic acid. Both ascorbic acid and dehydroxy ascorbic acid are highly volatile and unstable forms of vitamin C.

The ascorbic acid content of all roselle fruit blends decreased significantly during storage as the storage time progressed, possibly due to the fact that ascorbic acid being sensitive to oxygen, light and heat and is quickly oxidized by enzymatic and non-enzymatic catalysts in the presence of oxygen (Ziena 2000). The fact that ascorbic acid is particularly sensitive to high temperature may explain a decrease in ascorbic acid content, and the vitamins are easily lost during food processing and storage. The amount of ascorbic acid in the products dependent on the level of the pulp used (Brock *et al*., 1998). Similar pattern of decrease in ascorbic acid was observed in watermelon juice by Saini and Baine (1994), who recorded that more than 25-35 per cent of ascorbic acid was lost after 26 weeks of storage. Teotia *et al.*, (1997) reported a similar trend of decrease in content of ascorbic acid in muskmelon RTS beverage during storage. A decrease in ascorbic acid content

Table 4.9 : Changes in ascorbic acid (mg/100g) of roselle-aloe vera blended RTS during storage under ambient condition

Treatments	Ascorbic Acid (mg/100g)					
	Storage period (in days)					
	0	15	30	45	60	75
T_1 (100 parts Roselle juice + 0 parts Aloe vera juice)	3.00	2.700	2.700	2.467	2.400	2.400
T_2 (90 parts Roselle juice + 10 parts Aloe vera juice)	3.147	2.740	2.700	2.500	2.467	2.400
T_3 (80 parts Roselle juice + 20 parts Aloe vera juice)	3.147	2.800	2.720	2.600	2.500	2.467
T_4 (70 parts Roselle juice + 30parts Aloe vera juice)	3.220	2.867	2.800	2.700	2.700	2.500
T_5 (60 parts Roselle juice + 40 parts Aloe vera juice)	3.220	2.967	2.900	2.740	2.700	2.600
T_6 (50 parts Roselle juice + 50parts Aloe vera juice)	3.680	3.120	2.933	2.800	2.740	2.700
T_7 (40 parts Roselle juice + 60 parts Aloe vera juice)	3.680	3.220	3.000	2.800	2.800	2.760
T_8 (30 parts Roselle juice + 70parts Aloe vera juice)	3.680	3.223	3.000	2.900	2.800	2.800
T_9 (20 parts Roselle juice + 80 parts Aloe vera juice)	4.093	4.120	3.220	3.000	2.900	2.833
T_{10} (10 parts Roselle juice + 90 parts Aloe vera juice)	4.140	4.120	4.000	3.220	3.000	2.900
T_{11} (0 parts Roselle juice + 100parts Aloe vera juice)	4.600	4.200	4.100	4.000	3.500	3.220
Mean	3.600	3.279	3.097	2.884	2.773	2.689
SE (m)±	0.034	0.015	0.012	0.012	0.012	0.014
CV (%)	1.649	0.817	0.655	0.704	0.732	0.915
CD at 5%	0.101	0.046	0.035	0.035	0.035	0.042

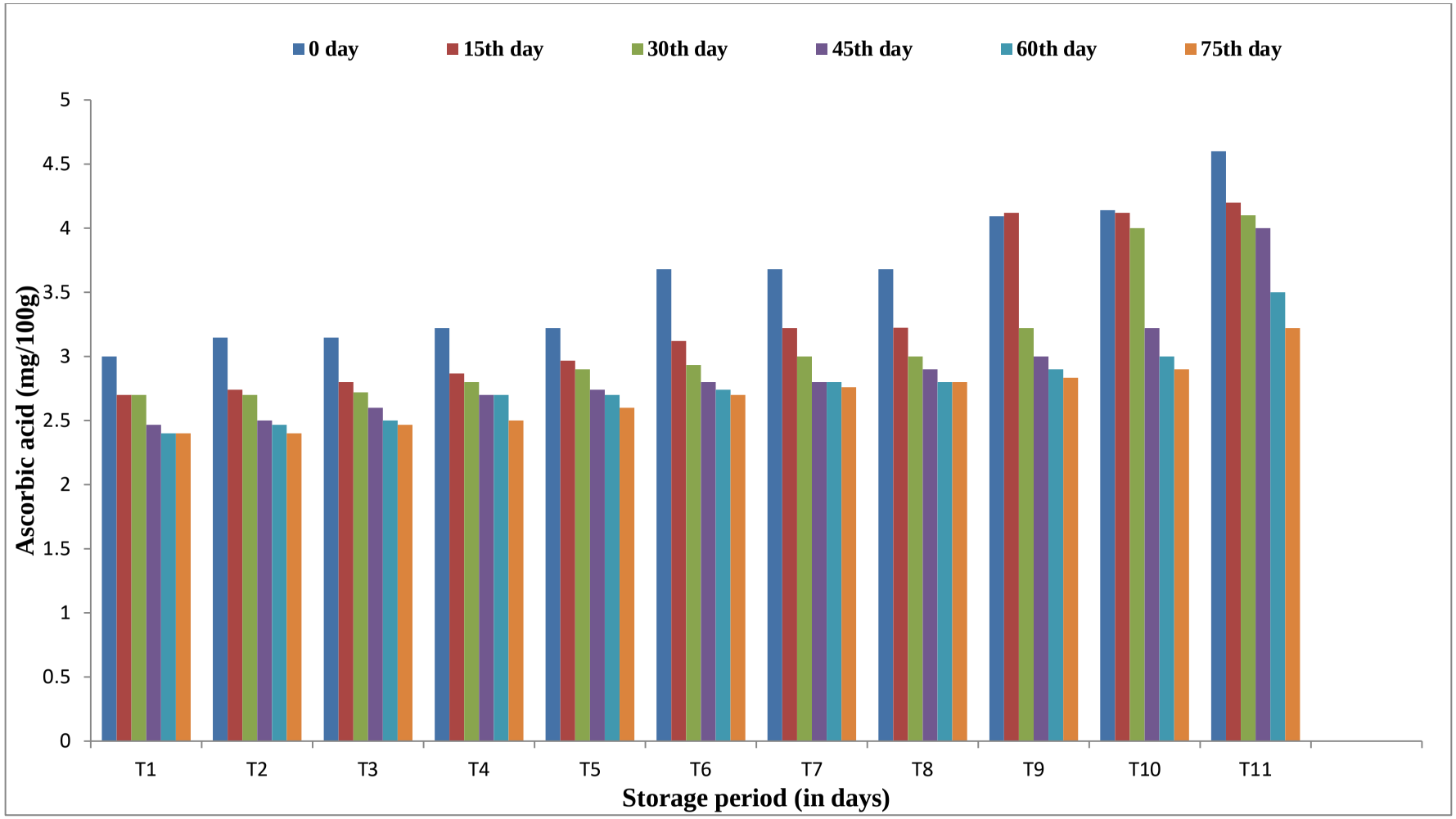

Fig 4.5 : Changes in ascorbic acid (mg/100g) of roselle-aloe vera blended RTS during storage under ambient condition

with storage period in kinnow RTS beverage and squash was reported by Sogi *et al,*. (2001)

4.3.5 Reducing Sugar

It is apparent from the present findings (Table 4.10 and Figure 4.6) that the reducing sugar decreased significantly during entire storage period.

The value of reducing sugar do not show much changes up to 0 to 30^{th} day

At 45^{th} days of storage maximum (1.9%) reducing sugar content was observed in treatment T_{11}. Whereas minimum (1.3%) reducing sugar was found in treatment T_1. At 60^{th} to 75^{th} days very slight changes were observed.

From the above result it is clear that maximum reducing sugar content was found in T_{11} and minimum was noted in T_1 during the entire period of storage. It was found that reducing sugar content decreased significantly with the advancement of storage period. T_{11} having maximum reducing sugar content decreased from (2.25% to 1.75%), while T_1 having minimum reducing sugar content decreased from (1.6% to 1.25%) from 0 to 75^{th} day of storage period.

From the above observations it is also found that T_7 is most stable as it shows minimum changes during storage, While maximum changes was observed in T_{11} as is least stable.

