ENCAPSULATION OF ESSENTIAL OIL FROM CRYOGROUND DILL SEED

A THESIS SUBMITTED TO THE

ANAND AGRICULTURAL UNIVERSITY

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE

OF

Master of Technology

IN

FOOD TECHNOLOGY

BY

ANITHA N

B. Tech (Food Science and Technology)

(Registration No. 2070918001)

COLLEGE OF FOOD PROESSING TECHNOLOGY & BIO ENERGY

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ABSTRACT

Dill seed is also known as “sowa”, is an annual herb cultivated throughout India. The essential oil of dill seed is rich in monoterpenes like carvone and limonene and are extensively used in food, pharmaceutical and cosmetics as well as for aromatherapy. Extraction of dill seed essential oil with higher bioactive compounds can be achieved by grinding of dill seed at sub zero temperature. Encapsulation of essential oil can be useful to prevent loss of volatile compounds and preserve them for further use in food fortification and medicinal purposes. Dill seed cultivar of Gujarat Suva-2 variety was used in the present study. Physicochemical characteristics of dill seed and solvent extracted dill oil was carried out. Cryogenic grinding of dill seed was carried out using liquid nitrogen at predefined temperatures (0 ° to -80°C), feed rate (6, 7 and 8 kg/h) and sieve size (0.8 and 1.0 mm). For optimization, observations such as grinding time, liquid nitrogen consumption, energy consumption, particle size and essential oil content were taken. Optimized conditions for cryogenic grinding of dill seed were -60 °C temperature at 6 kg/h feed rate and sieve size of 0.8 mm to obtain superior quality of dill seed powder. GC-MS analysis showed 27.63 % carvone and 7.21 % limonene in the essential oil from optimized dill seed powder. Encapsulation of essential oil of optimized dill seed powder was carried out with different carrier materials. Polymers namely gum tragacanth, maltodextrin, agar agar and gelatine at concentrations of 0.50 %, 1.00 % and 1.50 % were used to partially replace sodium alginate. 1 g of dill seed essential oil was added to each solution along with 100 µl of TWEEN 20. These oil-alginate-polymer mixtures were sprayed into a 5 % calcium chloride solution with continuous agitation. The beads formed were filtered and oven-dried at 40 °C for 24 h. Particle size and moisture content of encapsulated powder ranged from 35.01 to 44.07 µm and 2.01 to 3.94 %, respectively. The optimized polymeric blend for dill seed essential oil was sodium alginate-agar agar at a ratio of 75:25 with an encapsulation efficiency of 79.40 %.

Key words: dill seed, cryogenic grinding, essential oil, carvone, limonene, encapsulation

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CERTIFICATE

This is to certify that the thesis entitled **"ENCAPSULATION OF ESSENTIAL OIL FROM CRYOGROUND DILL SEED"** submitted by **Ms. Anitha N** (Reg. No. 2070918001) in partial fulfilment of the requirement for the award of degree of **Master of Technology** in the subject **Food Technology with specialization in FSQA** to Anand Agricultural University is a record of bonafide research work carried out by her under my guidance and supervision.

Place: Anand **(R. V. Prasad)**

Date: / / 2020 **Major Advisor**

ACKNOWLEDGMENT

I consider myself fortunate enough to have the opportunity to work on a topic as this which involves the fascinating concept of food technology.

Formal statement of acknowledgement will hardly meet the ends of justice in the matter of expression of my deeply felt sincere and heartiest gratitude to my Research Guide ***Dr. R. V. Prasad,*** *Head of the Department of Food Safety & Quality Assurance, College of Food Processing Technology & Bio Energy, Anand Agricultural University, Anand for his inspiring guidance, constant encouragement, constructive criticism and pains- taking efforts in suggesting, designing and improving the quality of study throughout the entire span of research work and finalizing this manuscript successfully.*

I would express my sincere thanks to ***Dr. R. F. Sutar****, Principal and Dean, College of Food Processing Technology and Bio-energy, AAU, Anand for his encouragement and guidance.*

Besides my advisor, I would like to thank the rest of my thesis committee: ***Dr. Amee Ravani, Mr. K. S. Damle and Dr. B. H. Joshi*** *for their encouragement, insightful comments, and hard questions.*

I express my sincere gratitude to ***Dr. H. G. Bhatt****, Head of Department of Food Safety & Testing, College of Food Processing Technology and Bio-energy, AAU, Anand for his ever-willing help, support, guide and blessing.*

I would like to thank ***Dr. Hetal Bhatt*** *and* ***Mr. Nilesh Sardar,*** *Senior Research Assistants, College of Food Processing Technology and Bio-energy, AAU, Anand for their guidance and support throughout the research work. Special thanks are due to my senior* ***Neha Kharkwal*** *and also my friends* ***Mehul Chudasama, Arunima Mukharjee, Atul Kansatwad*** *and* ***Manish Tiwari*** *for their help cooperation and encouragement at various stages for this research work.*

This acknowledgement would be meaningful if I do not remember my parents; to whom I owe all the purpose I have achieved so far, including work. In the course of research work, there were times when I felt low in spirits standing behind me rockhard, supporting me in every possible way. I am also grateful to whole faculty of the college whose help and guidance helped me to grow as a student and as a person.

Finally, I express my thanks to all staff members of College of Food Processing Technology and Bio-energy, Anand Agricultural University, Anand

Date: / /2020

Place: Anand ***(Anitha N)***

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LIST OF ABBREVIATIONS AND SYMBOLS

Particulars	Description
-	Minus
%	Percent
&	And
*	Significant
:	Is to
+	Plus
<	Lesser than
>	Greater than
±	Plus or minus
°C	Degree Celsius
µl	Microlitre
µm	Micrometre
A.O.A.C.	Association of Official Analytical Chemist
ANOVA	Analysis of Variance
b. p	Boiling point
$CaCl_2$	Calcium chloride
cm	Centimetre
cm^2	Square centimetre
CRD	Completely Randomized Design
$CuSO_4$	Copper Sulphate
CV	Coefficient of Variance
d.b.	Dry basis
df	Degree of freedom
DMSO	Dimethyl sulfoxide
et al.	Et Alia
etc.	Etcetera
FM	Fineness Modulus
F-value	Fisher value
g	Gram
g/l	Gram per litre

GC-MS	Gas Chromatography-Mass Spectroscopy
GLC	Gas Liquid Chromatography
h	Hour
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
i.e.,	That is
ID	Inner diameter
IU	International Unit
K_2SO_4	Potassium sulphate
K_2SO_4	Potassium Sulphate
kg	Kilogram
kg	Kilogram
kg/h	Kilogram per hour
Kg/m^3	Kilogram per meter cube
kJ	Kilojoule
KOH	Potassium Hydroxide
kWh	Kilo Watt hour
LN_2	Liquid nitrogen
M	Molar
m/s	Meter per second
m^2/s	Meter square per second
$meqO_2$	Milliequivalent oxygen
mg	Milligram
mg KOH/g	Milligram potassium hydroxide per gram
mg/100g	milligram per 100 grams
mg/kg	Milligram per kilogram
min	Minute
min	Minute
ml	Millilitre
mm	Millimetre
mm^2	Square millimetre
mmol/g	Millimolar per gram

MPa	Megapascal
N	Normal
NaOH	Sodium Hydroxide
nm	Nanometre
NS	Non-significant
PBS	Phosphate-buffered saline
pH	Potential Hydrogen
ppm	Parts per million
PUFA	Polyunsaturated Fatty Acid
p-value	Calculated probability
Pvt. Ltd.	Private Limited
rpm	Revolutions per minute
Rt	Retention time
SD	Standard deviation
SEm	Standard error of mean
SMP	Skim Milk Powder
TPC	Total polyphenolic content
v/v	Volume by volume
v/w	Volume by weight
viz.	Videlicet
w.b.	Wet basis
w/v	Weight by volume
w/w	Weight by weight
WPC	Whey Protein Concentrate
α	Alpha
β	Beta
µg	Microgram

CONTENTS

CHAPTER I

INTRODUCTION

Spices have been an important part of the human diet from ages which are used as adjuncts to season food to impart and enhance a characteristic taste and flavour in edibles and beverages and also sometimes added as preservatives. Spices are recognized for their therapeutic potential for many years as they have an extensive variability of collections of volatile and non-volatile compounds.

Despite remarkable advances in medicinal treatment and pharmaceutical development, the power of spices is now slowly re-emerging as an area of interest for improving human health quality. As a part of traditional medicine, the importance of spices has been amplified which not only provides nutrition upon its consumption but also helps in preventing and curing multiple symptoms of diseases and disorders with cost-effective, harmless and eco-friendly attributes due to the presence of various biochemical components. Today, awareness among the people has led to an increase in demand for the extraction of bioactive compounds from spices and condiments.

Spice extracts such as essential oils and oleoresins are hydrophobic liquids containing aromatic and volatile chemical compounds extracted from various plant parts like flowers, roots, bark, leaves, seeds etc. They are complex, multi-component systems composed mainly of hydrocarbons, terpenes (monoterpenes, sesquiterpenes), lactones, aldehydes, acids, alcohols, ketones, esters, phenol derived aromatic and aliphatic compounds. The secondary metabolites present in them exhibit antimicrobial, antiviral, antioxidant, hypolipidemic, hyperlipidaemic, anticancer, antiulcer properties.

Some of the common methods used for extraction of essential oils and oleoresins from spices include hydrodistillation, steam distillation, fractional distillation, expression, supercritical fluid extraction, maceration, ultrasonication, microwave extraction etc. Due to the increase in knowledge about the positive functions of essential oils, there is a growing interest among food and pharmaceutical industries in finding natural substitutes for synthetic flavours and preservatives.

Anethum graveolens, commonly known as dill is an annual aromatic herb which belongs to the family Umbelliferae (Apiaceae). It is the only spice in the species Anethum, originated from the Mediterranean and West Asia. The generic name of dill, “Anethum” is

derived from the Greek word “anethon” and the name “Dill” from the Norse word “dylla” or “dilla” which probably means to soothe. Dill is commonly known as dill (English), Shatpushpaa (Ayurvedic), Soyaa (Unani), Sadakuppai (Siddha), Sthatpushpi (Sanskrit), Sowa (Hindi) and Soya (Punjabi). The Indian dill plant is cultivated throughout India, chiefly in Punjab, Uttar Pradesh, Gujarat, Maharashtra, Assam and West Bengal, which is usually known as “sowa" (Chahal *et al.*, 2017).

Dill is an annual herb with pinnately divided leaves. The plant grows up to a height of 50-150 cm and 2-5 branches arise from the base of the stem and grow along with the main stem. Flowers are yellow in colour. The seeds, after-ripening attain light to dark brown colour and emit an aromatic odour. Seeds are broadly oval and compressed usually separate and free with 4 mm long, 2-3 mm broad and 1 mm thick. Both seeds and leaves of dill are valued as spices. Its essential oil is rich in terpenes like carvone and limonene which has got wide applications including food, flavour, pharmaceutical, detergent and soap, cosmetics and pesticide industry. It’s oil is used for aromatherapy in traditional medicinal treatment.

The quality of spices is assessed by the retention of essential oil, alkaloids, oleoresins, appearance, texture, shape, colour with superior quality yield, maintaining the desired appearance, medicinal and aromatic properties. Size reduction is a crucial aspect in the food industry as powders are considered as both end products and intermediate products. Since spices are valued for aroma and flavour, their preservation plays an important role during grinding.

Thermal damage is one of the main disadvantages in conventional grinding of spices that cause significant loss in their volatile oil, flavour compounds and natural colour that results in the production of an inferior quality product. These hurdles of the conventional grinding can be overwhelmed by cryogenic grinding that produces high-quality spice powder with fine and uniform particle size distribution. Size reduction at a low temperature environment produces desired material characteristics. The extreme low temperature in cryogenic grinding leads to solidification of oil present in the spices that makes them brittle and crisp in nature. The cryogens used in this process are liquids in the form of hydrogen, helium, oxygen, carbon dioxide etc. The low temperature maintained by the cryogens will captivate the heat generated in the pin mill during the grinding of the sample.

Encapsulation is a technique in which tiny droplets (solid, liquid or gas) are coated or entrapped within another homogeneous or heterogeneous polymeric continuous phase system. The material to be coated is called the core, active material, nucleus, fill or internal phase and the coating material used is called the shell, carrier, the wall material, matrix, encapsulant or external phase. Encapsulation creates a physical barrier to the core material and protects its sensitive components.

Food grade biopolymers like proteins and polysaccharides are used to create suitable system range for encapsulating, protecting and delivering lipophilic functional components. Encapsulation is of great importance and relevance in food and flavour industries due to the virtue of its ability to protect sensitive food components against degradation reactions and loss of volatiles. It is a common technique that is used to prolong the shelf life of the essential oils and oleoresins by preserving their biochemical characteristics. It is a process that converts a liquid into a free-flowing powder that allows alternative use of ingredients which is one of the largest food applications from the industrial and economic point of view.

It is necessary to understand the molecular structure of the biopolymer as it determines the physicochemical and functional characteristics of the final product. The coating material used can range from high molecular weight biopolymers like starch, modified starch and proteins to low molecular weight biopolymers such as cyclodextrin and maltodextrin. Other encapsulating agents include polysaccharides like gum arabic, sodium alginate, xanthan, carrageenan, chitosan etc. and proteins such as gelatine, casein, whey protein, soy protein etc. Typically, suitable materials based on the type of the core material used should be selected which are with high functional properties including good emulsification, film-forming, high solubility, low viscosity, stability to sterilization and environmental conditions, ease of use, consistency during and of low cost. The capsules produced during the encapsulation process can be used as a flavouring compound that can be added in functional, supplementary and nutraceutical foods.

Various techniques are employed to form microcapsules, including spray drying, fluidized bed coating, coacervation, freeze-drying, spray freeze drying, spray chilling and cooling, liposome entrapment, extrusion and inclusion complexation, co-crystallization, interfacial polymerization, molecular inclusion etc. Apart from protecting the core material from degradation, encapsulation also promotes ease of handling ability and control release of volatiles when they are used only in a very small quantity.

Looking at the present needs and applications of essential oils in food and pharmaceutical industries and demand for spice extracts among the public, a scientific study is undertaken with the following objectives:

Objectives

1. Physio-Chemical characterization of dill seed and solvent extracted dill seed oil
2. Optimization of cryogenic grinding parameters for the maximum retention of essential oil from dill seed
3. Optimization of encapsulation parameters for the extracted essential oil

CHAPTER II
REVIEW OF LITERATURE

This chapter illustrates the published literature related to the study. The review covers general information on significance of dill seeds, health benefits, cryogenic grinding and encapsulation of essential oil.

2.1 DILL SEED

Dill is also known as dill-weed or as shapt. It is cultivated to be used as a vegetable, spice seed and also as a source of essential oil. It is an erect, glabrous herb with furrowed, hallow and branched stems having alternate, multipennate and feathery leaves. It has yellowish flowers that are arranged in compound terminal umbels (Hemalatha *et al.*, 2011).

2.1.1 Physicochemical Characteristics of Dill Seed and Dill Seed Oil

Meena *et al.* (2019) reported that the proximate analysis of dill seed varies with variety, the region where it is grown and the stage of harvest. The detailed composition of dill seed reported is as shown in Table 2.1.