The decrease in reducing sugar may be due to the micro-organisms using sugars for their production. During the storage period the decrease in the reducing sugar may be due the use of sugars by the fermenting microflora, as microorganisms are known to depend on reducing sugar for their metabolic process. Adegoke (1995) reported a similar trend of decrease in reducing sugar in Sekete (fermented beverage) made out of maize. A similar result was observed by Kumar *et al.,* (2009) and Triphati *et al.,* (1988) reported that the variation in reducing sugars may be due to blending. This may be attributed to polysaccharide hydrolysis and inversion of non-reducing sugars during processing and storage (Roy and Singh, 1979).

Table 4.10 : Changes in reducing sugar (%) of roselle-aloe vera blended RTS during storage under ambient condition

Treatments	Reducing Sugar (%)					
	Storage period (in days)					
	0	**15th**	**30th**	**45th**	**60th**	**75th**
T_1 (100 parts Roselle juice + 0 partsAloe vera juice)	1.600	1.500	1.350	1.300	1.250	1.250
T_2 (90 partsRoselle juice + 10 partsAloe vera juice)	1.700	1.500	1.350	1.333	1.300	1.283
T_3 (80 parts Roselle juice + 20 partsAloe vera juice)	1.700	1.500	1.417	1.450	1.400	1.350
T_4 (70 parts Roselle juice + 30 parts Aloe vera juice)	1.767	1.567	1.500	1.500	1.433	1.400
T_5 (60 parts Roselle juice + 40 parts Aloe vera juice)	1.800	1.600	1.600	1.500	1.500	1.433
T_6 (50 parts Roselle juice + 50 parts Aloe vera juice)	1.850	1.633	1.650	1.583	1.550	1.500
T_7 (40 parts Roselle juice + 60 partsAloe vera juice)	1.883	1.700	1.683	1.650	1.600	1.550
T_8 (30 parts Roselle juice + 70 parts Aloe vera juice)	1.900	1.750	1.750	1.700	1.650	1.600
T_9 (20 parts Roselle juice + 80 parts Aloe vera juice)	2.000	1.800	1.800	1.750	1.683	1.650
T_{10} (10 parts Roselle juice + 90 parts Aloe vera juice)	2.100	2.000	1.900	1.800	1.750	1.700
T_{11} (0 parts Roselle juice + 100 parts Aloe vera juice)	2.250	2.100	2.000	1.900	1.800	1.750
Mean	1.868	1.695	1.636	1.592	1.547	1.496
SE (m)±	0.011	0.011	0.011	0.007	0.007	0.007
CV (%)	1.042	0.148	1.189	0.773	0.801	0.822
CD at 5%	0.033	0.033	0.033	0.021	0.021	0.021

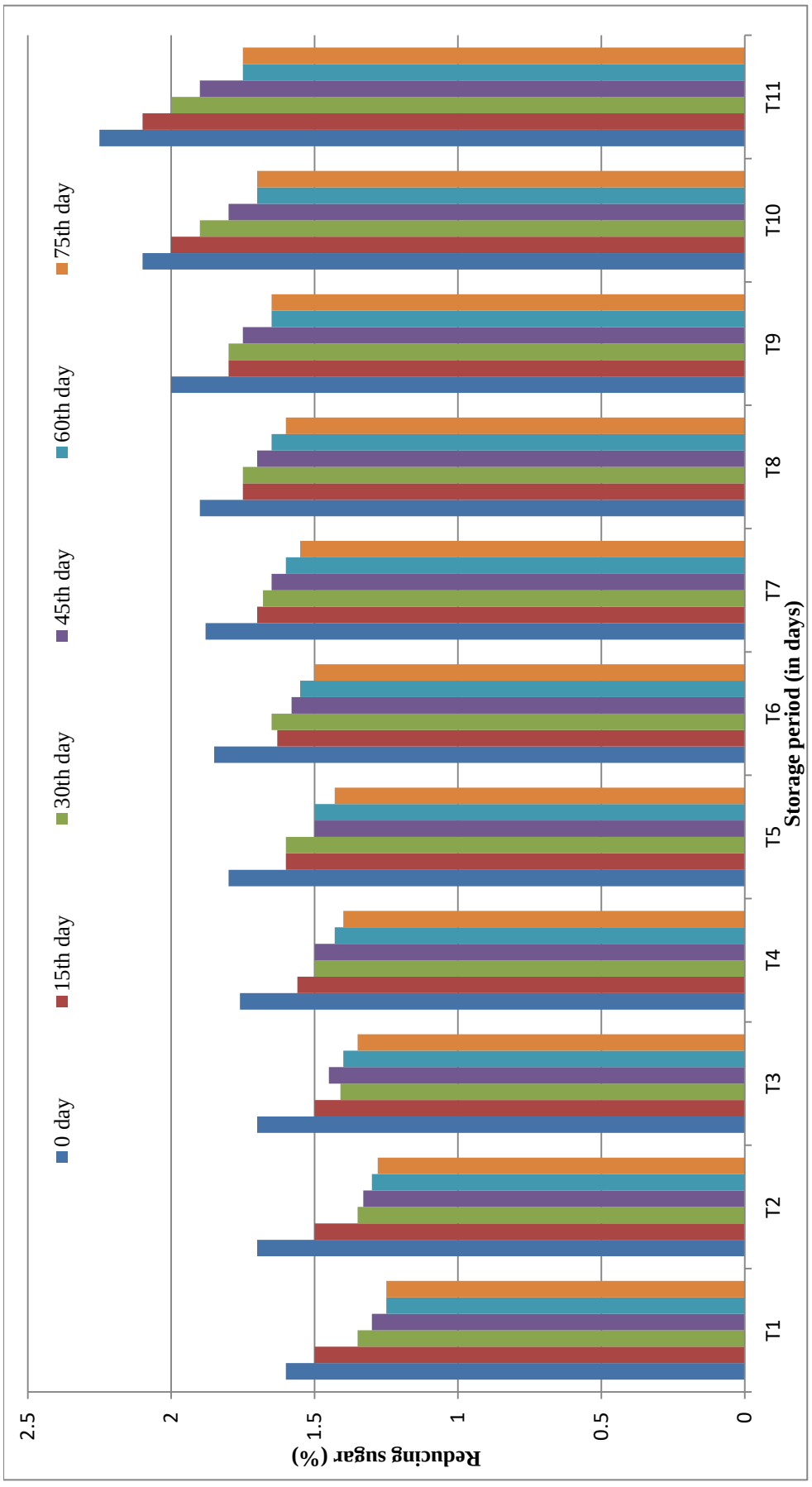

Fig 4.6: Changes in reducing sugar (%) of roselle-aloe vera blended RTS during storage period

4.3.6 Non-reducing sugar

The present study in (Table 4.11 and Figure 4.7) revealed that as the duration of storage advanced there was consistent decrease in non- reducing sugar content from 0 to 75th day of storage period.

At 0 days maximum non-reducing sugar content was noted on T_{11} (9.11%) and minimum was noted on (6.48%). Non-reducing sugar content was found maximum in T_{11} (8.51%) and minimum was recorded in T_1, T_2, T_3 (6.08%) at 15th days of storage.

Maximum non-reducing sugar content on 30th day was again noted on T_{11} (8.4%) , whereas minimum was noted for T_1 and T_2 (5.47%).The maximum and minimum non-reducing sugar content was observed on T_{11} and T_1 for 45th, 60th and 75th day of storage.

The non-reducing sugar content of RTS decreased significantly with advancement of storage period. The maximum non-reducing sugar content was noted in T_{11} during entire period which reduced from (9.11% to 7.09%) from 0 to 75th day of observation. Whereas minimum non-reducing sugar content was noted in T_1 during entire storage period and reduced from (6.48% to 5.07%) from 0 to 75th days.

Similar decreasing trend in non-reducing sugar were reported by Kannan and Thirumaran (2001) as a result of the conversion of non-reducing sugars into reducing sugar (Roy and Singh, 1979; Chauhan and Joshi, 2000) during storage. The decrease in non-reducing sugar content may be due to starch / sucrose hydrolysis in sugar (Attri *et al.*, 1991)

Table 4.7 : Changes in non-reducing sugar (%) of roselle-aloe vera blended RTS during storage under ambient condition

Treatments	Non-reducing sugar (%)					
	Storage period (in days)					
	0	15th	30th	45th	60th	75th
T_1 (100 parts Roselle juice + 0 parts Aloe vera juice)	6.480	6.080	5.470	5.270	5.070	5.070
T_2 (90 parts Roselle juice + 10 partsAloe vera juice)	6.880	6.080	5.470	5.430	5.270	5.203
T_3 (80 parts Roselle juice + 20 partsAloe vera juice)	6.880	6.080	5.743	5.880	5.670	5.470
T_4 (70 parts Roselle juice + 30 partsAloe vera juice)	7.153	6.347	6.080	6.080	5.810	5.670
T_5 (60 parts Roselle juice + 40 partsAloe vera juice)	7.290	6.480	6.480	6.280	6.080	5.810
T_6 (50 parts Roselle juice + 50 parts Aloe vera juice)	7.500	6.553	6.690	6.413	6.213	6.080
T_7 (40 parts Roselle juice + 60 parts Aloe vera juice)	7.633	6.880	6.817	6.690	6.480	6.280
T_8 (30 parts Roselle juice + 70 parts Aloe vera juice)	7.700	7.090	7.090	6.880	6.690	6.480
T_9 (20 parts Roselle juice + 80 partsAloe vera juice)	8.400	7.290	7.290	7.090	6.817	6.690
T_{10} (10 parts Roselle juice + 90parts Aloe vera juice)	8.510	8.400	7.700	7.290	7.090	6.880
T_{11} (0 parts Roselle juice + 100 partsAloe vera juice)	9.110	8.510	8.400	7.700	7.290	7.090
Mean	7.594	6.89	6.657	7.454	6.225	6.065
SE (m)±	0.046	0.058	0.045	0.028	0.035	0.029
CV (%)	1.046	1.447	1.182	0.764	0.970	0.832
CD at 5%	0.135	0.170	0.134	0.084	0.103	0.086

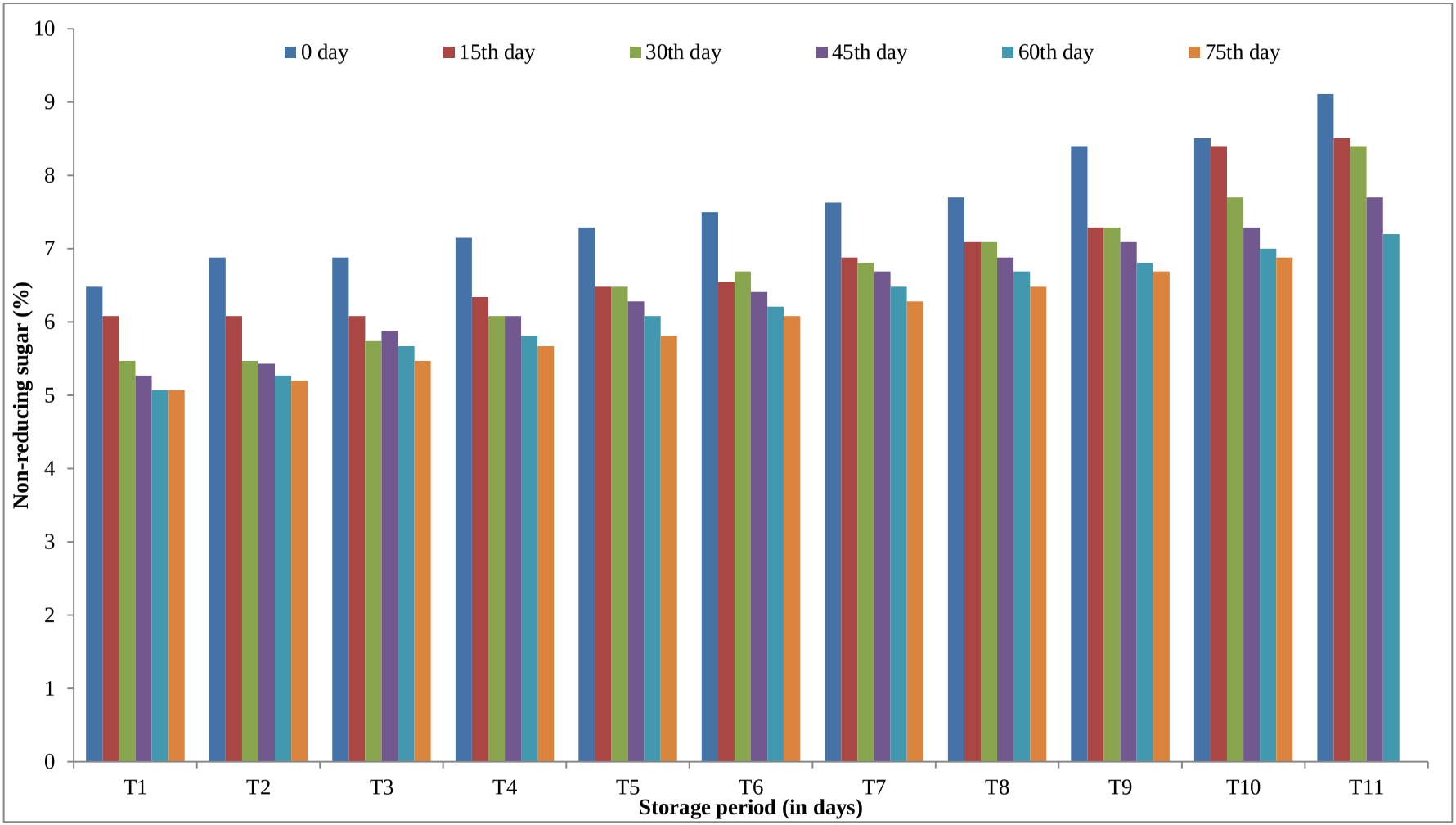

Fig 4.7 : Changes in non-reducing sugar (%) of roselle-aloe vera blended RTS during storage under ambient condition

4.3.7 Total sugar

It can be concluded from the results (Table 4.12 and Figure 4.8) of present experiment that changes in total sugar were found significant with the advancement of storage period. It was found that total sugar content in RTS showed decreasing trend throughout the storage period. It decrease significantly with increase in storage period.

The maximum total sugar content was noted on T_{11} (11.36%) while, the minimum (8.08%) was recorded on T_1 on 0 day.

At 15^{th} and 30^{th} days total sugar content do not show much changes

.The maximum and minimum total sugar content was observed for T_{11} and T_1 for 45^{th}, 60^{th} and 75^{th} day of storage.

The maximum total sugar content decreased from (11.36% to 8.84%) for T_{11} from 0 to 75^{th} day of storage, whereas the minimum total sugar content decreased from (8.08% to 6.32%) onT_1 from 0 to 75^{th} day of observation.

The explanation for a decreased total sugar could be attributed to the microbial development that can be observed by increased acidity and decreased pH. The reason for a decreased total sugar content may be explained by the fact that microorganisms break down these complex sugars into simpler ones and use them for their metabolic activities. Adegoke *et al.,* (1995) also revealed a decrease in total sugar as a result of use of sugars by the microflora. Similar finding were observed by Saini and Pal (1997) they found that total sugar content of kinnow juice decreased from (10.38 to 10.25 per cent) during storage at room temperature for six month. Prapha and Modgil (2010) observed the simlilar finding in Papaya-Mango (85:15) blended squash, they found that total sugar content decreased from (44.63 to 44.03 per cent) after 180 days of storage. They also stated that loss in total sugar content may be due to Maillard reaction and chemical reaction of sugar in presence of acid. Byanna and Gowda (2012) recorded that the total sugar content of blended RTS of sweet orange and pomegranate (90:10) decreased from 13.70 to 12.43 percent stored at room temperature for 180 days. They also stated

Table 4.12 : Changes in total sugar (%) of roselle-aloe vera blended RTS during storage under ambient condition