Table 2.1 Proximate analysis of dill seed

Constitutes	**Content (per 100 g)**
Moisture	6.6 g
Protein	13.1 g
Fat	17.9 g
Crude fibre	20.7 g
Carbohydrate	35.7 g
Minerals	6.0 g
Mineral matter	9.09 g
Calcium	1.6 g
Phosphorus	0.21 g
Iron	0.012 g
Sodium	0.01 g
Potassium	1.1 g
Vitamin A	175 IU
Vitamin B_1	0.42 mg
Vitamin B_2	0.28 mg
Niacin	2.8 mg
Vitamin C	12 mg
Volatile oil	2.7 mg
Energy	435 calories

Singh *et al.* (2016) characterized the physical properties of dill seed Ajmer Dill-1 (Rajasthan, India) variety based on different moisture content ranging from 4.85 % to 24.81 % (d. b.) and the results obtained are tabulated in Table 2.2. They reported that the seed's geometric parameters along with the angle of repose, coefficient of static friction and terminal velocity increased with increasing moisture content. Bulk density, true density and porosity of the seeds showed a decreasing trend with an increase in moisture content whereas sphericity remained unchanged.

Table 2.2 Physical properties of dill seed at different moisture contents

Parameter	**Moisture content (% d. b.)**				
	4.85	**10.11**	**14.91**	**19.86**	**24.81**
Length (mm)	3.86 ± 0.26	4.01 ± 0.24	4.08 ± 0.24	4.17 ± 0.28	4.31 ± 0.23
Width (mm)	1.83 ± 0.20	1.88 ± 0.22	1.96 ± 0.23	2.02 ± 0.23	2.13 ± 0.16
Thickness (mm)	1.18 ± 0.09	1.25 ± 0.11	1.25 ± 0.10	1.29 ± 0.11	1.33 ± 0.10
Arithmetic mean diameter (mm)	2.29 ± 0.13	2.38 ± 0.14	2.43 ± 0.15	2.49 ± 0.17	2.59 ± 0.12
Geometric mean diameter (mm)	2.02 ± 0.12	2.11 ± 0.13	2.15 ± 0.13	2.21 ± 0.14	2.30 ± 0.11
Sphericity	0.53 ± 0.03	0.53 ± 0.03	0.53 ± 0.02	0.53 ± 0.02	0.53 ± 0.02
Surface area (mm^2)	0.87 ± 0.09	0.87 ± 0.09	0.88 ± 0.08	0.89 ± 0.08	0.90 ± 0.07
Volume (mm^2)	2.72 ± 0.48	3.08 ± 0.57	3.27 ± 0.65	3.56 ± 0.71	3.99 ± 0.59

Desai *et al.* (2014) studied physico-chemical properties of essential oil from dill seed. The essential oil was extracted by hydrodistillation method from both whole seeds as well as powder sieved from the mesh number 8, 20, 35 and 65. 100 g of dill sample in 500 ml of water was extracted for 10-11 h until no more oil was collected in the trap. The specific gravity, refractive index, optical rotation of the oil extracted from whole dill seed was found to be 0.9521, 1.4933, and 57.11° respectively. It has been reported that the specific gravity, refractive index, optical rotation, solubility in alcohol and carvone content was higher for the oil extracted from the whole seed and decreased with decrease in particle size.

Desai *et al.* (2013) analysed the physical and chemical properties of dill seed GD-1 variety and reported that the seeds comprised 5.9 % moisture, 51.25 % carbohydrates, 2.63 % crude fat, 32.61 % crude fibre, 12.25 % protein and 7.66 % total ash. The physical

properties of dill seed measured at a moisture content of 5.9 % (d. b.) are tabulated in Table 2.3.

Table 2.3 Physical properties of dill seed at 5.9 % (d. b.) moisture content

Properties	**Range**	**Average value**
Length (mm)	4.99 - 5.12	5.06
Breadth (mm)	2.00 - 2.13	2.07
Thickness (mm)	1.13 - 1.21	1.17
Size (mm)	2.25 - 2.32	2.28
Sphericity	0.44 - 0.45	0.45
Bulk density (kg/m^3)	432.20 - 458.02	444.88
Porosity (%)	58.82 - 61.76	60.04
Angle of repose (°)	37.12 - 39.29	38.22
Co-efficient of static friction against the different surface		
Wood	0.69 - 0.76	0.74
Steel	0.58 - 0.60	0.59
Glass	0.43 - 0.49	0.46

Singh (2012) characterized hydrodistillated dill seed essential oil and reported that dill oil is pale yellow in colour with a pleasant aromatic odour and has an optical rotation of + 40º (at 25 °C), specific gravity of 0.971 (at 25 °C) and is soluble in 70 % ethanol. Badar *et al.* (2008) characterized dill seed essential oil extracted from steam distillation. The maximum yield obtained was 0.66 % after 13 h of distillation. The essential oil was pale yellow in colour with a pleasant odour and had watery viscosity. The specific gravity, refractive index (at 32.5 °C), and acid value of the oil were 1.51, 1.49 and 0.58 mg KOH/g respectively. Gas chromatography was performed to identify fatty acids present in the oil and the obtained results are given in Table 2.4.

Table 2.4 Fatty acids and their concentration in steam distilled dill seed essential oil

Name of the fatty acid	**Retention time**	**Concentration (%)**
Capric acid	0.882	5.97
Lauric acid	1.508	1.29
Myristic acid	1.96	0.25
Palmitic acid	3.043	4.66
Stearic acid	3.981	3.86
Oleic acid	5.913	37.05
Linoleic acid	7.798	45.13
Linolenic acid	13.636	0.26
Arachidonic acid	17.96	1.32

Saleh-E-In and Roy (2007) studied the fatty acid composition and proximate analysis of dill seed. The oil from crushed dill seed was extracted with petroleum ether (b. p. of 40-60 °C) for 72 h by using Soxhlet apparatus. The extracted oil was analysed for physico-chemical properties and proximate analysis which are given in Table 2.5 and 2.6, respectively. The oil was also used for identification and quantification of fatty acids and the results obtained are given in Table 2.7. They reported that the oil consisted of 6.22 % saturated fatty acids and 93.78 % unsaturated fatty acid among which oleic acid contributed the highest proportion of 87.10 %. Dill seed oil was found to be high in PUFAs that was advantageous over other vegetable oils for health benefits.

Table 2.5 Physico-chemical characteristics of dill seed oil

Characteristics	**Values**
Appearance	An opaque, viscous oily liquid with dark brown colour
Odour and taste	Unpleasant order with a bitter taste
Miscibility and solubility	Insoluble in water, freely miscible in chloroform, carbon tetrachloride, petroleum ether, hexane, diethyl ether and also miscible in alcohol on warming
Specific gravity at 30 °C	0.9231 ± 0.0012
Refractive index	1.4706 ± 0.0005
Optical rotation at 26 °C	+ 9.340 ± 2.0
Acid value (mg KOH/g)	15.67 ± 0.4
Iodine value (Hanus method)	98.72 ± 2.0
Saponification value (mg of KOH/g)	183.26 ± 2.5

Table 2.6 Proximate composition of dill seed

Parameters	**Values (%w/w)**
Moisture (fresh seed)	13.31
Dry matter (fresh seed)	86.69
Crude fibre	28.88
Nitrogen	3.01
Protein	18.87
Fat	9.36
Ash	6.98
Carbohydrate	22.58
Energy	250.13 (calories/g)

Table 2.7 Fatty acid composition of dill seed oil by Gas-Liquid Chromatography (GLC)

Name of fatty acids	Retention time	Peak area	Percent composition
Palmitic acid	17.68	33901	4.27
Linolenic acid	21.60	55313	6.68
Oleic acid	21.86	690820	87.10
Stearic acid	22.35	7559	0.95
Arachidic acid	26.68	1250	0.46
Behenic acid	30.70	4258	0.54
Total percentage			100.00

2.1.2 Extraction of Essential Oil

Stanojevic *et al*. (2015) extracted dill essential oil by Clevenger-type apparatus. The initial oil content in the dry dill seed was 4.0 ml/100 g. The sample and water ratios used for extraction were 1:10, 1:15, 1:20 and 1:25. The essential oil obtained was 2.12 ml, 2.41 ml, 2.80 ml and 2.73 ml respectively. The obtained yield of the essential oil increased with an increase in the hydro module, reached a maximum and then decreased. The highest oil yield obtained was 2.80 ml by application of 1: 20 (w/v) hydro module which was extracted for 180 min. The essential oils obtained from different hydro modules were subjected to Gas Chromatography-Mass Spectroscopy (GC-MS) analysis and the major compounds identified are listed in Table 2.8.

Table 2.8 GC-MS analysis of dill seed essential oil

Retention time/min	Retention index	Component	Percentage obtained for different hydromodules			
			1:10	**1:15**	**1:10**	**1:25**
5.92	1024	Limonene	5.1	2.1	0.9	1.4
9.89	1191	*cis*-Dihydro carvone	3.0	2.6	2.6	2.7
10.07	1200	*trans*-Dihydro carvone	2.7	2.5	2.7	2.7
10.40	1215	*trans*- Carveol	1.4	1.5	1.6	1.4
10.76	1226	*cis*-Carveol	1.8	2.3	2.4	2.0
11.13	1239	Carvone	85.9	88.8	89.0	89.3

Jianu *et al.* (2012) extracted dill essential oil using steam distillation for 4 h and the yield (% v/w) obtained was 2.91 % for mature seeds and 0.92 % for immature seeds. Simandi *et al.* (1996) extracted the essential oil from dill seed using hydrodistillation and

solvent (hexane) extraction. The yield obtained was 2.5 % from hydrodistillation and 12.8 % from solvent extraction.

Charles *et al.* (1995) reported that the oil content in dill seed ranged from 1.75 to 4 % (d. b.). The total oil was recovered in 3 h in a modified Clevenger trap. Ravid *et al.* (1987) extracted ground dill seed oil in a modified Clevenger type apparatus for 2 h and reported that dill seed contains 3.8 % oil. Embong *et al.* (1977) in a study stated that the oil content of dill seed varied according to the season as well as the country and the reported values were 2.1 % (India) and 5.6 % (Russia).

2.2 HEALTH BENEFITS AND APPLICATIONS OF DILL SEED

Meena *et al*. (2019) reported that the oil distilled from dill is an important ingredient in gripe water formulation which is administered to children suffering from bloat, stomach ache, hiccups and is used to soothe baby's colic. The decoction of dill seed is used to cure indigestion, stomach ache, flatulence, vomiting and to promote milk secretion in lactation mothers. A mixture of roasted dill seed and fenugreek seed cures diarrhea. Fresh dill seed powder consumed with ghee cures sterility in the woman and when consumed with milk cures jaundice. Leaf and root paste when applied to swelling of joints gives relief. Dill seed paste with sandalwood helps to release headaches. Dill is beneficial in stimulating and regulating menstrual flow. It effectively liberates spasmodic menstrual pain and the absence of menstruation due to anemia, exposure to cold and pregnancy. Blood pressure can be controlled when a mixture of dill and fennel seeds in equal proportions are consumed. D-limonene is used to dissolve cholesterol-containing gallstones. It has chemo preventive and chemo therapeutic activities. The flavonoids present in dill effectively controls pectic ulcer. The volatile oil of dill is carminative that improves appetite and relieves gas. Dill has been associated with managing insulin levels and it helps to reduce fluctuations of serum lipids in corticosteroid-induced diabetes.

Lal and Meena (2018) reported that the vitamin-B complex and flavonoids present in the essential oil of dill seed activates the secretion of some enzymes and hormones which creates calm and hypnotic effects, hence help people to get a good night's sleep. Dill seed has a good amount of calcium that prevents bone loss and bone mineral density reduction. Monoterpenes are chemo preventive in nature. They activate the secretion of an enzyme called glutathione-S-transferase (the radical glutathione is an effective antioxidant) which neutralizes the carcinogens specially cyano- and benzo-derivatives and free radicals,

thereby protecting the body from cancer. The other antioxidants present in the essential oils of dill also contribute to preventing cancer.

Dill oil has hypolipidemic activity and is used as a cardioprotective agent in decreasing blood cholesterol (Chahal *et al.,* 2017). It has been reported that chewing dill seeds decrease bad breath (Ebrahim *et al.,* 2015; Jana and Shekhawat, 2010). Pharmacological actions such as anti-inflammatory, antispasmodic, antidiabetic, antihypercholesterolemic, antimicrobial have also been reported (Orhan *et al.,* 2013).

2.2.1 Bioactive Compounds

The monoterpenes carvone and limonene are the main constituents of dill seed oil (Meena *et al.,* 2019). Kaptan and Sivri (2018) reported that the main components present in dill are monoterpenes containing 40 to 60 % D-carvone (23.1 %) and D-limonene (45 %). Nada *et al.* (2018) analysed dill oil by GC-MS and reported that the major compounds present are carvone (32.55 %), dillapiole (20.45 %) and D-limonene (16.06 %). Said-Al Ahl *et al.* (2016) reported that dill seed essential oil contains carvone (62.48 %), dillapiole (19.51 %) and limonene (14.61 %).

Ruangamnart *et al.* (2015) analysed bioactive compounds from both hydrodistillation and steam distilled essential oil by GC-MS and the identified compounds are listed in Table 2.9.

Table 2.9 GC-MS analysis of dill essential oil

Compound	**Relative content % [Rt (min)]**	
	Hydrodistillation	**Steam distillation**
β-pinene	-	0.79 (6.36)
β-myrcene	0.21 (6.60)	0.16 (6.61)
Decane	0.44 (6.87)	0.49 (6.87)
1,5,8-p-menthatriene	0.19 (7.04)	0.27 (7.02)
D-limonene	26.96 (7.65)	44.61 (7.66)
Undecane	0.34 (9.52)	0.38 (9.51)
Naphthalene	1.63 (12.30)	2.11 (12.30)
cis-dihydrocarvone	0.38 (12.78)	0.95 (12.79)
trans-dihydrocarvone	1.49 (13.03)	1.57 (13.04)
D-carvone	18.05 (14.56)	28.02 (14.56)
Myristicin	1.41 (25.12)	0.67 (25.13)
Dillapiole	48.9 (27.58)	19.98 (27.58)

Babri *et al.* (2012) in a study reported that the essential oil of dill seed contains R-(-) carvone (38.899 %), dillapiole (30.812 %), limonene (15.938 %) and *trans*-(+)-dihydrocarvone (10.999 %). Singh (2012) analysed hydrodistilled essential dill oil through GC-MS and the components identified are listed in Table 2.10.

Table 2.10 Bioactive compounds of hydrodistillated essential dill oil by GC-MS

Components	**Retention index**	**Content (%)**
Cyclohexane	-	0.5
α-Thujene	923	0.1
β-Myrcene	971	0.3
Limonene	1011	83.0
β-Phellandrene	1028	0.6
Thujyl alcohol	1100	0.7
Grandisol	1200	7.4
Carvone	1206	1.5
bis-1,2-Benzenedicarboxylic acid	-	5.7

Radulescu *et al.* (2010) performed GC-MS for hydrodistillated essential oil and the identified compounds are listed in Table 2.11.

Table 2.11 GC-MS analysis of dill essential oil

Compound	**Retention time/min**	**Retention index**	**Area or %**
α-pinene	6.328	930	0.02
α-phellandrene	8.839	1004	0.12
Limonene	9.531	1028	21.56
cis-dihydrocarvone	15.088	1201	0.04
trans-dihydrocarvone	15.154	1204	3.02
Carvone	16.481	1249	75.21

Jirovetz *et al.* (2003) reported that dill oil contains D-limonene (44.1 %) and D-carvone (50.1 %). Charles *et al.* (1995) reported that dill seed contains carvone and dihydrocarvone comprising 68 % to 83 % and limonene which ranges from 14.18 % to 21.43 % of the total oil constituents.

2.2.2 Antimicrobial, Antifungal and Antioxidant activity

Kaptan and Sivri (2018) reported that the essential oil of dill has antimicrobial activity against *Salmonella sp., Staphylococcus aureus* and *E. coli* and inhibits the growth

of *Listeria monocytogenes.* An inhibitory effect was also detected on *Penicillium verrucosum* which causes deterioration in cheese.