Treatments	Total sugars (%)					
	Storage periods (in days)					
	0	**15th**	**30th**	**45th**	**60th**	**75th**
T1 (100 parts Roselle juice + 0 partsAloe vera juice)	8.08	7.58	6.82	6.57	6.32	6.32
T2 (90 parts Roselle juice + 10 parts Aloe vera juice)	8.58	7.58	6.82	6.73	6.57	6.48
T3 (80 parts Roselle juice + 20 partsAloe vera juice)	8.58	7.58	7.16	7.33	7.07	6.82
T4 (70 parts Roselle juice + 30 parts Aloe vera juice)	8.92	7.91	7.58	7.58	7.24	7.07
T5 (60 parts Roselle juice + 40 parts Aloe vera juice)	9.09	8.08	8.08	7.83	7.58	7.24
T6 (50 parts Roselle juice + 50 parts Aloe vera juice)	9.35	8.25	8.17	7.99	7.83	7.58
T7 (40 parts Roselle juice + 60 parts Aloe vera juice)	9.51	8.58	8.58	8.34	8.08	7.83
T8 (30 parts Roselle juice + 70 parts Aloe vera juice)	9.60	8.84	8.84	8.58	8.34	8.08
T9 (20 parts Roselle juice + 80 parts Aloe vera juice)	10.40	9.09	9.09	8.84	8.50	8.34
T10 (10 parts Roselle juice + 90 partsAloe vera juice)	10.60	10.40	9.60	9.09	8.84	8.58
T11 (0 parts Roselle juice + 100 parts Aloe vera juice)	11.36	10.60	10.40	9.60	9.09	8.84
Mean	9.46	8.59	8.28	8.04	7.76	7.56
SE (m)±	0.057	0.057	0.072	0.036	0.036	0.036
CV (%)	1.045	1.142	1.515	0.765	0.793	0.830
CD at 5%	0.169	0.167	0.214	0.105	0.105	0.107

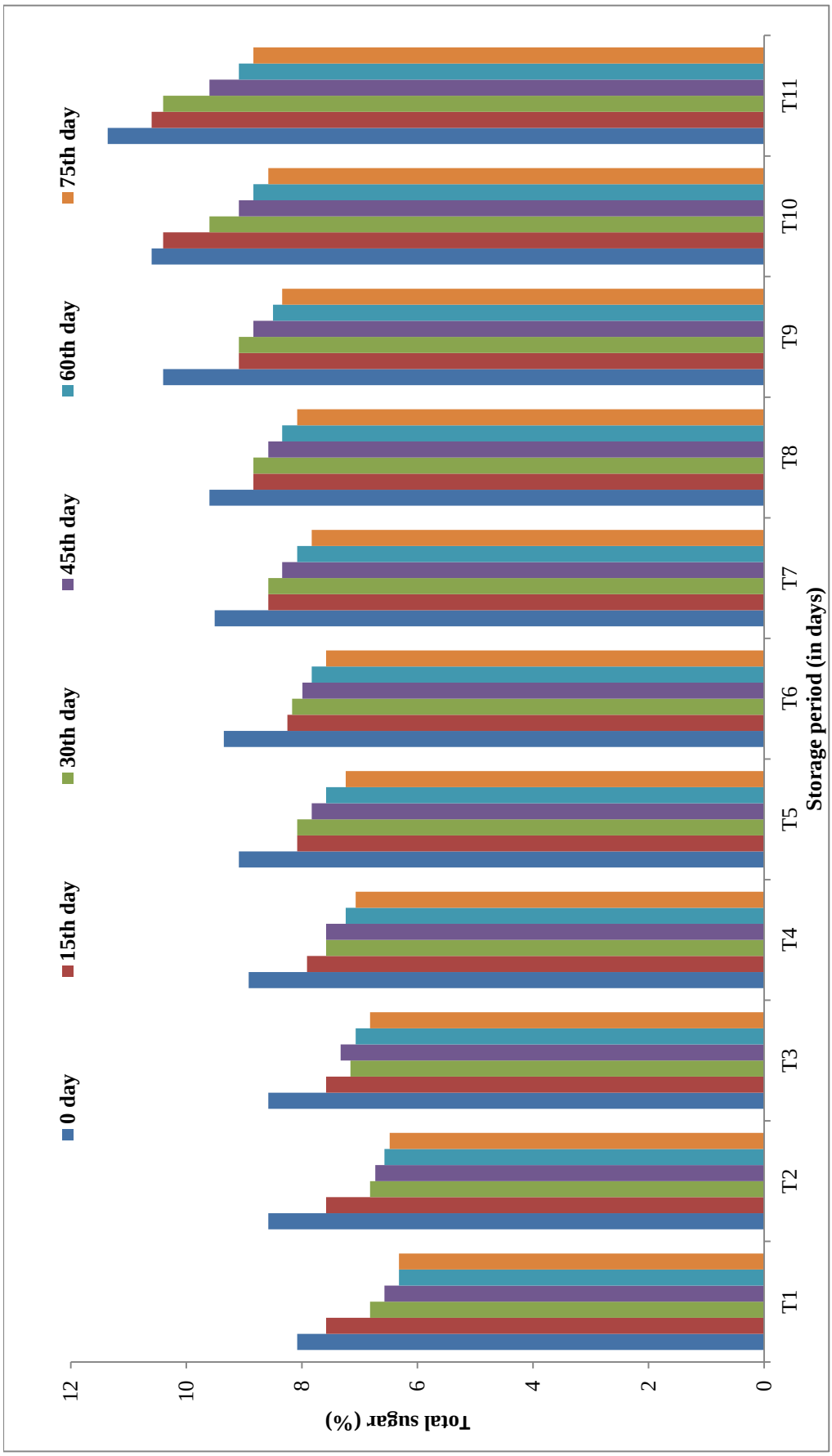

Fig 4.8 : Changes in total sugar (%) of roselle-aloe vera blended RTS during storage under ambient condition

that the decrease in total sugar could be attributed to the reaction of sugars with amino acids for their involvement in non-enzymatic browning, which was consistent with Sharma *et al.,* (2001) in hill lemon RTS beverage and Wasker (2003) in pomegranate and kokum blended juice.

4.3.8 Anthocyanin

It is apparent from the present finding (Table 4.13 and Figure 4.9) that anthocyanin content of the blended RTS showed non-significant decrease with the advancement of the storage period.

At 0^{th} day maximum anthocyanin content was found on T_1 (1.21 mg/100ml) while minimum anthocyanin content was found on T_{11} (0.00) followed by T_{10} (0.64 mg/100ml).

The values of anthocyanin show very less changes at 15^{th} to 45^{th} days of storage.

At 60^{th} and 75^{th} day maximum and minimum anthocyanin content was noted same on T_1 (1.18 mg/100ml) and T_{11} (0.00 mg/100ml) followed by T_{10} which also remain same (0.6 mg/100ml).

It is vivid from the above observation that T_2 shows minimum changes and was most stable treatment among all. While maximum changes was observed in T_4 which shows it is least stable treatment combination.

From the above findings it was clear that the maximum anthocyanin content was observed on T_1 having 100 per cent roselle juice which decreased non-significantly from (1.21 to 1.18 mg/100ml) from 0^{th} to 75^{th} day of storage, while minimum anthocyanin content was found on T_{11} (0.00mg/100ml) having 100 per cent aloe vera juice and no roselle juice showing at 0^{th} to 75^{th} day of storage followed by T_{10} having 90 per cent aloe vera and 10 per cent roselle decreased from (0.64 to 0.6 mg/100ml) with the advancement of storage period.

Anthocyanin degradation may have been accelerated due to presence of ascorbic acid and higher pH of the prepared roselle-fruit juice blends. It is also

Table 4.13 : Changes in anthocyanin (mg/100ml) of roselle-aloe vera blended RTS during storage under ambient condition

Treatments	Anthocyanin (mg/100ml) Storage periods (in days)					
	0	**15th**	**30th**	**45th**	**60th**	**75th**
T_1 (100 parts Roselle juice + 0 parts Aloe vera juice)	1.210	1.210	1.200	1.200	1.180	1.180
T_2 (90 parts Roselle juice + 10 parts Aloe vera juice)	1.180	1.173	1.170	1.170	1.170	1.160
T_3 (80parts Roselle juice + 20 parts Aloe vera juice)	1.150	1.140	1.130	1.120	1.120	1.100
T_4 (70 parts Roselle juice + 30 parts Aloe vera juice)	1.140	1.120	1.100	1.100	1.080	1.020
T_5 (60 parts Roselle juice + 40 partsAloe vera juice)	0.960	0.940	0.940	0.920	0.920	0.900
T_6 (50 parts Roselle juice + 50 partsAloe vera juice)	0.947	0.940	0.927	0.900	0.900	0.880
T_7 (40 parts Roselle juice + 60 parts Aloe vera juice)	0.940	0.930	0.920	0.887	0.840	0.833
T_8 (30 parts Roselle juice + 70 parts Aloe vera juice)	0.910	0.860	0.840	0.840	0.827	0.820
T_9 (20 parts Roselle juice + 80 parts Aloe vera juice)	0.757	0.740	0.730	0.720	0.700	0.700
T_{10} (10 parts Roselle juice + 90 parts Aloe vera juice)	0.640	0.620	0.610	0.610	0.600	0.600
T_{11} (0 parts Roselle juice + 100 parts Aloe vera juice)	0.000	0.000	0.000	0.000	0.000	0.000
Mean	0.894	0.879	0.869	0.860	0.848	0.835
SE (m)±	0.001	0.002	0.002	0.002	0.002	0.002
CV (%)	0.276	0.396	0.401	0.405	0.410	0.416
CD at 5%	0.004	0.006	0.006	0.006	0.006	0.006

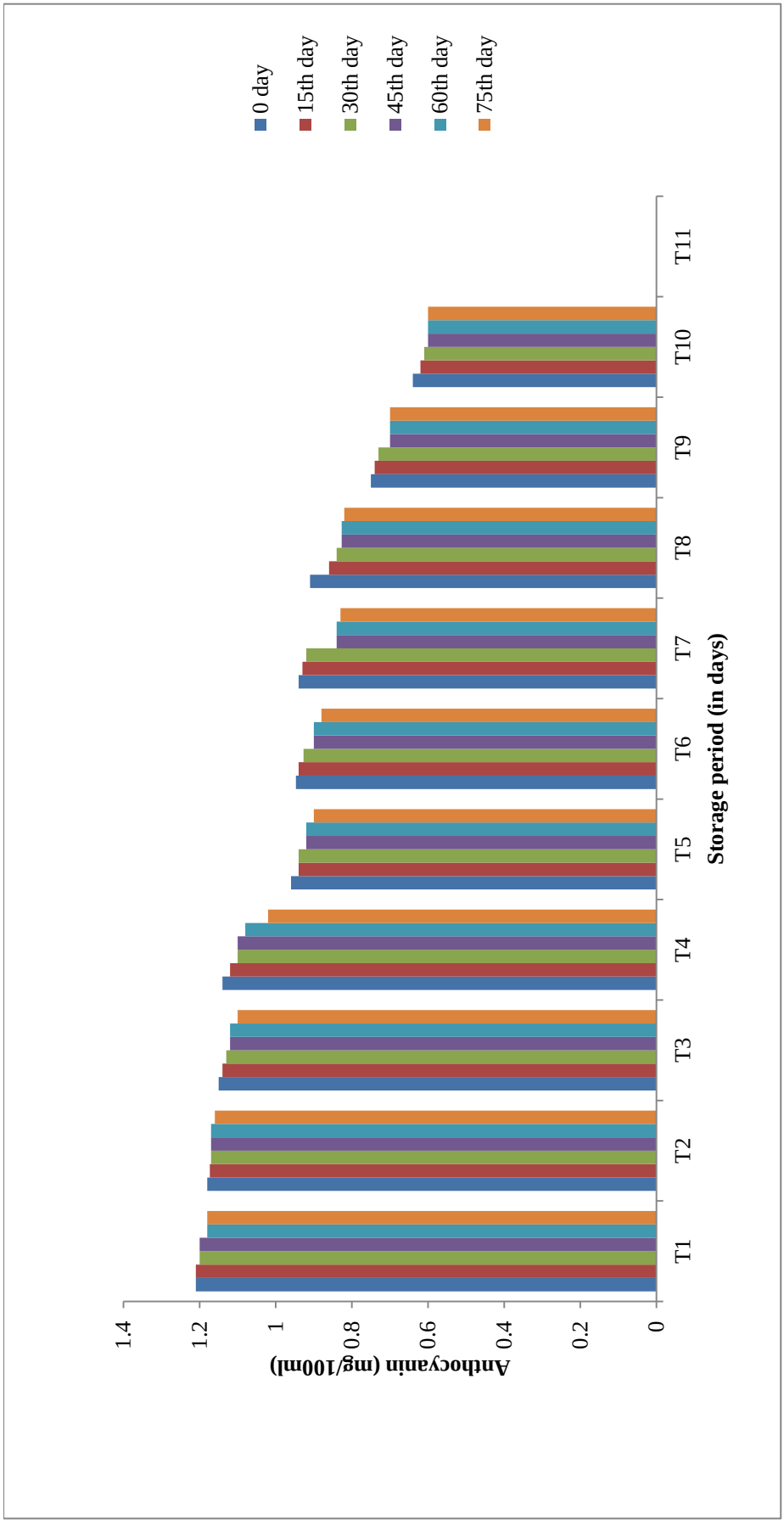

Fig 4.9 : Changes in anthocyanin (mg/100ml) of roselle-aloe vera blended RTS during storage under ambient condition

known that ascorbic acid interaction with anthocyanin can lead to the degradation of compounds by a condenastion reaction. Some monomeric anthocyanin may have been converted into polymeric compounds during storage (Iversen 1999; Ochoa *et al.*, 1999). That may be the explanation for the high losses of total monomeric anthocyanin in the blends.

CHAPTER -V
SUMMARY AND CONCLUSIONS

The present investigation entitled **" Standardization and Evaluation of RTS from blends of Indian Roselle (*Hibiscus sabdariffa L.*) and Aloe Vera (*Aloe barbadensis M.)*"** was carried out at the Laboratory of Vegetable Science Department, College of Agriculture, IGKV, Raipur (C.G.) during the year 2019-2020. The investigation was carried out to prepare RTS from the blends of roselle and aloe vera juice in different combinations to find out the acceptable recipe. The experiment was designed in Completely Randomized Block Design with total eleven combinations of treatment replicated 3 times.

The knowledge of raw material's physical and chemical properties helps in development of processing technology so, before preparing the RTS the physical and chemical composition of roselle and aloe vera were analyzed. The recipe was prepared to combine roselle and aloe vera concentration to make blended RTS beverage. The product was analyzed for their physico-chemical and sensory qualities at an interval of 15 days upto 75 days. The salient findings of the present investigation are summarized as follows:

1. Average weight, length and diameter of roselle were 4.25 g, 4.5 cm and 2.5 cm, respectively. The colour of roselle calyces and pulp were red wine and pinkish red, respectively. Weight of calyces, weight of seed, weight of waste from capsule, edible index and waste index were 2.42 g, 0.8 g, 1.83 g, 56.94 per cent and 43.05 per cent, respectively.
2. The total soluble solids, pH, acidity, ascorbic acid of roselle were 4.8 per cent, 2.34, 3.15 per cent and 17.68 mg/100g, respectively.
3. The reducing sugar, non-reducing sugar, total sugar recorded were 1.62 per cent, 1.66 per cent, 3.28 per cent, respectively.
4. Average weight of aloe vera leaves, length, width and pulp weight recorded were 450 g, 55 cm and 7 cm, 350 g, respectively. The leaf colour, gel colour and gel consistency recorded during investigation were translucent green,

translucent mass with light yellowish green colour and viscous and slimy, respectively.

5. The chemical composition of aloe vera leaves like moisture per cent, total soluble solids, pH, acidity, ascorbic acid, total sugar, reducing sugar and non-reducing sugar recorded were 97.34 per cent, 1.2 per cent, 4.33, 0.32 per cent, 3.89 per cent, 1.52 per cent, 0.036 per cent and 1.484 per cent, respectively.
6. The treatment having 80 per cent roselle juice and 20 per cent aloe vera juice scored highest for the overall acceptability at the time of preparation of blended RTS.
7. The TSS content decreased with an advancement in storage days. TSS was recorded highest for the treatment T_1 having 100 per cent roselle juice.
8. pH is inversally associated with the acidity and decreases with the increase in acidity during storage days. The maximum pH was recorded on treatment T_{11} having 100 per cent aloe vera juice.
9. The acidity per cent increased with increasing period of storage and was recorded maximum in T_1 having 100 per cent roselle juice.
10. The ascorbic acid content decreases with an advancement in storage days and T recorded the maximum ascorbic acid content having 100 per cent aloe juice.
11. The reducing sugar, non-reducing sugar, total sugar content decreased significantly throughout the storage period.