Nada *et al*. (2018) reported in a study that the essential oil of dill seeds showed antimicrobial and antifungal activity against four fungi strains *Aspergillus niger, Aspergillus flavus*, *Penicillium expansum* and *Penicillium italicum* and five bacterial strains *Staphylococcus aureus, Bacillus cereus*, *Bacillus subtilis, Escherichia coli* and *Salmonella typhimurium.*

Mohamed *et al.* (2013) studied the impact of antimicrobial properties of some essential oils as antibacterial agents on the quality of cheese yoghurt. Cheese yoghurt was manufactured from milk which was previously added with five strains of pathogens and was incorporated with caraway and dill seed essential oils. They were then stored at 7 °C ± 2 for 14 days. Cheese yoghurt samples were examined for microbial growth at period of 0, 7 and 14 days of cold storage. They found that the gram-positive bacteria were more susceptible compared to gram-negative bacteria. The most sensitive microorganism reported was *Listeria monocytogenes*, while the most resistant microorganism was *E. coli* O157:H7.

Mousavi *et al.* (2013) obtained dill extract using a cold solvent method by three solvents i.e., methanol, hexane and acetone to examine the antioxidant potency of dill extract on sunflower oil. Dill extracts were added to sunflower seed oil at concentrations of 0.5, 0.3, 0.1 and 0.05 % w/w to determine the oxidative stability of sunflower seed oil. The antioxidant potential of the samples containing extracts was evaluated by measuring the peroxide value and the induction period using the rancimat apparatus. The sample containing 0.5 % methanolic extract of dill was found to have the lowest peroxide value and the highest oxidative induction period that improved the stability of sunflower oil.

Jianu *et al*. (2012) studied the antimicrobial activity of essential oil obtained from dill seed and reported that the essential oil from immature dill seeds possesses more efficient antimicrobial activity than mature seeds. It strongly inhibited the development of *E. coli, Salmonella typhimurium, Klebsiella pneumonia* and *Clostridium perfringens.* The essential oil from the mature seeds showed higher antimicrobial activity against *C. perfringens and S. typhimurium.*

2.2.3 Applications in Food Industries

Meena *et al.* (2019) reported that carvone is industrially used for fragrance and

flavour. It is also used as a potato sprout inhibitor and as an antimicrobial agent. Kaptan and Sivri (2018) reported that dill contains essential oil in its leaves, stem, flowers as well as fruits. Chopped fresh dill leaves are used for flavour and aroma in kefir, yoghurt and sour cream.

Zaky *et al.* (2013) evaluated the quality of salt-free labneh prepared using dill and caraway essential oils. Salt-free labneh products were prepared from buffalo milk using 2 µl/100 ml milk of both dill and caraway essential oils. Chemical, anti-oxidant and organoleptic properties of samples stored at 7 ºC ± 2 were evaluated for 28 days. Microbiological examinations were periodically carried out until 14 days of sample storage. They reported that the presence of dill and caraway essential oils enhanced the anti-oxidant activity and increased the scavenging of free radicals in treated samples compared with control ones. Starter culture was not much affected when added with essential oils. But the total bacterial viable counts were found to be increased for all samples till they reached their maximum counts after 7 days. Coliform bacteria as well as yeasts & molds were not detected in all samples during storage. Using dill and caraway essentials oils improved the sensory properties of both fresh and stored salt-free labneh samples. Their body & texture were soft with desirable spreadable properties. Essential oils enhanced the taste, flavour and odour of labneh and also created a refreshing mouth feeling.

Dill leaves and seeds have a strong spicy odour, therefore used as a flavouring agent in the food industry for salads, sauces, soups, cakes, bread loaf, pastries, preserves, tea, seafood, meat and pickles (Babri *et al*., 2012; Dahiya and Purkayastha, 2012).

2.3 CRYOGENIC GRINDING

Cryogenic grinding is also known as cryomilling or freeze milling where the particle size of the material is reduced at low temperature using cryogens by the act of cooling or chilling the material. Pre-cooling of raw spices before grinding conserve the volatile components from loss. Consequently, the integrity and quality of the spices remain intact. Thus, it removes the drawbacks of ambient grinding.

The oil content of spices generally poses the problem, whereas other factors considered are product particle size, yield, uniformity, free form contamination, economy, and dust from grinding operation. The flavour strength per unit mass of the cryogenically ground product is twice as that of the conventionally ground spices. A schematic diagram of the cryogenic grinding system is shown in Figure. 2.1.

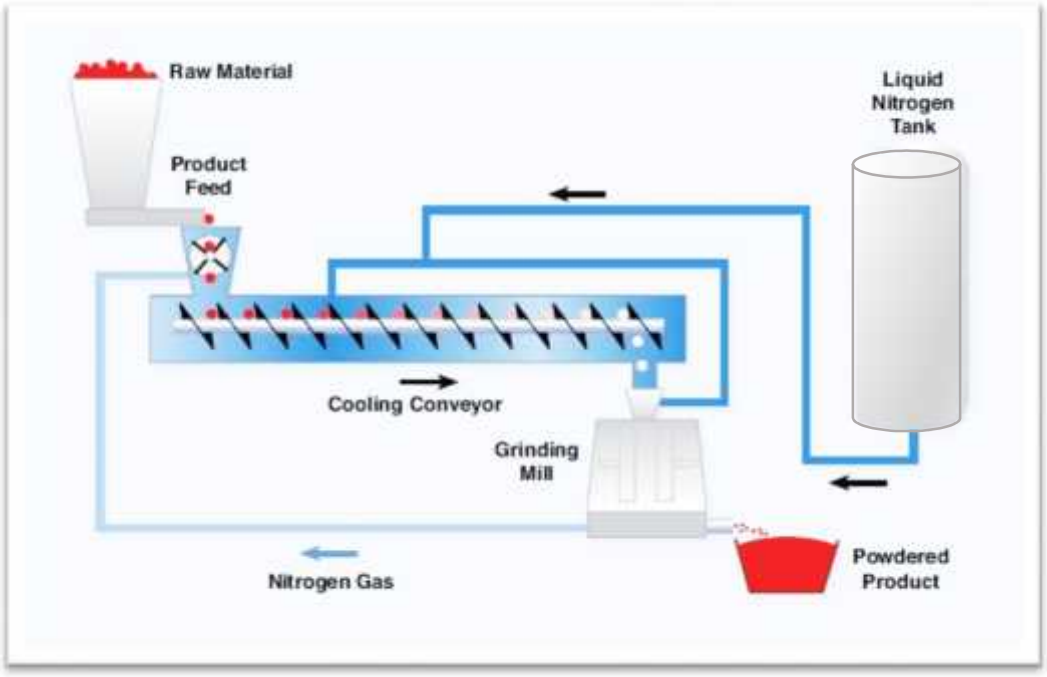


Figure. 2.1 Schematic diagram of cryogenic grinding system

2.3.1 Advantages of Cryogenic Grinding Over Ambient Grinding

Many researchers had summarized the advantages of cryogenic grinding over traditional-ambient grinding techniques (Pesek and Wilson, 1986), (Pesek *et al.*, 1985) and (Anon, 1962). Advantages are as follows:

a) Cryogenic grinding prevents the loss of volatile compounds and retains their original flavour strength and weight. On an average, 7g of cryoground spices have the same flavouring potential as 10g of ambient ground products.

b) Cryogrinding minimizes the oxidation of spice oils because of the expelling air in the mill due to the evaporation of liquid nitrogen in the grinding zone. During cryogrinding, spices became very brittle and spice oil was found to be solidified. These finely grounded spices give more flavour uniformly throughout the final product.

c) Cryoground spices disperse better flavour and uniformity in the preparation of liquids or solutions. It virtually prevents specking problems in sausages.

d) Cryoground spices were more stable as compared to ambient ground spices. It might be due to the absorbance of some quantity of nitrogen by spices.

e) The lower temperature during grinding minimizes the “gumming up” of

surfaces and screens of the mill which gradually increases the grinding rate.

f) The process is applicable to many foods other than spices i.e., coffee, tea, cocoa, coconut and dehydrated meat.

Advantages of cryogenic grinding over traditional grinding are simplified in Table 2.12 (Morrison, 2011).

Table 2.12 Comparison between traditional and cryogenic grinding

Parameter	**Cryogenic grinding**	**Traditional grinding**
Energy consumption	Low	High
Throughput	High	Low
Mill clogging	No clogging	Frequent
Volatile losses	Minimum	Higher
or capacity	Low	High
Control on particle size	Effective	No control
Grinding of soft material	Possible	Very difficult
Fire risk	No	High
Air pollution	No	Yes
Microbial load	Does not exist	Possible

2.3.2 Cryogenic Grinding of Different Spices

Ghodki *et al.* (2019) studied the influence of cryogenic grinding on microstructural characteristics of Indian spices i.e., black pepper, cinnamon, king chili and fenugreek. In the cryo-grounded samples, total pore volume (except for king chili), total sample volume, and total solid volume were found to be higher up to 100 – 526.83 %, 5.83 – 120.90 %, and 4.41 – 120.12 %, respectively with the prominent size distribution of pores. An increase in microstructural properties also resulted in 2.31–10.45 % higher volatile oil extraction yield. Anti-inflammatory properties of cumin (*Cuminum cyminum L.*) and ajwain seed extracts were increased by cryogenic grinding technology (Saxena *et al*., 2018).

Barai *et al.* (2017) examined cryogenic and conventional grinding of cumin and coriander seeds. They have reported that the moisture content of the spice powder obtained was 10.93 % and 7.00 % lesser in cryogenic grinding than that of conventional grinding for cumin and coriander seeds respectively. There was 43.3 % and 172.7 % increase in the volatile oil content in cumin and coriander respectively due to cryogenic grinding. The non-

volatile was increased in cumin by 107.7 % and 45.4 % in case of coriander when compared to conventional grinding.

Saxena *et al.* (2017) conducted a study to improve the quality of cumin powder by using cryogenic grinding technology at different temperature i.e., -30 °, -20 °, -10 °, 0° and 10 °C with three different feed rates i.e., 5, 6 and 7 kg/h and particle size of 0.8, 1.0 and 1.5 mm. The volatile content was ranged between 2.1 to 3.3 % in cryoground cumin powder. They found that the cryogenic grinding retained 62.56 % more volatile oil as compared to ambient grinding. Cumin powder with superior quality was obtained at a grinding temperature of -30 °C with a feed rate of 7 kg/h and a sieve size of 0.8 mm, based on the retention of volatile oil. They also reported that the cryoground cumin powder retained the highest volatile oil of 3.3 %. Cryoground cumin powder was stored in airtight, moisture-proof zip lock aluminium foil pouches under refrigerated (4 ± 1 °C) and ambient conditions. The stored samples were analysed for volatile oil content for a period of three months at 30 days intervals. Under both storage conditions, the loss in total volatile oil content was observed with an increase in the storage period. After 90 days, the loss of volatile oil was found to be 7.58 % and 12.25 % under refrigerated and ambient storage conditions, respectively.

Sharma (2017) conducted experiments with methanol and hexane crude seed extract of cumin and ajwain genotypes obtained after grinding with cryo and non-cryo ground technology. He evaluated crude seed extract of cumin and ajwain for anti-microbial, anti-inflammatory, anti-diabetic, hepatoprotective, and diuretic activities. Findings were suggestive for the superiority of cryogenic grinding technology over traditional grinding for retention of medicinal properties of seed spices. Seed extracts of cryoground technology were proved better than non-cryogenic grinding for both activities. As cryogenic grinding was able to retain more phenolic, flavonoid, and essential oil contents compared to non-cryoground seeds, they showed considerably more antibacterial and antifungal properties.

Ghodki and Goswami (2016) studied the effect of grinding temperature on particles and physicochemical properties of black pepper. Grinding was carried out at five different temperatures i.e., -120 °, -80 °, -40 °, 0 ° and 40 °C with a feed rate of 1.5 kg/h. Rotor speed and grinding time were set at 26 m/s and 10 min, respectively. It was found that lower grinding temperature resulted in higher volatile oil recovery of a maximum amount i.e., 1.79 ml/100g at -120 °C compared to 0.81 ml/100g at 40 °C. An increase in moisture content and water activity in black pepper powder was also found with a decrease in the

grinding temperature. However, the pH of the powder decreased with a decrease in the grinding temperature. The mineral composition of the cryoground powder significantly increased with a decrease in the grinding temperature.

Table 2.13 Physicochemical properties of black pepper powder with different grinding temperatures

Grinding temperature (°C)	Moisture content (% d. b.)	pH	Water activity	Volatile oil (ml/100g)
-120	4.10 ± 0.03	6.07 ± 0.04	0.64 ± 0.00	1.79 ± 0.02
-80	3.91 ± 0.05	6.14 ± 0.01	0.59 ± 0.00	1.55 ± 0.03
-40	3.86 ± 0.02	6.20 ± 0.03	0.54 ± 0.00	1.21 ± 0.09
0	3.82 ± 0.03	6.24 ± 0.04	0.53 ± 0.00	0.98 ± 0.05
40	3.79 ± 0.01	6.30 ± 0.04	0.52 ± 0.00	0.90 ± 0.05
Hand-pounded	3.71 ± 0.07	6.22 ± 0.02	0.58 ± 0.00	0.81 ± 0.02

Mekaoui *et al*. (2016) reported that cryogenic grinding of cumin seeds increased the yield of volatile oil (6.22-14.5 %) when compared to classical grinding. Sharma *et al.* (2016), in a study found that cryogenic grinding enhanced the recovery of volatiles by 33.9 % in the GC 4 genotype of cumin and 43.5 % in the RZ 209 genotype of cumin.

Sharma *et al*. (2016) compared volatile oil and fatty oil constituents of cumin seed oil showing a significant effect of cryogenic grinding. The compound cuminaldehyde responsible for typical cumin oil flavour was found to increase from 48.2 % to 56.1 % on cryogenic grinding. Overall, the oil extracted from cryoground seed powder was more fresh and pleasant as shown in Table 2.14. They analysed the technology by grinding seeds of two contrasting genotypes of cumin, Gujarat Cumin-4 and RZ-209 at ambient and cryogenic temperatures. They reported that cumin seeds lose a significant proportion of volatile oil (18–19 %) while ground at ambient temperature which could be minimized in cryogenic grinding. The recovery of the volatiles enhanced by 33.9 % in Gujarat Cumin-4 and 43.5 % in RZ-209 by cryogenic grinding.

Table 2.14 Essential oil and oleoresins percentage in cumin variety

Variety	Essential oil (%)		Oleoresins (%)	
	Cryoground	Non-cryoground	Cryoground	Non-cryoground
GC 4	4.1 ± 0.15	3.0 ± 0.15	17.7 ± 0.43	15.3 ± 0.73
RZ 209	3.6 ± 0.09	2.5 ± 0.15	14.9 ± 0.13	11.5 ± 0.49

Ghodki and Goswami (2015) optimized the cryogenic grinding parameters for cassia (*Cinnamomum loureirii nees* L.). The initial moisture content of cassia bark was maintained at 11.1 ± 0.1 %. Before cryogenic grinding, the cassia bark was broken into pieces of length approximately equal to 50 mm. The grinding was carried out with four independent variables: feed rate of 2 to 10 kg/h, the grinding temperature of -132 º to 30 ºC, the peripheral speed of 7.77 to 18.13 m/s and a moisture content of 6 to 14 % (d. b.) The optimized cryoground sample had a moisture content of 9.1 %, the feed rate of 2 kg/h per with desirability value of 0.91 which was grounded at the temperature of -97 ºC with a peripheral speed of 10.5 m/s. The final particle size of the optimized cryo-ground sample was 60 µm yielding volatile oil of 2.9 ml/100 g of powder.

Saxena *et al*. (2015) studied the effect of cryogenic grinding on volatile oil and oleoresins content of nine different coriander genotypes. Feed rate was set at 1 kg/h with a screw speed of 3 rpm. Pin mill speed was set at 10,000 rpm. The inlet temperature was -50 °C with the product particle size of 50 µm. Volatile oil and oleoresin content were found to be higher in cryoground samples i.e., 0.14 % in genotype RCr 436 to 0.39 % in genotype Sindhu. Oleoresin content was found to be 13.80 % in ACr 1 to 19.58 % in Australia as shown in Table 2.15. The amount of total antioxidant content in cryoground seeds was significantly high in all genotype, ranging from a minimum of 5.09 mg BHT E/g crude seed extract in Sindhu to a maximum of 10.85 mg BHT E/g crude seed extract in Sadhna followed by genotype Sudha i.e. 10.21 mg BHT E/g crude seed extract.

Table 2.15 Effect of grinding methods on the recovery of essential oil and oleoresin content in coriander genotypes

Genotypes	Volatile oil (%)			Oleoresins (%)	
	Intact seeds	Cryoground	Non cryoground	Cryoground	Non cryoground
Acr 1	0.25	0.23	0.10	13.80	11.77
RCr 41	0.32	0.21	0.14	19.32	12.48
Australia	0.32	0.30	0.18	19.58	10.38
RCr 435	0.11	0.14	0.10	19.52	13.75
Sindhu	0.32	0.39	0.32	17.66	15.53
Swati	0.34	0.37	0.29	19.25	5.39
Sadhna	0.29	0.38	0.23	17.48	11.21
Sudha	0.32	0.29	0.24	18.16	9.53
RCr 436	0.11	0.14	0.08	18.81	12.83

Sharma *et al.* (2015) studied the effect of cryogenic grinding on oil yield, phenolics and antioxidant properties of two ajwain seed genotypes i.e., AA 2 and AA 93. Cryogenic grinding was carried out at -30 °C with a feed rate of 1 kg/h at 3 rpm. The particle size of the powder was set to 50 µm. Oleoresins were collected using accelerated solvent extraction for 30 g of seed powder and then the solvent was evaporated by using a rotary evaporator. The essential oil was collected utilizing 25-30 g of sample using a Clevenger apparatus. Total Phenolic Content (TPC) was determined by using Folin-Ciocalteu assay and the absorbance was measured at 710 nm. It was found to be higher in seed extracts of cryo ground samples of both the genotypes. TPC was maximum i.e., 109.75 mgGAE/ml in crude seed extract of AA 2 in methanol extract and 168 mgGAE/ml in crude seed extract of AA 93 in DMSO (Dimethyl sulphoxide) extract. Maximum total flavonoid content was found in hexane crude extract for AA 2 i.e., 797.17 mgQE/ml that was determined by measuring absorbance at 517 nm. Both genotypes of ajwain showed maximum antioxidant activity in methanol seed extract i.e., 151.61 and 135.38 mg BHTE/ml for AA 2 and AA 93 respectively. It was found that cryogenic grinding was superior to obtain a higher quality of volatile oil compared to traditional grinding.

Barnwal *et al.* (2014) studied the effect of cryogenic and ambient grinding on cinnamon and turmeric. Cinnamon bark and turmeric rhizomes with an initial moisture content of 8.8 % and 7.3 % (w. b.) respectively were used for the study. A laboratory grinder with a pin mill attached with liquid nitrogen (LN_2) dewar of 50 L capacity was used for cryogenic grinding by maintaining the temperature of less than -50 ºC. Grinding of spices was performed with a screw speed of 4 rpm and pin mill speed of 10,000 rpm. The obtained spice powder was analysed for various physical parameters and the results obtained are mentioned in Table 2.16 below. Specific energy consumption was lower for cryogenic grinding when compared to that of conventional grinding. The physical parameters i.e. average particle size, volume surface mean diameter, mass mean diameter and volume mean diameter were found to be lower in cryogenic grinding than in ambient grinding. The average particle size of cryoground cinnamon and turmeric was 0.356 mm and 0.336 mm respectively and in ambient grinding it was 0.454 mm and 0.407 respectively. The colour values of cryoground spices were found to be better than ambient ground spices as shown in Table 2.16.

Table 2.16 Physical properties of ambient and cryoground cinnamon and turmeric

Parameters	Cinnamon		Turmeric	
	Ambient grinding	**Cryogenic grinding**	**Ambient grinding**	**Cryogenic grinding**
Average particle size	0.454	0.356	0.407	0.336
Volume surface mean diameter	0.360	0.351	0.351	0.335
Mass mean diameter	0.374	0.300	0.379	0.328
Volume mean diameter	0.309	0.277	0.270	0.259
Colour value				
L	48.0	55.8	36.0	35.00
a	19.6	9.6	27.4	28.6
b	67.3	22.3	58.0	58.7

Meghwal and Goswami (2014) studied the cryogenic grinding of fenugreek and black pepper seeds using rotor, ball, hammer and pin. They reported that the time required for grinding of the sample was higher in ball mill > rotor mill > pin mill > hammer mill. The power consumption was higher in pin mill > hammer mill > rotor mill > ball mill. The consumption of liquid nitrogen was found to be higher in the ball mill > hammer mill > pin mill > rotor mill. The temperature raised during grinding was higher in pin mill > hammer mill > rotor mill > ball mill.

Saxena *et al.* (2014) studied the effect of cryogenic grinding on volatile oil yield of coriander seeds genotypes i.e., Sudha and RCr-436. Cryogenic grinding was carried out at -50 °C using liquid nitrogen with a feed rate of 1 kg/h at 3 rpm. Pin mill speed was set to 10,000 rpm. It was found that volatile oil yield was higher in cryoground samples i.e., 0.294 % (Sudha) and 0.100 % (RCr-436) compared to conventional grinding i.e., 0.240 % (Sudha) and 0.080 % (RCr-436).

Sharma *et al*. (2014) conducted a study on cryogenic grinding technology to enhance the volatile oil, oleoresin and antioxidant activity of two cumin genotypes. They have reported that cryogenic grinding not only retained the volatiles in both the genotypes but enhanced the recovery too. Total phenolic and flavonoid content was also more in cryogenically ground seeds for both the genotypes and non-cryogenic or ambient grinding temperature caused 18-19 % loss of volatile oil in both the genotypes.

Liu *et al*. (2013) stored black, white, and green pepper powders separately in black packaging bags at 4 °C for 6 months and found that cryogenic grinding was found to be

better compared to hammer milling in preserving the aroma constituents but their concentrations were reduced during storage.

Meghwal and Goswami (2013) performed ambient and cryogenic grinding of fenugreek seeds. In ambient grinding, the sample was manually fed through the feed hopper at 0.348-0.414 kg/h whereas in cryogenic grinding the samples were fed along with liquid nitrogen at 0.458 - 0.580 kg/h. They reported that the cryogenic grinding temperature of -28 º to -70 ºC solved the problems of ambient grinding such as protein structure damage, volatile oil loss and undesirable changes in colour of the final product.

Meghwal and Goswami (2010) studied ambient and cryogenic grinding of black pepper to test the novelty of cryogenic grinding and pinpoint the drawbacks of ambient grinding. It was found that ambient grinding needs more power (8.92 %) and specific energy (14.5 %) than cryogenic grinding. Cryogenic grinding produced coarser particles. The higher amount of volatile oil i.e., (2.15 ml/100g) was found in cryogenic grinding. Powder with freshness and lower whiteness (40 %) and higher yellowness (14 %) for cryogenic grinding was reported.

Murthy and Bhattacharya (2008) carried out cryogenic grinding of black pepper using a pilot plant pin mill at different temperatures and feed rates. They reported that there was no significant difference in the geometric mean particle diameter of both ambient and cryo-ground black pepper powder. The yield of volatile oil ranged between 1.42 and 1.91 ml/100g in cryoground powder whereas it was between 0.78 and 0.98 ml/100 g in ambient ground powder. The average volatile oil yield was 1.7 ml/100 g in the cryoground sample whereas it was 0.9 ml/100 g in the normal ground sample. The highest volatile oil was found to be at -40 °C and 35 kg/h feed rate. The gas chromatographic analysis found that the ratio of monoterpenes to sesquiterpenes was between 1.35 to 1.85 in cryogenic grounded samples which were higher compared to ambient grounded samples. Limonene content was found highest i.e., 16.82 % in cryoground sample at -54 °C and 52 kg/h feed rate. The quantity of monoterpenes in the cryoground sample varied from 40.5 two 53.5 % whereas it was only 25.7 % in the case of ambient grinding.

Singh and Goswami (2000) carried out cryogenic grinding of cloves using sieve size of 0.5 mm and 1 mm, feed rate of 1.5, 3, 4.5 and 6 kg/h and grinding temperature of -110 º, -90 º and -50 ºC. The initial moisture content of the cloves was 9 % (d. b.). 200 g of clove was ground at the temperature ranging from -110 º to -50 ºC with grinder speed maintained

at 69 to 92 m/s. They reported that there was an insignificant variation in volatile oil content from 13.31 to 13.16 ml/100g with the rise in the grinding temperature from -110 º to -50 ºC. The volatile oil content declined from 11.0 to 9.3 ml/100 g in ambient grinding conditions as the grinding temperature increased from 55 º to 85 ºC. It has been reported that deposition of powder over sieve surface was negligible at -50 ºC and deposition increased with an increase in temperature. Problems such as blockage of sieve perforations, overloading of material in grinder with incoming raw material was encountered during grinding at temperatures above -50 ºC. The particle size of the ground powder was found to be increased with increase in the temperature from -110 º to -50 ºC. Specific energy consumption increased with an increase in grinding temperature, rotor speed and feed rate. The yield of volatile oil was 29.5 % higher in the cryoground sample as compared to sample grounded at ambient temperature.

Singh and Goswami (1999) studied on grinding of cumin seed having an initial moisture content of 9.5 % (d. b.) and fat content of 15 % at various temperatures to observe its effect on volatile oil content and its components. An increase in temperature in the cryogenic range (-160 ° to -70 °C) had no significant effect on volatile oil content, whereas an increase in temperature in the ambient range (40 ° to 85 °C) significantly reduced the volatile oil content from 2.86 to 2.26 ml/100g. The volatile oil components were not significantly affected by grinding temperature in the cryogenic temperature range. Volatile oil retention was 31 % more in cryogenic grinding than conventional grinding. But there was a significant reduction with an increase in grinding temperature from 55 º to 85 ºC under ambient grinding conditions. With the increase in temperature from -160 ° to -70 °C, the volume mean diameter and specific energy consumption of cumin powder increased from 129 to 164 µm and 72 to 108 kJ/kg, respectively for 12 number of rotor ribs.

Pesek & Wilson (1986) and Pesek (1985) worked on grinding of spices i.e., white pepper, nutmeg, cinnamon, oregano and cumin. Cryoground products were found to be superior to ambient grounded spices. The results suggested that the light and volatile compounds were retained higher through cryogrinding compared to ambient grinding of spices. GC-HS (Head Space) analysis of spice volatiles obtained by cryogenic and ambient grinding is shown in Table 2.17.

2.4 ENCAPSULATION OF OILS

Encapsulation is one of the best techniques to protect volatile components from

adverse environmental conditions and to achieve controlled release of the sensitive components at controlled conditions.

Table 2.17 GC-HS analysis of different spice volatile oils obtained by cryogenic and ambient grinding

Spice	Volatile oil compound	Grinding techniques		
		Ambient	Cryogenic	% Increase due to cryo-grinding
White pepper	α-Pinene	4.86	9.50	95.5
	β-Pinene	6.95	10.63	53.0
	Unidentified	9.64	13.36	38.6
	Limonene	5.59	6.76	21.9
Nutmeg	α-Pinene	16.75	19.38	15.7
	β-Pinene	8.84	9.34	5.7
Cinnamon	α-Pinene	1.15	8.32	623.5
	Camphene	0.40	2.53	532.5
	β-Pinene	0.45	2.42	437.8
	Cineole	0.11	0.47	327.3
	Limonene	0.24	0.54	125.0
Cumin	α-Pinene	0.24	0.42	75.0
	β-Pinene	3.23	4.44	39.0
	γ-Terpinene	1.24	1.39	12.1
Oregano	Unidentified	0.62	2.86	361.3
	α-Pinene	1.49	5.71	283.2
	Camphene	0.82	2.44	197.6
	β-Pinene	1.40	4.10	192.9
	Myrcene	0.00	3.98	Very high
	Cymene	8.32	17.31	108.1
	γ-Terpinene	4.92	12.87	16.6

Bastos *et al.* (2020) encapsulated black pepper essential oil with gelatine and sodium alginate. Gelatine (GE) and sodium alginate (NaAlg) solution was prepared by dissolving in water using a magnetic stirrer for 24 h (NaAlg) and 15 min (GE) at 50 °C. The pH of the mixture was adjusted to 4 using NaOH and HCl solutions. Black pepper essential oil was initially mixed with 40 % Tween 20 and then added to GE/NaAlg solution by continuously agitating with a magnetic stirrer for 3 min. Further 25 ml of calcium chloride ($CaCl_2$) solution was added and dissolved using a magnetic stirrer. Capsules were stored at 10 °C for 48 h and then the supernatant was removed. Final capsules were frozen at -80 °C. The terpenes in black pepper essential oil after encapsulations were then determined for their chemical composition as shown in Table 2.18. As compared to 28 % in original essential oil, 22.9 % of β-caryophyllene was retained after encapsulation.

Limonene was found to be 12.10 %, sabinene 9.13 %, β-pinene 8.73 %, α-pinene 8.15 %. The main terpenes in the essential oil were preserved with 80 % of their original content (unencapsulated essential oil).