Conclusions:

Conclusions drawn from the findings obtained from this investigation are as follows:

1. The study revealed blended RTS with 80 per cent roselle and 20 per cent aloe vera ranked first in the recipe standardization of blended RTS with various combinations of roselle and aloe vera juice.
2. The study also revealed that non-significant changes were observed when stored up to 45 days but after that a considerable changes were observed in chemical properties of blended RTS when stored up to 75 days at room temperature under ambient storage condition.

3. On the basis of chemical analysis T_2 having 90 per cent roselle and 10 per cent aloe vera was found to be most stable treatment showing minimum changes followed by T_1 having 100 per cent roselle juice.

Suggestions for Future Research:

Following suggestions for future line of work are given on the basis of the experienced gained and the findings obtained after the completion of present investigation.

1. Since the conclusion of this research is focused on 75 days of testing. Therefore it can be replicated across successive days in order to draw some definitive conclusions and recommendations.
2. So far, very little work has been done in the field of roselle and aloe vera in Chhattisgarh as well as in India for the production of processed products made from these crops. Therefore, special attention should be paid to the creation of new products based on roselle and aloe vera, particularly beverages.
3. Following specific guidelines, value-added products of roselle and aloe vera need to be developed for commercial use.
4. Roselle is one of the crops which is under utilized in India, Since leaves are used and calyces are non utilized focus should be given to produce new processed products from the roselle calyces.
5. Since the calyces are rich in anthocyanins, more value added products such as jam, jellies can be made and also it can be used for preparing natural colourant juice.
6. The potential of this medicinal and nutritional fruit for the preparation of various added items should be investigated.

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APPENDIX-1

Weekely Meteorological Data During Storage Period of Blended RTS of Roselle and Aloe Vera (Nov 2019 to Feb 2020)

Week No.	Month & Date	Max. T(˚C)	Min. T(˚C)	RF(mm)	RH(%)		WS(kmph)	SS(hrs)
					I	II		
45	Nov 05-11	30.6	23.0	81.6	91	65	3.0	5.5
46	12-18	29.6	15.5	0.0	90	38	1.8	8.7
47	19-25	30.2	15.2	0.0	89	38	1.4	8.2
48	26-02	29.7	16.3	0.0	90	43	2.0	6.3
49	Dec 03-09	28.0	13.3	0.0	84	34	2.4	7.6
50	10-16	29.5	15.3	0.0	91	48	1.7	5.2
51	17-23	26.7	14.1	0.8	88	42	1.9	4.9
52	24-31	26.1	11.9	0.0	81	35	2.5	5.7
01	Jan 01-07	23.2	13.0	2.7	83.5	54.7	4.0	3.5
02	08-14	25.1	10.8	3.2	89.7	46.3	2.1	6.2
03	15-21	28.6	14.1	0.0	87.9	47.9	1.8	5.7
04	22-28	28.8	13.4	0.0	86.7	38.7	1.8	7.3
05	Jan-Feb 29-04	26.1	13.9	0.0	75.9	45.7	2.9	4.3
06	Feb 05-11	21.3	13.6	7.1	93.6	66.1	4.1	2.4
07	12-18	29.7	11.9	0.0	88.1	27.3	2.2	9.8

RESUME

Name	:	Gunjeshree Gond
Date of Birth	:	4 May 1994
Present Address	:	Sai Mandir, Sai Colony, Zora Raipur, (C.G.) 492001
Phone	:	9755531402
E-mail	:	gunjangond77@gmail.com
Permanent Address	:	House No. 2825, Arjuni School Para, Vill Post-Arjuni, Teh-Sakti, District- Janjgir Champa (C.G.) Pin Code-495689

Academic Qualification:

Degree	Year	University/Institute
Higher Secondary School	2012	Carmel Convent Higher Secondary School, Amlai, (M.P.)
B.Sc. (Ag.)	2014	College of Agriculture and Station Raigarh, (C.G.)
M.Sc. (Hort.) Vegetable Science	2020	IGKV, College of Agriculture Raipur, (C.G.)

Professional Experience (if any)	:	Rural Agricultural Work Experience
Programme		
Membership of Professional Societies (if any)	:	No
Awards /Recognition (if any)	:	No
Publication (if any)	:	1

Signature

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Gunjeshree Gond
Department of Vegetable Science, CoA, I.G.K.V. Raipur, Chhattisgarh, India

Annu Verma
Department of Vegetable Science, CoA, I.G.K.V. Raipur, Chhattisgarh, India

Oshin Pali
Department of Vegetable Science, CoA, I.G.K.V. Raipur, Chhattisgarh, India

Corresponding Author:
Gunjeshree Gond
Department of Vegetable Science, CoA, I.G.K.V. Raipur, Chhattisgarh, India

Evaluation of anthocyanin content in blended roselle and aloe vera ready to serve during storage

Gunjeshree Gond, Annu Verma and Oshin Pali

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Abstract
The present study entitled "Evaluation of anthocyanin content in blended roselle and aloe vera Ready To Serve during storage" was conducted in the Laboratory of Vegetable Science of Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) during the year 2019-2020. The blended RTS beverage was prepared with different concentrations of roselle and aloe vera juice and was evaluated for anthocyanin at an interval of 15 days upto storage period of 75 days with the help of Spectrophotometer. Roselle extracts have potential use as valuable source of anthocyanin, it attracted attention as a potential source of natural colourants in food applications. Among 11 treatments the maximum anthocyanin content was noted for T_1 having 100 per cent roselle juice and minimum was noted for T_{11} having 100 per cent aloe vera juice having no anthocyanin content. It is evident from the present findings that anthocyanin content decreased non-significantly with the advancement of storage period. The concentration of maximum anthocyanin in T_1 decreased from (1.21mg/100ml to 1.18mg/100ml) upto 0 to 75 days of storage, while minimum anthocyanin concentration in T_{11} having 100 per cent aloe vera juice and no roselle followed by T_{10} which decreased from (0.640mg/100ml to 0.6mg/100ml) with the advancement of storage period.

Keywords: Aloe vera, anthocyanin, *Hibiscus sabdariffa* L., RTS, spectrophotometer

Introduction
Roselle, *Hibiscus sabdariffa Linn*. (2n=4x=72) is a shrub belonging to the family Malvaceae. It is a Hibiscus plant native to India and Malaysia that has been introduced to other parts of the world, including Central America, West Indies and even Africa (Bruke,1975) [2]. Roselle is a multipurpose plant and is used as a folk remedy in the treatment of abscesses, bilious conditions, cancer, cough, dyspepsia, fever, heart ailments, hypertension and strangury (Duke, 1985) [4]. They are recorded to be antihypertensive, antiseptic, sedative, diuretic, digestive, purgative, emmolient, demulcent and astringent (Odigie *et al*., 2003) [10].
Red colour of roselle is due to pigment known as "Anthocyanin". The anthocyanin found in roselle calyces have been reported to contain delphinidin-3-sambubioside, cydine-3-sambubioside, delphinidin-3-monoglucoside and cynidin-3-monoglucoside (Hong and Wrostland, 1990) [7]. Anthocyanins the biggest group of water soluble natural pigments, are the glycosides of polyhydroxy and the polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts shown in Fig 1. Colour is considered as one of most vital properties determining the acceptability of foods and drinks and is essential element for their identification. The matured, dried calyces or flower pod is used to prepare tea, wine, cocktail, sauce, jam, marmalade, candy, pickles, and curry due to its distinctive scarlet red color and sweet aroma but sour taste. Roselle is one of the under exploited food crops with the potential for processing by the dietry and food industries. The extract of roselle calyces has a distinctive red colour, good flavour, low sugar and high acidic content. But due to its high acidity and strong astringent taste, it is not consumed fresh. However, roselle calyces have unlimited potential in their processed form in world trade.