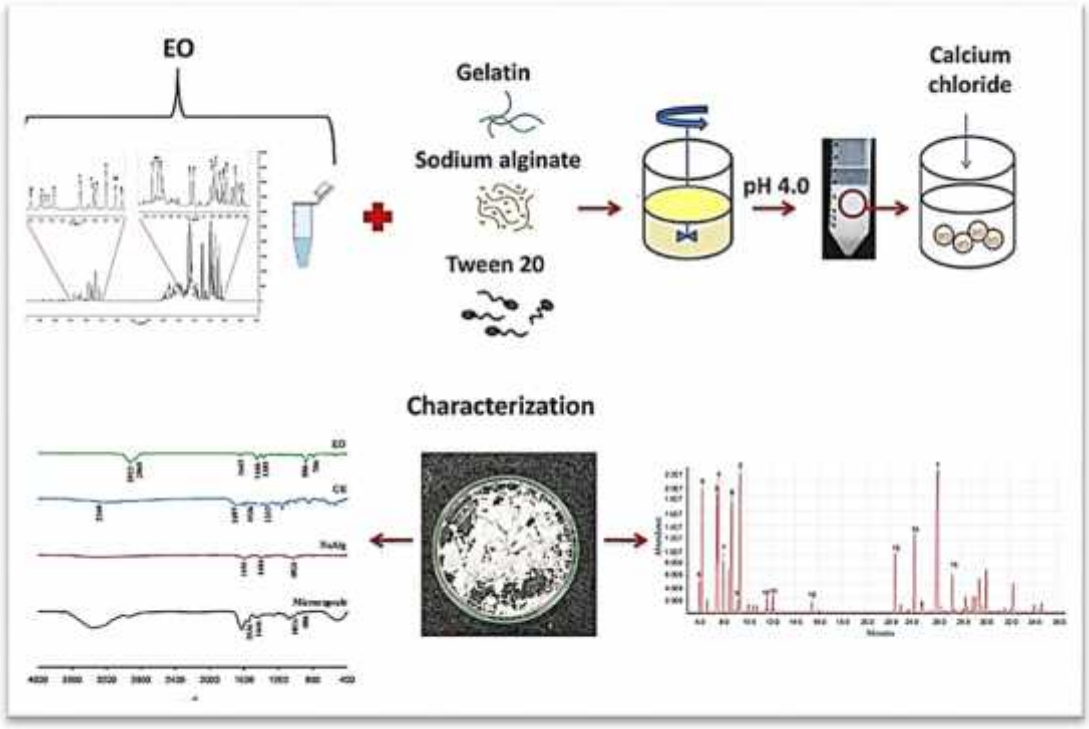


Figure 2.2 Formulation of the black pepper essential oil capsules

Table 2.18 Chemical compositions of the encapsulated black pepper essential oil by GC

Component	Essential oil (%)	Encapsulated essential oil (%)
β-caryophyllene	28.00	22.90
Limonene	15.00	12.10
Sabinene	11.40	9.13
β-pinene	11.00	8.73
α-pinene	10.50	8.15
3-carene	6.00	0.81
α-copaene	3.60	1.83
Elemene	2.00	0.18
Mircene	1.60	0.98
α-capriophylene	1.00	0.62

Yazhini *et al.* (2017) encapsulated probiotic bacteria, *Lactobacillus lactis and Bifidobacterium bifidum*. 100 mg of the probiotic culture of each bacterium were inoculated in 5 ml of broth and incubated overnight. The incubated culture was then centrifuged at

4,000 rpm for 30 min. Then the bacterial pellets were washed thrice using phosphate-buffered saline (PBS) solution and were resuspended in l ml of PBS and mixed in 10 ml of 4 % autoclaved sodium alginate solution. The mixture was extruded into 100 ml of sterile 2.5 % $CaCl_2$ solution using a sterile insulin syringe with the help of a magnetic stirrer. The distance upheld between the tip of the syringe and $CaCl_2$ solution was 30 cm. the beads formed were left in the solution for 30 min for hardening and then transferred into 100 ml of 0.4 % sterile chitosan solution and left for 30 min. The formed beads were washed and placed in the incubator for 24 h at 37 ºC for drying. These beads were later tested for viability efficiency by exposing them to three different temperatures of 37 ºC, 42 ºC and 50 ºC and the observations are given in Table 2.19.

Table 2.19 Survival percentage of non-encapsulated and encapsulated probiotic bacteria

Temperature (ºC)	*Lactobacillus lactis*		*Bifidobacterium bifidum*	
	Non encapsulated	Encapsulated	Non encapsulated	Encapsulated
37	96.36 ± 0.15	97.56 ± 0.39	92.90 ± 0.277	99.35 ± 0.61
42	88.67 ± 0.03	92.62 ± 0.12	92.46 ± 0.08	94.15 ± 0.11
50	84.90 ± 0.52	90.04 ± 0.64	76.62 ± 0.45	90.61 ± 0.18

Vasile *et al.* (2016) encapsulated fish oil in the alginate-chitosan polymer. Two ratios of emulsions were studied. Emulsion 1 with 10 % fish oil, 1 % alginate and 89 % water w/w. Emulsion 2 with 10 % fish oil, 1 % alginate, 2 % gum and 87 % water w/w. one g of oil was added into 9 ml of aqueous polyelectrolyte solution and was mixed at 20,000 rpm for 3 min. The emulsion was then sprayed into $CaCl_2$ solution (20 g/l) using a peristatic pump fitted at 9 ± 0.1 rpm. The tip of the needle was fixed at a distance of 6 cm above the gelling solution. The beads were left in the gelling solution for 10 min to harden and then transferred into a 2 % w/v chitosan solution (prepared in 0.1 M HCl) and were left for 10 min. Lastly, the beads were separated and washed with $CaCl_2$ solution and vacuum dried for 24 h at 30 ºC in an oven chamber functioning at a pressure of 700 mbar. Alginate-chitosan and alginate-chitosan-gum beads were spherical in shape with uniform size (1.13 ± 0.07 mm and 1.49 ± 0.06 mm respectively). Alginate-chitosan beads were oily and sticky whereas alginate-chitosan-gum beads were free-flowing. Both the type of beads was stored in controlled conditions to evaluate primary and secondary lipid oxidation. Pure fish oil stored under the same conditions was used as control. They found that the beads with gum decreased oxidative damage of oil during storage in comparison to alginate-chitosan beads.

Chranioti and Tzia (2014) studied freeze-drying of fennel oleoresin products using gum arabic (GA) as an encapsulating agent combined with modified starch (MS), maltodextrin (MD) and chitosan (CH). 15 g modified starch, maltodextrin and gum arabic of coating materials were individually dispersed in water and the final volume was made up to 100 ml whereas, 2 % of chitosan was dispersed in 1 % glacial acidic acid solution and kept at room temperature for 24 h to ensure complete dispersion. 0.33 g of oleoresin was added into the aliquot and the emulsions were homogenized for 5 min at 10,000 rpm. Further, one ml of Tween 80 was added to advance emulsification. The emulsion was then frozen overnight and was freeze-dried. They reported that substituting gum arabic with modified starch provided a good encapsulation in terms of storage stability, redispersibility and microencapsulating efficiency (74.88 %).

Fernandes *et al.* (2014) encapsulated rosemary essential oil using gum arabic, starch, maltodextrin and inulin (IN) as wall materials. The wall materials were dissolved in distilled water and kept overnight at room temperature to ensure full saturation of polymer molecules. Rosemary essential oil was then added to the aliquot by stirring at 3,500 rpm for 10 min. The emulsion was then spray dried. The effects of total or partial replacement of gum arabic by modified starch, maltodextrin and inulin on the characteristics of rosemary essential oil were studied. The emulsion ratios used along with the moisture content of the microcapsules and oil retention (%) are given in Table 2.20.

Table 2.20 Moisture content and oil retention values for spray-dried rosemary microcapsules

Wall material	Wall material: essential oil (g/100 g of solution	Moisture content (%)	Oil retention (%)
GA	20:5	1.64 ± 0.17	56.83 ± 3.21
MS	20:5	1.55 ± 0.15	56.79 ± 0.00
MS:MD	10:10:5	2.25 ± 0.85	60.22 ± 1.59
MS:IN	10:10:5	3.48 ± 0.08	45.45 ± 0.01
GA:MD	10:10:5	2.05 ± 0.45	45.45 ± 0.00
GA:IN	10:10:5	3.27 ± 0.08	29.53 ± 3.22

Liakos *et al.* (2014) encapsulated different essential oils. 0.3 g of sodium alginate was dissolved in 10 ml of distilled water and was added with 0.1 ml of glycerol as a plasticizer. The essential oils with different concentrations were blended with the sodium alginate/glycerol solutions and igepal (100 µl) was added as a surfactant into the solution to maximize the miscibility of essential oil in the sodium alginate/glycerol matrix. They

studied three different combinations of emulsions for each essential oil. The lowest essential oil concentration used was 16 % along with 48 % sodium alginate (NaAlg), 20 % glycerol and 16 % igepal. The intermediate essential oil concentration used was 50 % along with 30 % NaAlg, 10 % glycerol and 10 % igepal and the highest essential oil concentration used was 66 % along with 20 % NaAlg, 8 % glycerol and 6 % igepal. 200 µl of the prepared emulsion was drop cast on glass slides to form films and were left to dry at room temperature. They suggested that these films can be used as a disposable wound dressing and in food packaging materials. The films formed were tested for their antimicrobial activity against *Escherichia coli* and *Candida albicans.* Essential oils except chamomline blue were found to inhibit the growth of *C. albicans*. Cinnamon, lemongrass and peppermint oils were effective against the growth of *E. coli.*

Carneiro *et al*. (2013) studied encapsulation and oxidative stability of flaxseed oil by a spray dryer. The wall materials used were maltodextrin mixed with gum arabic, whey protein concentrate (WPC) and two types of modified starch (Hi-Cap 100TM and Capsul TA) at a ratio of 25:75. The superior encapsulation efficiency was obtained for maltodextrin: Hi-Cap followed by the maltodextrin: Capsul combination. The lowest encapsulation efficiency was obtained for maltodextrin: WPC with poor emulsion stability. Maltodextrin: WPC combination protected the active material well as compared to other combinations against lipid oxidation.

Durante *et al.* (2012) encapsulated durum wheat bran oil extracted from supercritical carbon dioxide. The alginate/oil emulsion was obtained by mixing 2 % (w/v) of sodium alginate aqueous solution with wheat bran oil (1:5 v/v) by continuous mechanical stirring at 350 rpm for 60 min. The obtained alginate/oil emulsion was sprayed into 100 ml of 0.05 M $CaCl_2$ gelling solution using a needle with constant stirring at room temperature. The obtained spherical beads of particle size approximately 2.7 mm were washed in distilled water and freeze-dried overnight. Further, the dried sample was subjected to storage study at 4 ºC and 25 ºC for over 90 days. The samples were analysed for stability based on fatty acid hydroperoxide production and tocopherol (α, β, and γ), tocotrienol (α, β, and γ) and carotenoid (lutein, zeaxanthin, and β-carotene) degradation. Stability of encapsulated beads increased when stored at 4 °C, maintaining high levels of isoprenoids and low content of fatty acid hydroperoxides during 30 days of storage.

Hill *et al.* (2013) encapsulated cinnamon bark extract, trans-cinnamaldehyde, clove bud extract and eugenol by freeze-drying. Beta-cyclodextrin and essential oil in the

molecular ratio of 1:1 was mixed at the concentration of 16 mmol/l using a magnetic stirrer. The emulsion was then frozen and lyophilized for approximately 48 h. The encapsulation efficiency of the extracts was measured using a spectrophotometer at 280 nm and the results found are listed in Table 2.21.

Table 2.21 Encapsulation efficiency of beta-cyclodextrin inclusion microcapsules

Entrapped molecule	**Encapsulation efficiency (%)**
trans-cinnamaldehyde	84.70
Eugenol	90.15
trans-cinnamaldehyde:eugenol (2:1)	82.00
Cinnamon bark extract	41.72
Clove bud extract	77.74

Nayak *et al.* (2012) encapsulated glibenclamide, a drug used to treat non-insulin-dependent diabetes mellitus using a blend of sodium alginate/gum arabic polymers. Sodium alginate and gum arabic aqueous dispersions were prepared by using a magnetic stirrer for 10 min running at 1,000 rpm. Glibenclamide was then added to the polymer mixture at the ratio of 1:4 and the emulsion was thoroughly homogenized. The emulsions were finally ultrasonicated for 5 min to enhance dispersion. The emulsion was further added drop-wise into 100 ml of 10 % (w/v) $CaCl_2$ solution using a 21-gauge needle. The droplets were left in the solution to complete the curing reaction and to produce rigid beads for 15 min. Then the beads were decanted from the solution, washed two times with distilled water and dried overnight at room temperature. The ratio of the polymer mixture used in this study with obtained encapsulation efficiency and the percentage of drug released from the encapsulated beads for 7 h measured at 228 nm using UV-VIS spectrophotometer is mentioned in Table 2.22. They have reported that the increment in both the amounts of sodium alginate and gum arabic in the emulsion led to an increase in the encapsulation efficiency and a decrease in the drug release. The average size of obtained dried beads ranged from 1.15 ± 0.11 to 1.55 ± 0.19 mm.

Chan (2011) encapsulated palm oil using two types of sodium alginates, one with high guluronic acid content and the other with a high mannuronic acid content using the extrusion technique. It was found that the alginate concentration, alginate type and oil volume fraction had a significant influence on the encapsulation efficiency and bead properties. 100 ml of sodium alginate/oil emulsion was prepared at different concentrations of alginate ranging from 5-45 g/l and oil ranging from 10 to 60 % volume to the total volume of emulsion using impeller at a speed of 300 rpm for 45 min. The emulsion was

extruded through a 0.55 mm needle and was dripped into a $CaCl_2$ solution of 15 g/l using a magnetic stirrer. The tip of the needle was placed 15 cm above the surface of the gelling solution. The beads were left in the gelling solution for 30 min to harden, separated from the solution using a sieve and rinsed with distilled water. Drying was carried out using two methods. Freeze-dried under vacuum pressure of 0.015 kPa at a condenser temperature of -55 ºC for 24 h and oven-dried at 70 ºC. The alginate/oil emulsion, with an oil loading up to 30 % vol and 25 g/l of high guluronic acid alginate solution (equivalent to oil to alginate ratio of up to 15 g/g), was found to be stable with an encapsulation efficiency of 90 % before drying. The overall encapsulation efficiency after freeze-drying and oven-drying were 90 % and 79 %, respectively. Freeze-dried beads were free-flowing and non-oily whereas the oven-dried beads were sticky and oily.

Table 2.22 Levels of polymers used with their encapsulation efficiency and percentage of drug released.

Sodium alginate (mg)	**Gum arabic (mg)**	**Encapsulation Efficiency (%)**	**Drug release for 7 h (%) (absorbance at 228 nm)**
200.00	100.00	54.17 ± 1.54	53.23 ± 2.22
200.00	200.00	67.36 ± 2.15	44.24 ± 2.03
300.00	100.00	60.25 ± 2.12	49.76 ± 2.16
300.00	200.00	70.28 ± 3.04	43.75 ± 1.77
179.29	150.00	59.13 ± 2.22	50.81 ± 1.97
320.71	150.00	65.80 ± 1.98	46.36 ± 2.03
250.00	220.71	73.93 ± 3.37	40.54 ± 1.54
250.00	79.29	55.38 ± 1.76	53.06 ± 1.98
250.00	150.00	58.40 ± 2.50	51.27 ± 2.07

Kadam *et al.* (2010) encapsulated ginger oil by using gum acacia by a spray dryer. 80 g of gum acacia was dissolved in 200 ml of distilled water and kept overnight to enhance hydration. 20 g of ginger oil was added to the polymeric solution and the emulsion was homogenized at 10,000 rpm for 5 min. spray drying of the emulsion was carried out using different combinations of inlet and outlet temperatures. The observed effect of temperature on the physico-chemical properties of the microencapsulated ginger oil powder is given in Table 2.23.

Kanakdande *et al.* (2007) studied the stability of cumin oleoresin microencapsulated in different combinations of gum arabic, maltodextrin and modified starch. 30 % w/v solution of different blends of gum arabic, maltodextrin and modified starch (HiCap 100)

were dispersed in water and the final volume was made up to 100 ml. 3 g of oleoresin was added into the former solution and homogenized at 3,000 rpm for 5 min. Two drops of Tween 80 were added for emulsification. Then the emulsion was spray dried at inlet and outlet temperature of 160 ± 2 º and 120 ± 2 ºC. The microcapsules were analysed for their content and stability of volatiles. They reported that gum arabic offered greater protection as compared to maltodextrin and modified starch. Although, a blend of gum arabic, maltodextrin and modified starch offered greater protection when compared to gum arabic alone.

Table 2.23 Effect of different inlet and outlet temperature in spray dryer on physico-chemical properties of ginger oil microcapsules.

Parameter	Inlet and outlet temperature combination (ºC)			
	150 & 99	**160 & 108**	**170 & 112**	**180 & 126**
Moisture content (%)	5.90	4.01	3.67	2.21
Bulk density (g/ml)	0.79	0.74	0.71	0.69
Encapsulation efficiency (%)	92.00	91.00	87.00	82
Average particle size (µm)	55.90	41.20	37.80	32.1
Overall acceptability	7.90	8.50	8.10	7.8

Kaushik and Roos (2007) studied limonene encapsulation by freeze-drying using different combinations of gum arabic, sucrose and gelatine systems. The study was carried out for two different levels of limonene in the weight ratios (w/w) of 9:1 and 8.5:1.5 (matrices: limonene). Polymeric gums were dissolved in the mineral water at 45 ºC to obtain 20 % total solids concentration. Different ratios of matrices were prepared and were subjected to two-stage homogenization at 25 MPa in the first stage and 4 MPa in the second stage using a laboratory homogenizer. The emulsion was then frozen and subjected to freeze-drying. The detailed formulation ratios along with their retention (%) are given in the table below. They reported that the mixture containing gelatine, gum arabic and sucrose in the w/w ratio of 0.66:0.17:0.17 retained the highest amount of limonene in the sample containing matrices: limonene ratio (w/w) of 8.5:1.5 and emulsion containing only gum arabic has retained the highest amount of limonene in the ratio of 9:1 as shown in Table 2.24.

Baranauskiene *et al.* (2006) studied encapsulating properties of skimmed milk powder (SMP) and whey protein concentrate (WPC) for the coating of the essential oil of oregano (*Origanum vulgare* L.) and aroma extracts of citronella (*Cymbopogon nardus* G.)

and sweet marjoram (*Majorana hortensis* L.) using the spray-drying technique. They found that the efficiency of microencapsulation of flavour entrapped into the microcapsules varied from 54.3 % (marjoram in WPC) to 80.2 % (oregano in SMP). The content of flavour residual on the surface of encapsulated oregano essential oil was remarkably lower (1.1 % and 1.4 %) as compared with citronella (11.2 % and 15.2 %) and marjoram (16.7 % and 22.1 %) encapsulated in SMP and WPC matrixes respectively. The particle size of microencapsulated products was found to be 6 to 280 µm for SMP and 2 to 556 µm for WPC.

Table 2.24 Retention levels of limonene by freeze-drying in microcapsules

Matrix components	**Retention (%) in ratio (9:1)**	**Retention (%) in ratio (8.5:1.5)**
Sucrose	4.2 ± 0.1	6.4 ± 0.4
Gum arabic	75.3 ± 0.3	60.2 ± 0.2
Gelatine	66.0 ± 0.4	65.1 ± 0.2
Sucrose + gelatine (1:1)	36.4 ± 0.2	49.8 ± 0.3
Sucrose + gum arabic (1:1)	57.1 ± 0.2	27.2 ± 0.2
Gum arabic + gelatine (1:1)	54.2 ± 0.1	41.7 ± 0.1
Sucrose + gum arabic + gelatine (1:1:1)	50.9 ± 0.2	41.1 ± 0.2
Gum arabic + sucrose + gelatine (0.66:0.17:0.17)	64.2 ± 0.2	40.2 ± 0.2
Sucrose + gum arabic + gelatine (0.66:0.17:0.17)	47.2 ± 0.1	33.4 ± 0.2
Gelatine + gum arabic + sucrose (0.66:0.17:0.17)	67.7 ± 0.2	71.8 ± 0.1

Shaikh *et al.* (2006) studied microencapsulation of black pepper oleoresin by spray drying using gum arabic and modified starch as wall materials. 40 g of gum arabic and modified starch were dispersed individually in distilled water at 60 º - 70 ºC and the final volume was made up to 100 ml. One g of black pepper oleoresin (2.5 % based on carrier) was added to the mixture. It was then homogenized at 3,000 rpm for 5 min and added with two drops of Tween 80 to aid emulsification. Further spray drying was carried out with air pressure adjusted to 5 bars with inlet and outlet temperatures maintained at 178 ± 2 ºC and 110 ± 5 ºC respectively. The microcapsules were evaluated for the content and stability of volatile and non-volatile components. It was found that the gum arabic offered greater production to the pepper oleoresin compared to modified starch.

Yoo *et al.* (2006) optimized microencapsulation of α-tocopherol using sodium alginate and studied its controlled release properties. Different ratios of coating material, emulsifying agent concentration and $CaCl_2$ concentration were prepared using a stirrer at 2,000 rpm. The resulting emulsion was sprayed into $CaCl_2$ solution by using an air optimizing system. The beads formed were hardened for 30 minutes at room temperature, washed with distilled water, sieved and then lyophilized. The combinations used and their retention quantified by measuring UV absorbance at 285 nm are shown in Table 2.25. The encapsulation yield of α-tocopherols in sodium alginate microcapsules ranged from 31.2 % to 58.3%. The optimal parameters obtained for the microcapsules was from the ratio 6.6:3.4 (w/w) of sodium alginate: α-tocopherol with 1.35 % (v/v) emulsifier and 4.3 % (w/v) $CaCl_2$ concentration.

Table 2.25 Retention (%) of α tocopherol in microcapsules.

α-tocopherol:sodium alginate (%, v/v)	**Emulsifier (% v/v)**	**$CaCl_2$ (%, w/v)**	**Retention at 285 nm (%)**
5:5	0.5	3.5	53.86
5:5	0.5	4.5	54.25
5:5	1.5	3.5	51.94
5:5	1.5	4.5	53.43
7:3	0.5	3.5	39.57
7:3	0.5	4.5	43.81
7:3	1.5	3.5	51.24
7:3	1.5	4.5	54.81
6:4	1.0	4.0	53.07
6:4	1.0	4.0	58.32
4:6	1.0	4.0	53.99
8:2	1.0	4.0	50.99
6:4	0.0	4.0	31.15
6:4	2.0	4.0	50.33
6:4	1.0	3.0	46.98
6:4	1.0	5.0	52.84

Soottitantawat *et al.* (2005) studied the influence of emulsion and powder size on the stability of encapsulated D- limonene by spray drying. The matrices used were gum arabic, maltodextrin and modified starch (Hi-Cap 100). Two emulsions with 20 % w/w solution of gum arabic and 20 % w/w of Hi-Cap were prepared. The third emulsion was prepared by using a combination of 10 % w/w of gum arabic and 10 % w/w of maltodextrin. 20 g of D-limonene was added to the matrices and emulsions were homogenized at 8,000 rpm for 3 min. Spray drying of the emulsions was carried out with a feed rate of 45 ml/min, the airflow rate of 110 kg/h, inlet and outlet temperature of 200 ºC and 110 ± 10 ºC respectively.

It was found that the oxidation decreased with an increase in the concentration of polymers and emulsion particle size for gum arabic and modified starch. However, the modified starch showed higher stability of encapsulated D-limonene compared to others.

Beristain *et al.* (2001) studied encapsulation of steam distillated cardamom essential oil with mesquite gum by spray-dryer. Aqueous solutions containing 300 g of mesquite gum/kg were prepared using distilled water. Emulsions with oil: gum ratios of 1:5, 1:4 and 1:3 w/w were used for the study. The emulsions were homogenized at 5,000 rpm for 15 min and were spray-dried at inlet and outlet temperature of 200 ± 5 ºC and 110 ± 5 ºC respectively. The highest encapsulation efficiency i.e., 83.6 % was obtained for an oil: gum ratio of 1:4. Moisture content was found to be similar for all three ratios.

CHAPTER III
MATERIALS AND METHODS

The entire study pertaining to encapsulation of essential oil from cryoground dill seed was carried out at the college of Food Processing Technology and Bioenergy, Anand Agricultural University, Anand. The particulars of the materials and techniques adopted during the investigation are described in this chapter.

3.1 DILL SEED

Fresh, mature and uniform quality dill seed cultivar of variety Gujarat Suva-2 were procured from Seed Spice Research Station, Jagudan, Sardarkrishinagar Dantiwada Agricultural University, Dantiwada. The seeds were manually cleaned to remove extraneous and unwanted materials. Further, the seeds were packed in 1 kg aluminium pouches and were stored under ambient conditions before being used for experimentation.

3.2 PHYSICO-CHEMICAL CHARACTERIZATION OF DILL SEED AND DILL SEED OIL

3.2.1 Physical Characterization of Dill Seed

The physical properties of the material denotes the physical state of the material whose knowledge is useful in designing machinery for harvesting, drying, freezing etc. The following parameters provide information about the effect of processing on the raw material. All physical properties of dill seed were determined as per the methods cited by Singh *et al.* (2016).

Plate 3.1 Dill seed (Gujarat Suva – 2)

3.2.1.1 Size and sphericity

To measure size and shape, 20 seeds were randomly chosen from the bulk material and the principle dimensions namely length, width and thickness were measured using Vernier calliper with an accuracy of ± 0.01. The arithmetic mean diameter (D_a), geometric mean diameter (D_g) and sphericity (φ) were calculated by using the following mentioned relationship between length, width and thickness.

$$\text{Arithmetic mean diameter (Da)} = (L + W + T)/3 \quad \ldots (3.1)$$

$$\text{Geometric mean diameter (Dg)} = (L \times W \times T)^{1/3} \quad \ldots (3.2)$$

$$\text{Sphericity } (\varphi) = \frac{(L \times W \times T)^{1/3}}{L} \quad \ldots (3.3)$$

Where,

L = Longest intercept of length (mm)

W = Longest intercept of width (mm)

T = Longest intercept of the thickness (mm)

3.2.1.2 Mass of 1000 seeds

To determine the mass of 1000 seeds (M_t), about 300 seeds(n) were randomly picked and weighed (M) on an electronic balance. The mass of 1000 seeds was calculated by using the following formula.

$$M_t = \frac{M}{n} \times 1000 \quad \ldots (3.4)$$

3.2.1.3 Bulk density

Bulk density (ρ_b) was determined by filling a 500 ml cylinder with dill seeds at a constant rate without compressing and then weighing the contents of the cylinder. Bulk density was calculated by using the equation mentioned below.

$$\rho_b = \frac{M_X}{V_C} \quad \ldots (3.5)$$

Where,

ρ_b = Bulk density (kg/m^3)

M_X = Weight of sample (kg)

Vc = Capacity of the cylinder (m^3)

3.2.1.4 True density

True density (ρ_t) was determined by using the toluene displacement method as toluene is less absorbed by the seeds when compared to that of the water. A known quantity of seeds were immersed in toluene and the volume displaced by toluene was recorded. True density was calculated by using the equation mentioned below.

$$\rho_t = \frac{M_X}{Vc} \qquad \ldots (3.6)$$

Where,

ρ_t = True density (kg/m^3)

M_X = Weight of sample (kg)

Vc = Volume of toluene displaced in the cylinder (m^3)

3.2.1.5 Porosity

The porosity (ε) of the material is the ratio of spaces in the bulk to its bulk volume. The porosity was calculated by means of the equation given below.

$$\varepsilon = 1-\left[\frac{\rho_b}{\rho_t}\right] \times 100 \qquad \ldots (3.7)$$

Where,

ε = Porosity

ρ_b = Bulk density (kg/m^3)

ρ_t = True density (kg/m^3)

3.2.1.6 Angle of repose

The angle of repose was determined by using a squared box containing a vertical cylinder covered by a metal funnel with a discharge hole. The box was filled with the seeds and were allowed to flow freely outside the box which forms a cone shape of grains on the vertical cylinder. The height and diameter of the formed slope were recorded and the angle of repose was calculated by using the following formula.

$$\text{Angle of repose } (\theta) = \tan^{-1} \frac{2h}{d} \quad \ldots (3.8)$$

Where,

h = Height of the formed cone (mm)
d = Diameter of the formed cone (mm)

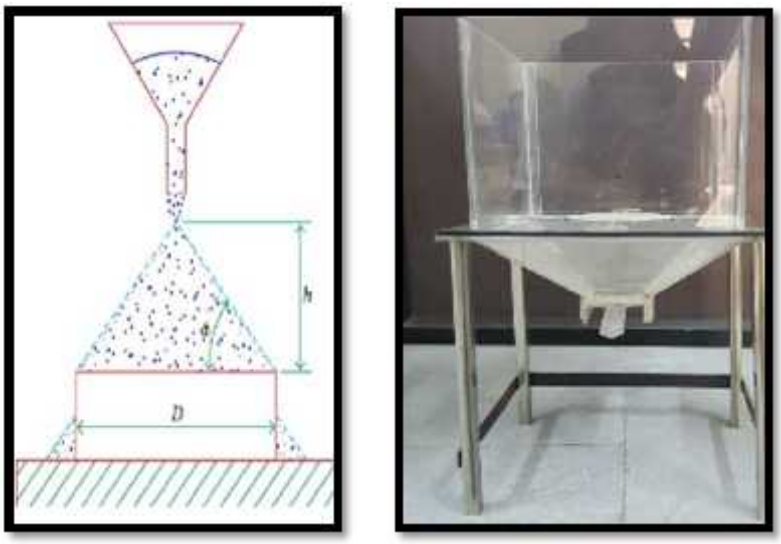


Plate 3.2 Angle of repose

3.2.1.7 Coefficient of friction

The static coefficient of friction for dill seed was determined on a galvanized iron. The seeds were filled in a cube and was placed on an adjustable tilting Plate. The plate was then elevated steadily till the filled cube just begins to slide down. The angle at this point was recorded and the coefficient of static friction was calculated by using the given equation.

$$\text{Coefficient of friction } (\mu) = \tan \theta \quad \ldots (3.9)$$

Plate 3.3 Coefficient of friction

3.2.2 Chemical Characterization of Dill Seed

Knowledge of the chemical composition of seed is essential as they are an important source of nutrients. The moisture content (%) of the dill seed was determined as per the procedure cited by Pruthi (1988). Protein (%), crude fat (%) and ash content (%) were determined as per the procedure described in AOAC (2012). Fiber (%) and essential oil (%) were determined as per the procedure described in Ranganna (2007) and Stanojevic *et al*. (2015) respectively.

3.2.2.1 Determination of moisture

The moisture content of the material was determined by using the toluene distillation method. Coarsely ground dill seed of about 40 g was taken in a round bottom flask. Toluene was added to cover the sample completely (not less than 75 ml). The top of the condenser was closed using a cotton plug to prevent condensation of atmospheric moisture into the tube. The sample was heated using a heating mantle and distillation was carried out. Distillation was continued till there was no change in the volume of the water collected in the trap. The water droplets held upon the condenser were removed using a wire loop and the condenser was rinsed with 5 ml toluene. The trap was cooled to room temperature and the volume of water in the trap was recorded. The moisture content of the seed was calculated by using the given equation below.

$$\text{Moisture \% (w. b.)} = \frac{V_W}{W_S} \times 100 \quad \ldots (3.10)$$

$$\text{Moisture \% (d. b.)} = \frac{V_W}{W_S - V_W} \times 100 \quad \ldots (3.11)$$

Where,

V_W = Volume of water collected in the trap (ml)

W_S = Weight of the sample (g)

Plate 3.4 Dean and Stark apparatus

3.2.2.2 Determination of protein

Protein content was determined by using Kjeldhal method. Kjeldahl assembly used is as shown in Plate 3.4. About 2 g of sample was digested with concentrated sulphuric acid (H_2SO_4) containing a pinch of catalyst mixture (K_2SO_4:$CuSO_4$ at a ratio of 5:1). The digested solution was then distilled with 40% NaOH and liberated ammonia was trapped in 4% boric acid solution, using a mixed indicator (methyl red: bromocresol green, 1:5). The condensate was titrated against 0.1 N hydrochloric acid (HCl) until the blue colour disappears. Percent nitrogen was calculated by using the following equation and protein percentage was quantified by multiplying with a factor of 6.25.

$$\text{Total Nitrogen (\%)} = \frac{(\text{Burette reading} - \text{Blank reading}) \times N \times 14.007}{W} \times 100 \quad \ldots (3.12)$$

Where,

N = Normality of HCl

14.007 = Atomic weight of nitrogen

W = Weight of the sample

$$\text{Total protein (\%)} = \text{Total Nitrogen (\%)} \times 6.25 \quad \ldots (3.13)$$

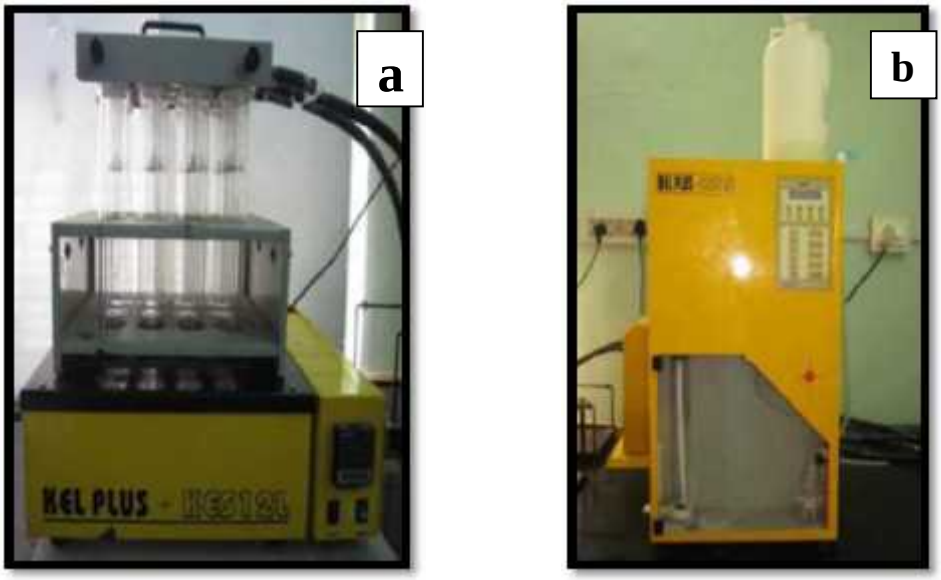


Plate 3.5 (a) Digestion assembly (b) Distillation unit

Plate 3.6 Fibra plus

3.2.2.3 Determination of crude fiber

Fibra Plus instrument (make: Pelican Equipments, Chennai, Plate 3.6) was used for estimation of crude fiber. About 2 g of defatted sample was taken in a thimble and digested with 1.25 % H_2SO_4. Boiling was carried out for 30 min and the acid was drained. The residue was washed with hot distilled water till free from acid. The same procedure was carried out using 1.25 % NaOH. Again, washing was carried out with distilled water to make it free from alkali.