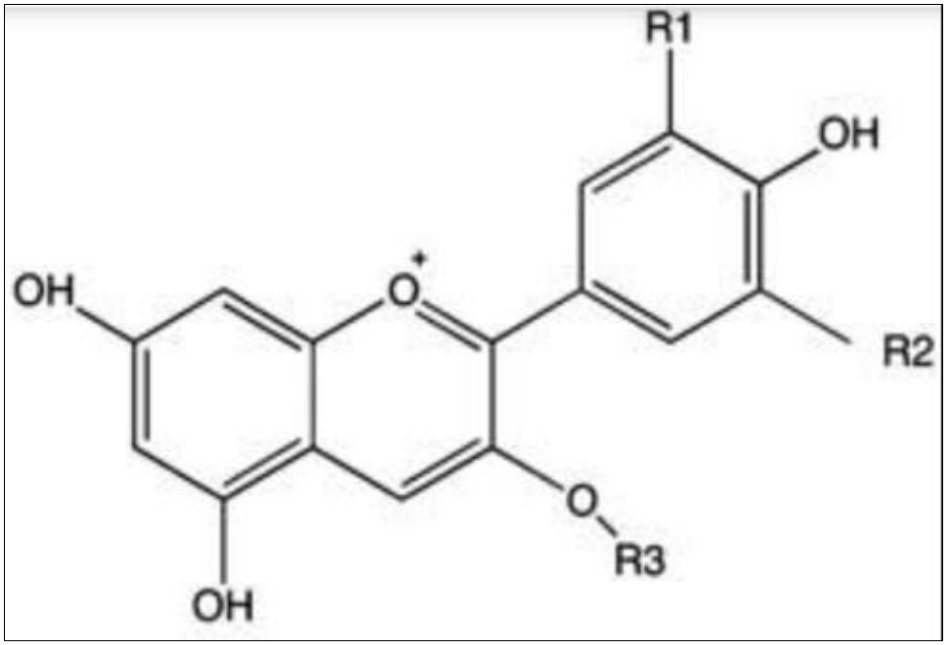

Fig 1: Anthocyanin Structure

Aloe vera (*Aloe barbadensis Miller*.) is a perennial succulent plant belonging to family Liliacea. It was used as naturally occurring medicinal and therapeutic product (Dutt, 2002) [5]. It has also been used as traditional contemporary folk remedy (Volger and Ernst, 1999) [11]. Aloe vera contains aloins, a small quantity of volatile oil, resin, gum, anthraquinone, chrysophanic acid and coumarins. Aloe vera is unpleasant in its raw state and has bitter taste so its palatibility can be improved by adding some other fruit juices.

Material and Methods

The present investigation is carried out in the Laboratory of Vegetable Science of Indira Gandhi Krishi Vishwavidyalaya Raipur (C.G.) during the year 2019-2020. The present study is designed to prepare blended roselle and aloe vera RTS and evaluation of anthocyanin during storage period of 75 days with the help of spectrophotometer. A blended RTS is designed in CRD (Completely Randomized Design) with different concentration of roselle and aloe vera having 11 treatments and 3 replications. Different blended concentrations of roselle and aloe vera are T_1 having (100) per cent roselle juice, T_2 (90:10), T_3 (80:20), T_4 (70:30), T_5 (60:40), T_6 (50:50), T_7 (40:60), T_8 (30:70), T_9 (20:80), T_{10} (10:90), T_{11} (100) per cent aloe vera juice.

For this investigation roselle calyces were collected from the Dantewada, seeds were planted at the month of June during rainy season and was harvested in mid November onwards when the flower has dropped that time calyces were fully matured and dark pink in colour. Aloe vera was collected from IGKV campus.

Extraction of Roselle juice by Hot Water Extraction Method (HWE)

Fresh and Healthy diseased free mature calyces was selected, peeled and washed with water after that it was ready for the juice extraction. For the preparation of Roselle-Aloe vera blended RTS, the Roselle juice was extracted by using the Hot Water Extraction (HWE) method described by (Wong *et al.* 2003) and was performed by using 1kg dried calyces per 5 litre of distilled boiling water (100 ^{0}C) for 20-25 minutes as described by (Aina and Odipe, 2006) [1]. The HWE method was the most effective extraction method resulting in high anthocyanin and ascorbic acid contents of 43g/l (as delphinidin-3-glucoside) and 234g/kg respectively. Fresh Roselle extract were filtered with a muslin cloth.

Extraction of Aloe Vera Juice

Fresh aloe leaves free from disease and pests of uniform size with no mechanical damage is selected. The conventional hand- filleting method of processing aloe vera was created to prevent contaminating the inner fillet with the yellow sap. In this method, the lower (25mm) portion of the leaf base and tapering point (50-100mm) of the leaf top is removed. Spines along the leaf margin, top and bottom rind of the leaves were removed with sharp knife and leaves were blanched for 10 minutes at 60-65 ºC.

Preparation of RTS

According to the treatments the ratio of 10 per cent roselle and aloe vera juice is blended with measured quantity of water and TSS is checked, and then the treatment is boiled for 2-3 minutes and sugar is added to it until TSS reaches to 10 per cent and then sodium benzoate 1gm/lt of RTS and citric acid 3gm/lt was added as a preservative. At last when the prepared RTS get cooled it is filled in glass bottles and sealed properly with crown corking sealing machine and kept at ambient temperature.

Table 1: Different combinations of blended roselle and aloe vera RTS

Treatments	Proportion of juice in the Blends	
	Roselle juice	Aloe vera juice
T_1	100 parts	0 parts
T_2	90 parts	10 parts
T_3	80 parts	20 parts
T_4	70 parts	30 parts
T_5	60 parts	40 parts
T_6	50 parts	50 parts
T_7	40 parts	60 parts
T_8	30 parts	70 parts
T_9	20 parts	80 parts
T_{10}	10 parts	90 parts
T_{11}	0 parts	100 parts

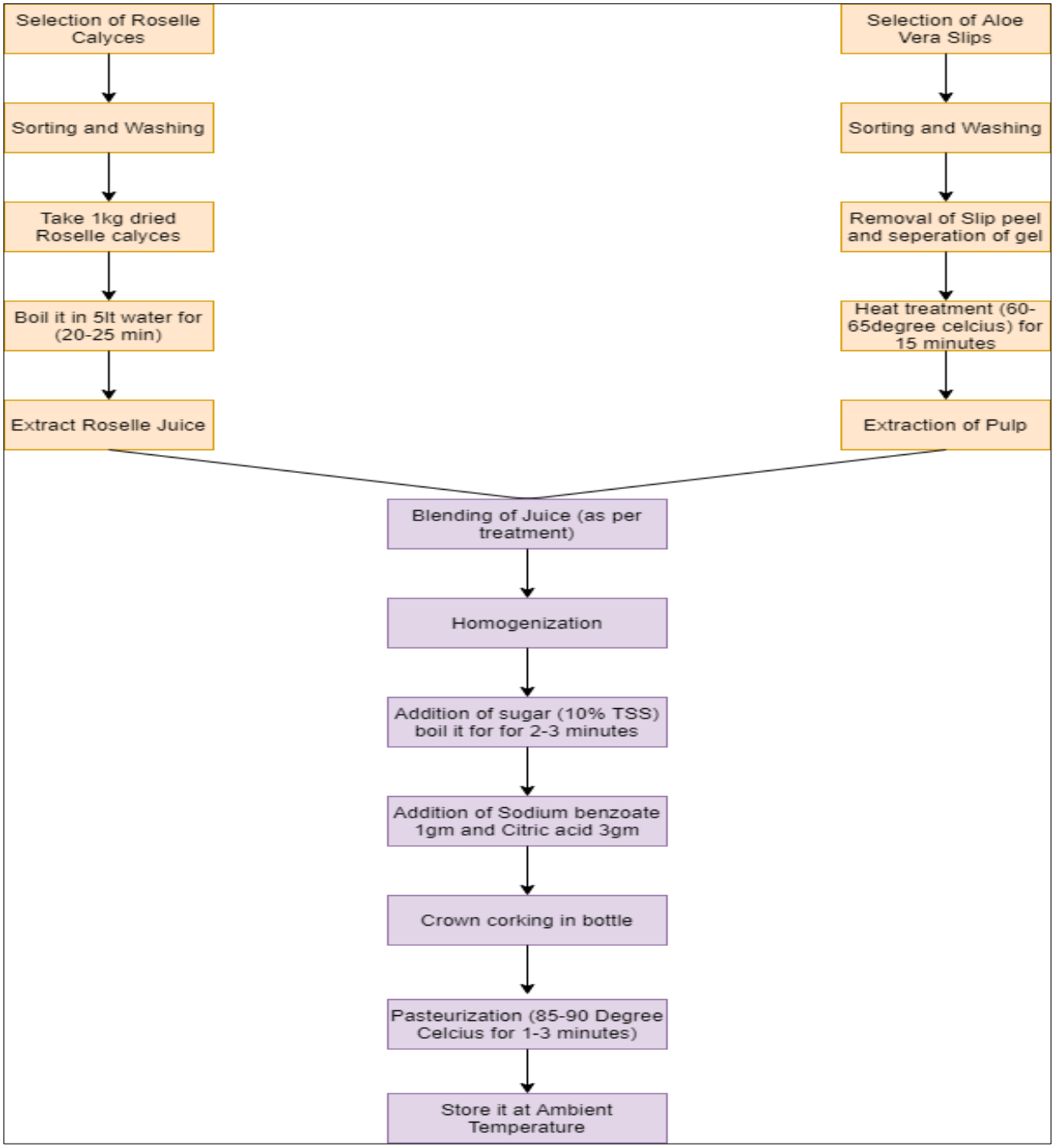

Fig 2: Flowchart of preparation of RTS

Determination of Anthocyanin

Reagents: 0.1 N HCL

Preparation of Standard: Standard was prepared by taking 10 ml of sample and diluted with 50 ml of distilled water.

Procedure: 10 ml of sample was diluted with 50 ml of 0.1 N HCL and was allowed to equilibrate in the dark for 1 hour. Absorbance was recorded at the optical density (O.D.) 510 nm in spectrophotometer. Anthocyanin is determined by the formula:

$$\text{Total O.D/100 ml} = \frac{\text{O.D} \times \text{Volume made up}}{\text{ml. of juice taken}} \times 100$$

$$\text{Total anthocyanin (mg/100ml)} = \frac{\text{Total O.D/ 100 ml}}{87.3.}$$

The changes in concentration of anthocyanin during storage in different treatment is observed upto 75 days at an interval of 15 days. Different treatments are individually analyzed. The data were subjected without transformation and statistically analyzed with CRD using OPSTAT.

Results and Discussion

It is apparent from the present finding (Table.2 and Fig.3) that anthocyanin content of the blended RTS showed non-significant decrease with the advancement of the storage period. The concentration of anthocyanin was found to be maximum in treatment T_1 having 100 per cent roselle juice with the advancement of the storage period. Whereas minimum anthocyanin concentration was found in treatment T_{11} having 100 per cent aloe vera juice followed by T_{10} having 10 per cent roselle juice and 90 per cent aloe vera juice. The concentration of maximum anthocyanin in T_1 decreased from (1.21mg/100ml to 1.18mg/100ml) from 0 to 75th days of

storage, while minimum anthocyanin content was observed in T_{11} having 100 per cent aloe vera during entire period of observation followed by T_{10} where concentration of anthocyanin decreased from (0.640mg/100ml to 0.6mg/100ml) from 0 to 75th day of storage period.

Anthocyanin degradation may have been accelerated due to presence of ascorbic acid and higher pH of the prepared roselle-fruit juice blends. It is also known that ascorbic acid interaction with anthocyanin can lead to the degradation of compounds by a condenastion reaction. Some monomeric anthocyanin may have been converted into polymeric compounds during storage (Iversen 1999; Ochoa *et al.*, 1999) [8, 9]. That may be the explanation for the high losses of total monomeric anthocyanin in the blends.

Table 2: Changes in Anthocyanin content of blended RTS during storage under ambient condition.

Treatments	Anthocyanin (mg/100ml)						
	Storage periods (in days)						
	0	15th	30th	45th	60th	75th	Mean
T_1 (100% Roselle juice + 0% Aloe vera juice)	1.210	1.210	1.200	1.200	1.180	1.180	1.181
T_2 (90% Roselle juice + 10% Aloe vera juice)	1.180	1.173	1.170	1.170	1.170	1.160	1.170
T_3 (80% Roselle juice + 20% Aloe vera juice)	1.150	1.140	1.130	1.120	1.120	1.100	1.126
T_4 (70% Roselle juice + 30% Aloe vera juice)	1.140	1.120	1.100	1.100	1.080	1.020	1.093
T_5 (60% Roselle juice + 40% Aloe vera juice)	0.960	0.940	0.940	0.920	0.920	0.900	0.93
T_6 (50% Roselle juice + 50% Aloe vera juice)	0.947	0.940	0.927	0.900	0.900	0.880	0.916
T_7 (40% Roselle juice + 60% Aloe vera juice)	0.940	0.930	0.920	0.887	0.840	0.833	0.891
T_8 (30% Roselle juice + 70% Aloe vera juice)	0.910	0.860	0.840	0.840	0.827	0.820	0.849
T_9 (20% Roselle juice + 80% Aloe vera juice)	0.757	0.740	0.730	0.720	0.700	0.700	0.724
T_{10} (10% Roselle juice + 90% Aloe vera juice)	0.640	0.620	0.610	0.610	0.600	0.600	0.613
T_{11} (0% Roselle juice + 100% Aloe vera juice)	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mean	0.894	0.879	0.869	0.860	0.848	0.835	0.86
SE (m) ±	0.001	0.002	0.002	0.002	0.002	0.002	_
CV (%)	0.276	0.396	0.401	0.405	0.410	0.416	_
CD at 5%	0.004	0.006	0.006	0.006	0.006	0.006	_

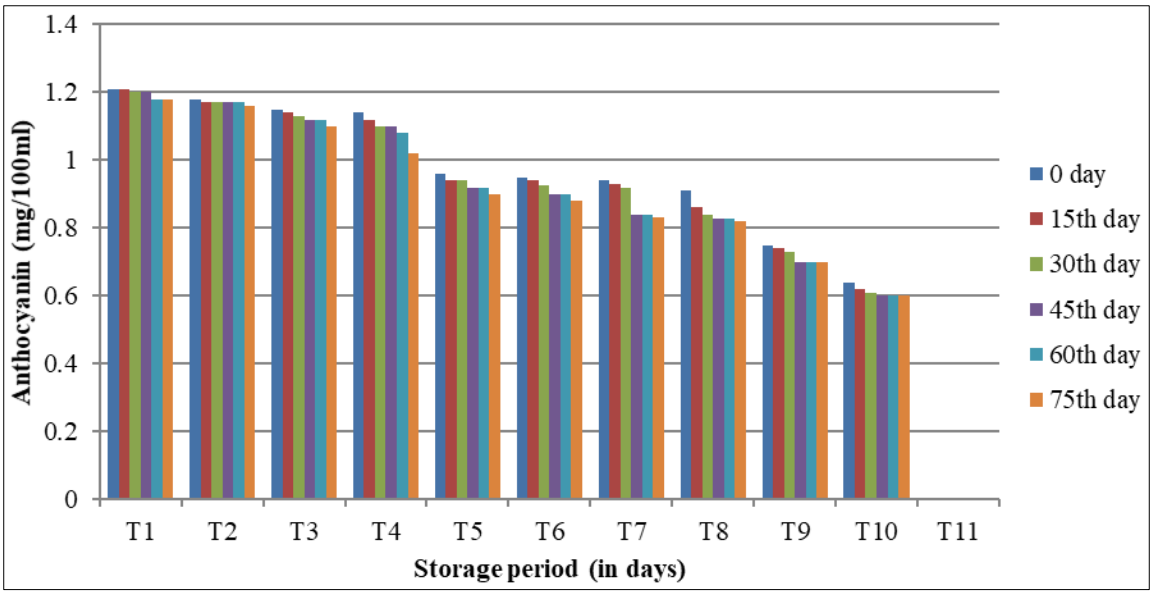

Fig 3: Changes in Anthocyanin content of blended RTS during storage under ambient condition

Conclusion

The present findings confirm that concentration of anthocyanin decreased non- significantly from 0 to 75th day of storage period. The maximum anthocyanin content was noted in T_1 which changes from (1.21 to 1.18 mg/100ml) during storage, whereas minimum anthocyanin content was noted in T_{11} having no anthocyanin content followed by T_{10} which changes from (0.640 to 0.6mg/100ml). These results are in agreement that the concentration of anthocyanin decreased from T_1 to T_{11} due to decrease in concentration of roselle with increase in concentration of aloe vera in each treatment.

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