The left-over neutral residue was dried in the oven at 105 °C to constant weight and ignited in a muffle furnace for 4 h at 550 to 600 °C. The loss in weight of the residue was noted. The percentage of crude fiber was calculated by using the formula given below.

$$\text{Crude fiber (\%)} = \frac{(W_1 - W_2)}{W} \times 100 \qquad \ldots (3.14)$$

Where,

W_1 = Weight of crucible and contents before ashing (g)

W_2 = Weight of crucible containing ash (g)

W = Weight of the sample (g)

3.2.2.4 Determination of crude fat

Fat content was determined by using Soxhlet extractor (Plate 3.7). About 3 g of sample was weighed accurately and defatted using petroleum ether for 6 h. The resultant ether extract was then evaporated to remove solvent residues. The crude fat was determined by using the formula given below.

$$\text{Crude fat (\%)} = \frac{(W_1 - W_2)}{W} \times 100 \qquad \ldots (3.15)$$

Where,

W_1 = Initial weight of the flask (g)

W_2 = Final weight of the flask (g)

W = Weight of the sample (g)

3.2.2.5 Determination of ash

About 5 g sample was accurately weighed and ignited over the burner. A smoke-free sample with silica crucible was kept in a muffle furnace (Plate 3.8). Ashing was carried out at the temperature of 550 °C until white ash is obtained. The sample was cooled in a desiccator and weighed. The percent ash was calculated by the following equation.

$$\text{Ash (\%)} = \frac{(W_1 - W_2)}{W} \times 100 \qquad \ldots (3.16)$$

Where,

W_1 = Weight of the empty crucible (g)

W_2 = Weight of the crucible containing ash (g)

W = Weight of the sample (g)

Plate 3.7 Soxhlet apparatus

Plate 3.8 Muffle furnace

3.2.2.6 Determination of carbohydrate

Carbohydrate content was computed by the difference method using the following equation.

Carbohydrate (%) = 100 - % (Moisture + Protein + Ash + Crude fat + Crude fiber) . . . (3.17)

3.2.2.7 Determination of essential oil

The essential oil content of dill seed was determined by steam distillation process using a Clevenger apparatus. About 50 g of coarsely ground dill seed was taken into a round bottom flask and 500 ml distilled water was added. The assembly was set on a heating mantle as shown in Plate 3.9. Distillation was carried out until the volume of oil remains constant in the trap. The essential oil content was calculated by using the equation mentioned below.

$$\text{Essential oil (\%)} = \frac{\text{Volume of essential oil (ml)}}{\text{Weight of the sample}} \times 100 \qquad \ldots (3.18)$$

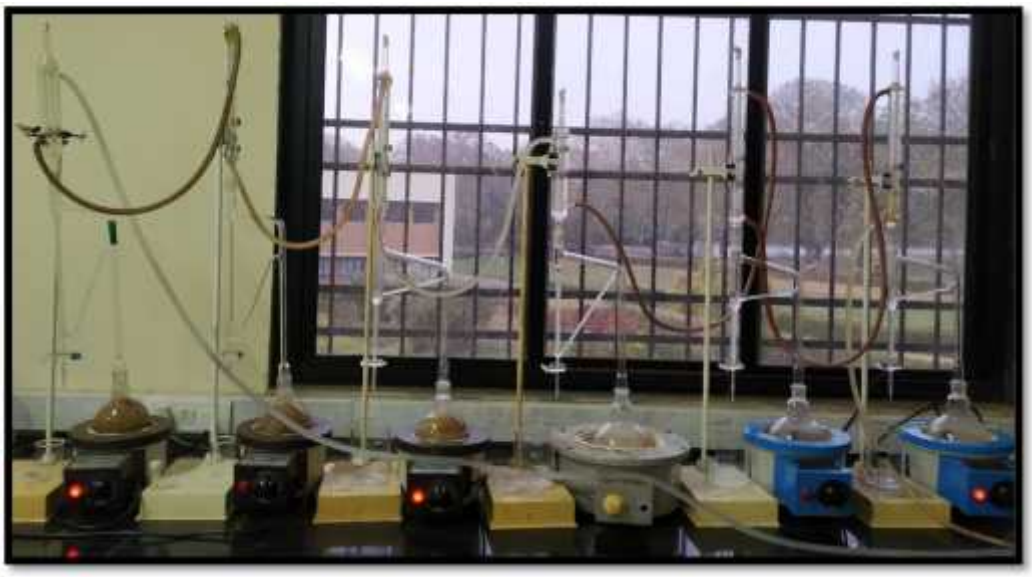


Plate 3.9 Clevenger assembly

3.2.3 Chemical Characterization of Dill Seed Oil

Dill seed oil was extracted using petroleum ether and hexane as solvents using the procedure mentioned in 3.1.2.4. The oil obtained was analysed for specific gravity, saponification value, acid value, iodine value and peroxide value as per the procedure cited in

FSSAI (2016). The refractive index and the induction period were determined by digital refractometer and rancimat apparatus respectively.

3.2.3.1 Specific gravity

Clean and dry pycnometer of 10 ml was filled with dill oil and carefully stoppered to prevent entrapment of air bubbles. The oil that has come out of the capillary opening was wiped off. The pycnometer was immersed in the water bath at 30 °C for 30 minutes and then the surface was dried. The weight of the dried pycnometer was noted. A similar procedure was carried out for water using the same pycnometer that was used for oil. The specific gravity of the oil was calculated by using the given formula.

$$\text{Specific gravity at 30 °C} = \frac{\text{A-B}}{\text{C-B}} \qquad \ldots (3.19)$$

Where,

A = Weight of specific gravity bottle with oil at 30 °C

B = Weight of empty specific gravity bottle at 30 °C

C = Weight of specific gravity bottle with water at 30 °C

3.2.3.2 Refractive index

The refractive index of a material is defined as the ratio of the sine of the angle of incidence to the sine of the angle of refraction. It was determined by using a digital refractometer (Atago®). Few drops of oil were placed on the surface of the prism at 20 °C and 40 °C and the refractive index was recorded.

Plate 3.10 Refractometer

3.2.3.3 Saponification value

About 1.5 to 2.0 g of the dry sample was taken into a conical flask. 25 ml of alcoholic potassium hydroxide solution was added to the flask. The flask was connected with an air condenser and was kept in the water bath to complete saponification. The flask was then cooled. The excess amount of potassium hydroxide present in the solution was determined by titrating against 0.5N hydrochloric acid, using about 1.0 ml phenolphthalein indicator. A similar process was followed for blank where distilled water was used instead of the sample. The saponification value of the oil was calculated from the below mentioned equation.

$$\text{Saponification Value} = \frac{56.1\times (B-S) \times N}{W} \quad \ldots (3.20)$$

Where,

B = Volume of standard hydrochloric acid consumed by blank (ml)

S = Volume of standard hydrochloric acid consumed by the sample (ml)

N = Normality of the standard hydrochloric acid

W = Weight of the oil sample (g)

Plate 3.11 Saponification assembly

3.2.3.4 Acid value

About 1.5 to 2.0 g of the dry sample was taken into a conical flask and was added with 50 ml of freshly neutralized hot ethyl alcohol. The mixture was boiled for 5 min and the hot mixture was titrated against a standard sodium hydroxide solution by adding 1 ml of phenolphthalein indicator to the mixture. The acid value was calculated using the following formula.

$$\text{Acid value} = \frac{56.1 \times V \times N}{W} \quad \ldots (3.21)$$

Where,

V = Volume of standard sodium hydroxide consumed by the sample (ml)

N = Normality of the sodium hydroxide (ml)

W = Weight of the sample (g)

3.2.3.5 Iodine value

About 1.5 to 2.0 g of sample was taken into a conical flask with 25 ml of carbon tetrachloride and 25 ml of Wijs solution (1 ml of iodine monochloride in 180 ml of glacial acetic acid). The solution was mixed well and kept in dark for 30 min. Further, it was added with 15 ml of potassium iodide followed by 100 ml of boiled and cooled water. The mixture was titrated against standardized sodium thiosulphate using starch as an indicator until the blue colour disappears. A similar procedure was followed for blank using distilled water instead of sample and the iodine value was calculated by using the following formula.

$$\text{Iodine value} = \frac{12.69\,(B - S)\,N}{W} \quad \ldots (3.22)$$

Where,

B = Volume of standard sodium thiosulphate consumed by the blank (ml)

S = Volume of standard sodium thiosulphate consumed by the sample (ml)

N = Normality of sodium thiosulphate (ml)

W = Weight of the sample (g)

3.2.3.6 Peroxide value

About 5g of oil was taken into a conical flask and was added with a 30 ml mixture of acetic acid and chloroform in a ratio of 3:2. Then the mixture was added with 0.5 ml of saturated KI (Potassium Iodide) solution and was allowed to stand for 1 min in dark with occasional shaking. The mixture was added with 30 ml water and 3-4 drops of starch indicator. The solution was mixed well and titrated against 0.1N sodium thiosulphate until the blue colour disappeared. A similar process was carried out for blank using distilled water instead of sample and the peroxide value was calculated by using the formula given below.

$$\text{Peroxide value (meq } O_2\text{ /kg oil)} = \frac{(B- S) \times N}{W} \times 100 \qquad \ldots (3.23)$$

Where,

B = Volume of standard sodium thiosulphate consumed by the blank (ml)

S = Volume of standard sodium thiosulphate consumed by the sample (ml)

N = Normality of sodium thiosulphate (ml)

W = Weight of the sample (g)

3.2.3.7 Induction period

The highly volatile oxidative products that are formed during the process are absorbed by distilled water and the time required to get these reaction products is referred to as the induction period. The induction period of the oil was determined by using rancimat apparatus (Metrohm Ltd., Switzerland) as shown in Plate 3.12. About 5 g of the sample was taken in a test tube and was exposed to airflow at a constant temperature of 130 °C.

3.3 CRYOGENIC GRINDING OF DILL SEED

Cryogenic grinding of dill seed was carried out using a cryogenic grinder (Spectra Cryogenics System Pvt. Ltd., Kota, Rajasthan) as shown in Plate 3.13.

The cryogenic grinding of dill seed was carried out using a pilot-scale cryogenic grinder. The grinding system consists of the following components.

a) A liquid nitrogen tank of 185 cm^3 capacity with pressure maintained at 2 MPa to supply liquid nitrogen.

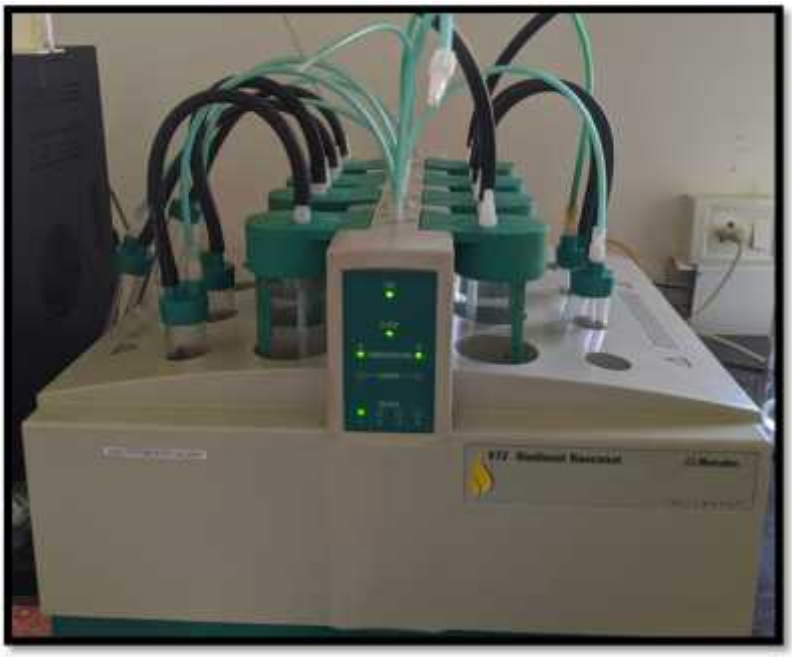


Plate 3.12 Rancimat apparatus

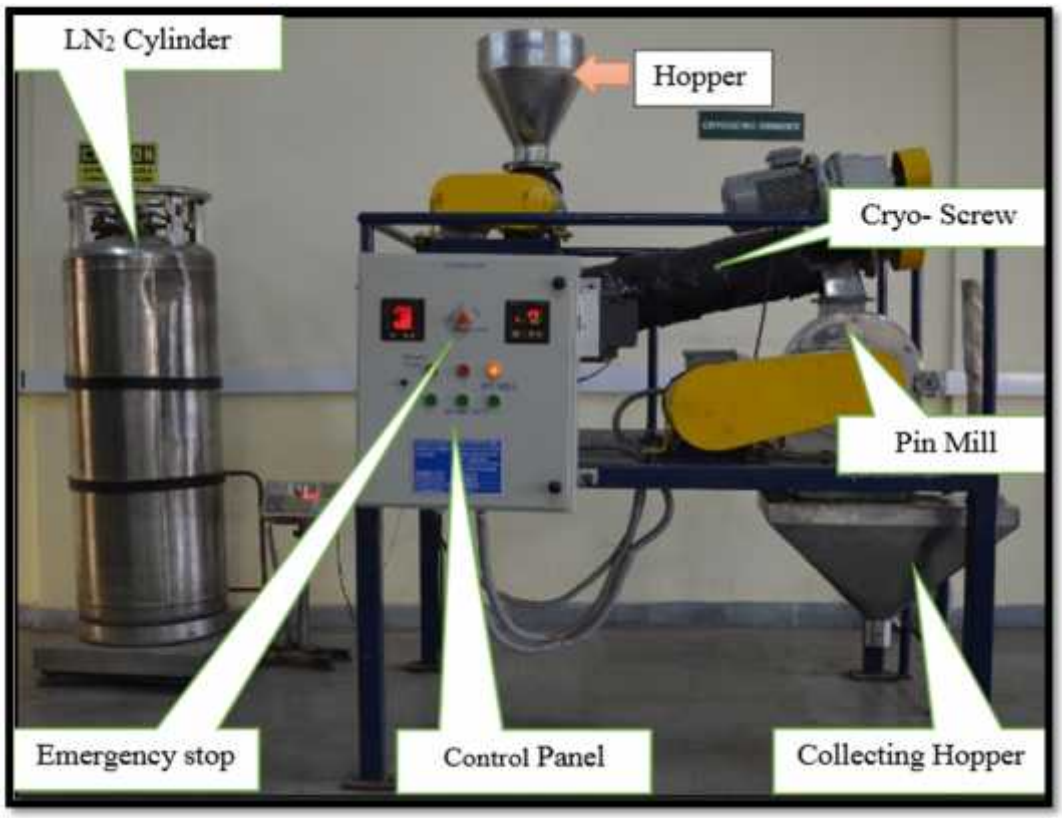


Plate 3.13 Cryogenic grinding system

b) A rotary valve located just below the hopper to convey the raw material towards the cryogenic screw conveyor. The feet rate of the material can be adjusted by using VFD (Variable Frequency Drive).
c) A cryogenic screw conveyor of 80 cm long to convey the material into the pin mill.
d) Pin mill that works based on attrition and friction forces to reduce the particle size of the material with a fixed rotation speed of 4500 rpm.
e) Sieves of 0.8 mm, 1 mm and 1.5 mm that can be fitted into the pin mill to obtain a powder of the desired particle size.
f) A collection hopper to collect the ground material from the pin mill.
g) A control panel to control operating conditions of the machine with on and off switches for rotary valve, pin mill and screw conveyor. It has a knob to adjust the speed of the screw conveyor and an emergency knob to stop the machine.

The grinding was carried out at a temperature range of 0 ° to -80 °C in three replications with a feed rate of 6, 7 and 8 kg/h using a sieve size of 0.8 mm and 1 mm. 500 g of dill seeds were fed into the machine through the hopper and the ground material obtained was packed immediately into aluminium laminated pouch of 110 µ thickness. The obtained powder was then analysed for moisture, particle size and essential oil contents.

3.3.1 Experimental Design

Factorial CRD (Completely Randomized Design) was used as an experimental design to optimize the cryogenic grinding parameters for dill seed. Statistical analysis of data in terms of ANOVA and correlations between independent and dependent variables was obtained using Design-Expert version 12 statistical software.

The independent and dependant variables chosen for the experimentation are given below.

a) Independent variables:

1. Temperature (°C): 0, -20, -40, -60, -80
2. Feed Rate (Kg/h): 6, 7, 8
3. Sieve Size (mm): 0.8, 1.0

b) Dependent variables:

1. Grinding Time (min)
2. Liquid Nitrogen consumption (Kg)
3. Energy consumption (kWh)
4. Essential oil (%)
5. Moisture (%)
6. Particle size (mm)

3.3.2 Observations

The following methodology was adopted to record the data for all the dependent parameters mentioned above.

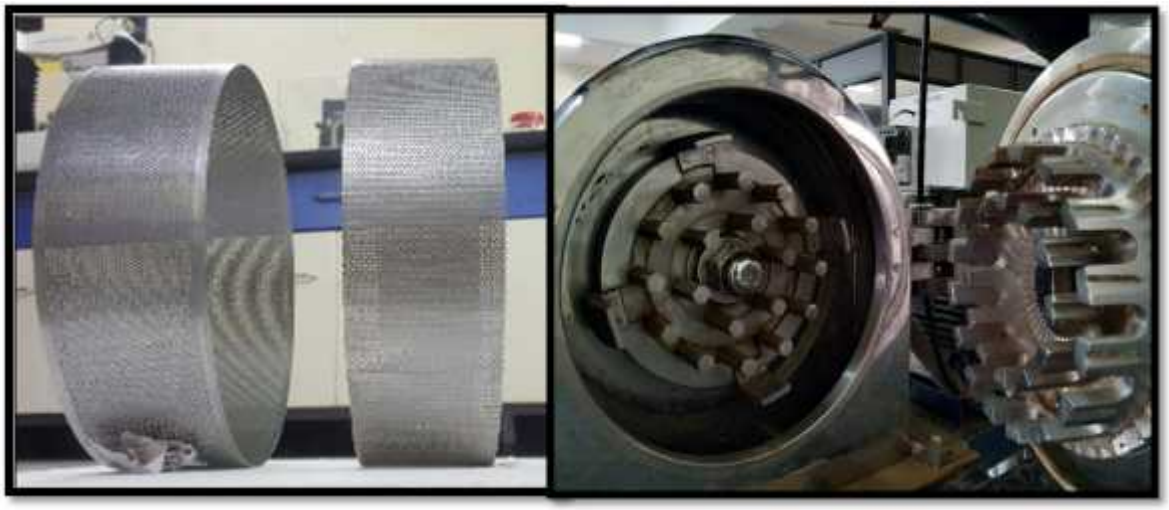


Plate 3.14 Sieves of 0.8 mm and 1 mm **Plate 3.15 Inside view of pin mill**

Plate 3.16 Cryoground dill seed powder in aluminium laminated pouches

3.3.2.1 Grinding time

The grinding time in min was recorded from the time when the raw material enters into the hopper till the ground material stops flowing from the collecting hopper.

3.3.2.2 Liquid nitrogen consumption

The initial weight of the liquid nitrogen cylinder and empty weight (kg) was noted in order to calculate the amount of liquid nitrogen consumed for grinding of the sample at each temperature in the experimentation.

Plate 3.17 Liquid nitrogen cylinder

3.3.2.3 Energy consumption

The energy consumption for grinding each sample was measured by the difference between the initial and final reading in the energy meter (kWh).

3.3.2.4 Determination of essential oil

The essential oil content of 50 g of cryoground pounder was determined by Clevenger apparatus as described in section 3.2.2.7.

3.3.2.5 Determination of moisture

The moisture content of the cryoground powder was determined as described in section 3.2.2.1.

Plate 3.18 Energy meter

3.3.2.6 Mean particle size

The mean particle size of cryoground dill seed powder was determined by manual sieving. Different sieves of the size 0.850 mm, 0.710 mm, 0.500 mm and 0.425 mm were arranged in a series with a pan from top to bottom. 250 g of ground dill seed powder was oven-dried to constant weight and the dried sample was sieved for 5 min and the individual weight of the powder retained on each sieve was recorded. The fineness and the mean particle size were calculated by using the below formula.

Cumulative % mass retained = Sum of cumulative mass retained on each sieve

$$\text{Fineness Modulus (FM)} = \frac{\text{Cumulative \% mass retained}}{100} \quad \ldots (3.24)$$

$$\text{Mean particle size (Dp)} = 0.135 \times (1.336)^{FM} \quad \ldots (3.25)$$

3.3.3 Quantification of Bioactive Compounds in Essential Oil

The essential oil of the optimized sample along with control was analysed for its major bioactive compounds i.e. carvone and limonene by using GC-MS (Parkin Elmer limited). The Certified Reference Material (CRM) i.e. carvone and limonene were procured from Dutt Enterprise, Anand, Gujarat. Marker compounds were diluted 1000 times in hexane at different concentrations viz. 25, 50, 100, 250, 500, 750 and 1000 ppm and were quantified. An elite 5 MS capillary column of 30 cm length, 0.25 mm ID and 0.25 µDF was used as a stationary phase with helium as a carrier gas with a flow rate of 1 ml/min. The initial oven temperature was programmed at 50 °C with a holding time of 7 min. The temperature was

raised to 210 °C at the rate of 7 °C/min with a holding time of 10 min. The injector split ratio and temperature was maintained at 25:1 and 230 °C respectively. The sample was injected at a volume of 4 µl.

Plate 3.19 GC-MS

3.4 ENCAPSULATION OF ESSENTIAL OIL

The essential oil obtained from the optimized parameters was encapsulated using sodium alginate as a coating material with other polymers like maltodextrin, gelatine, agar agar and gum tragacanth. Encapsulation of essential oil was carried out as per the method suggested by Martins *et al.* (2009) with slight modifications. The experiment was conducted to find out the effect of partial replacement of sodium alginate polymer with other polymers on encapsulation efficiency.

3.4.1 Preparation of Polymeric Solution

2% polymeric solution (i.e. 1 g of sodium alginate) was dissolved in 30 ml of distilled water and the polymer was completely dissolved at 60 °C temperature. The volume was made up to 50 ml and the solution was left for four hours to attain complete saturation. This was used as a control. Polymers namely gum tragacanth, maltodextrin, agar agar and gelatine at concentrations of 0.5 %, 1 % and 1.5 % were used to partially replace sodium alginate.

3.4.2 Preparation of Emulsion and Formation of Encapsulated Beads

1 g of optimized dill seed essential oil was added in 2 % polymeric solution along with 100 µl of TWEEN 20 emulsifier. The emulsion was thoroughly mixed and then sprayed into

100 ml of 5% calcium chloride solution that was agitated using a magnetic stirrer. The beads formed during the process were left in the calcium chloride solution for 30 minutes for curing and then were filtered using Whatman filter paper (125 mm). The filtered beads were washed three times with distilled water and then dried in an oven at 40 °C for 24 h. The dried beads were uniformly powdered with motor and pestle. The powder was analysed for moisture, particle size, encapsulation efficiency and colour value.

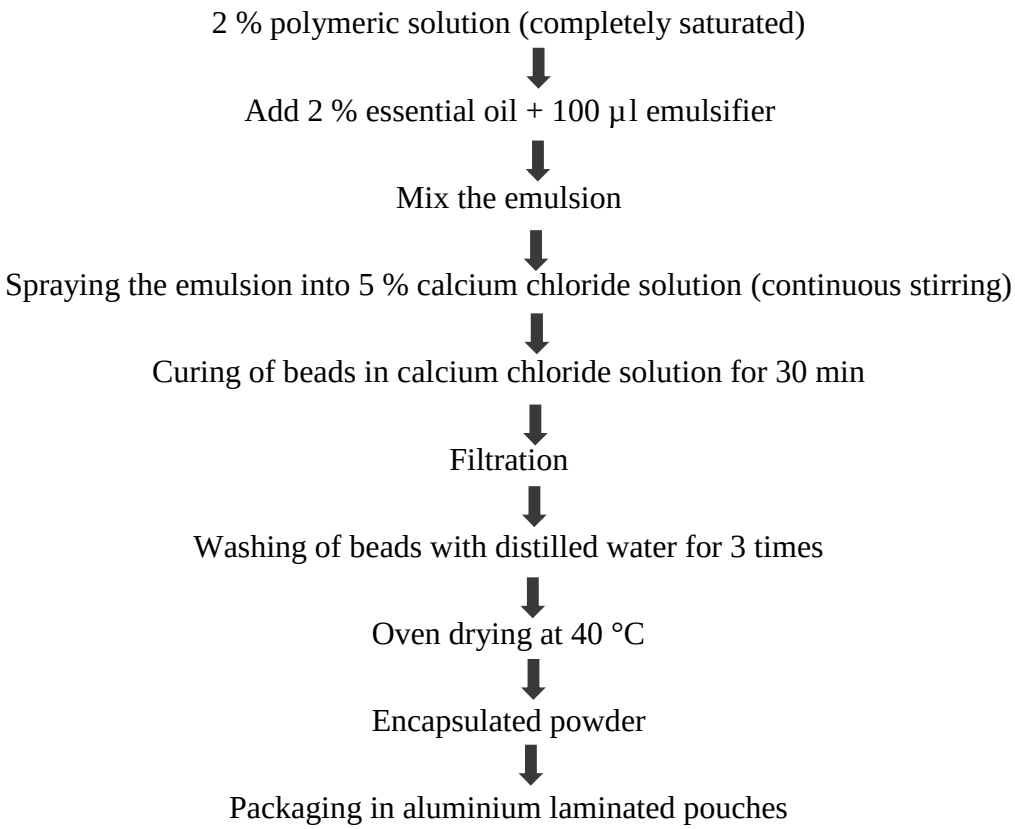


Figure 3.1 Process flow chart for encapsulation

3.4.3 Characterization of Encapsulated Powder

3.4.3.1 Moisture

The moisture of encapsulated powder was determined gravimetrically as cited by Carneiro *et al.* (2013). About 1 g of sample was weighed in an empty petridish and dried in an oven maintained at the temperature 70 °C till constant weight and the moisture of the powder was determined by using the formula mentioned below.

$$\text{Moisture (\%)} = \frac{(W_1 - W_2)}{W_1 - W} \times 100 \qquad \ldots(3.26)$$

Where,

W = Weight of the empty petridish (g)

W_1 = Weight of the petridish with sample before drying (g)

W_2 = Weight of the petridish with sample after drying (g)

3.4.3.2 Encapsulation efficiency

Encapsulation efficiency was determined according to the method described by Carneiro *et al.* (2013). About 5 ml of hexane was added to 0.5 g of encapsulated powder. The solution was mixed for 2 min to extract free oil at room temperature. The mixture was filtered and the powder collected was rinsed with 7 ml of hexane for 3 times. The solvent was evaporated at 60 °C till constant weight. The non-encapsulated oil was determined by the weight difference between the initial empty flask and the extracted oil residue. The encapsulation efficiency was calculated by using the below equation.

$$\text{Encapsulation efficiency (\%)} = \left[\frac{TO - SO}{TO}\right] \times 100 \qquad \ldots(3.27)$$

Where,

TO = Total oil content

SO = Surface oil content

3.4.3.3 Particle size

The particle images of the encapsulated powder were captured using an optical microscope (Motic image plus). Small amount of sample was placed on a transparent microscopic glass slide with two drops of glycerol in order to facilitate the free flow of the individual particle. Images of particles were captured using a 10x microscopic lens and analysed for the average particle size of the sample in Biovis Particle Plus software v. 4.58.

3.4.3.4 Colour value

The colour value of the encapsulated powder was measured using colour lab (make: Lovibond RT850i). Colour representation by the L*, a*, b* notations were recorded. L* is the degree of lightness of the colour. a* value equals to zero represents the black colour and 100

represents the white colour. a* (red-green) represents the degree of redness of the samples if the value lies between 0 to 60 or greenness if the value lies between 0 to -60. b* (yellow-blue) colour value indicates the amount of yellowness in the sample if the value lies between 0 to 60 or blueness if it lies between 0 to -60. The colour measurement is based on the principle of reflection of light.

Plate 3.20 Encapsulation process

Plate 3.21 Motic image plus

Plate 3.22 Colour lab

3.4.4 Experimental Design

Factorial CRD was used as an experimental design to optimize the polymeric blend of encapsulated powder. Statistical analysis of data in terms of ANOVA and correlations between independent and dependent variables was obtained using Design-Expert version 12 statistical software.

CHAPTER IV

RESULTS AND DISCUSSION

This chapter deals with the results obtained from various experiments carried out throughout the investigation. Data composed during the investigation of cryogenic grinding and encapsulation of optimized essential oil from cryogenic grinding were statistically analysed and the results were discussed and interpreted.

4.1 PHYSICO-CHEMICAL CHARACTERIZATION OF DILL SEED AND DILL SEED OIL

4.1.1 Physical Characterization of Dill Seed

Physical properties of dill seed viz. size, sphericity, bulk density, true density, porosity, angle of repose and coefficient of friction were determined as per the procedures mentioned in the sections 3.2.1.1 to 3.2.1.7 in the previous chapter. The experimental data is presented in Table 4.1 below.

Table 4.1 Physical properties of dill seed

Parameter	**Mean values ± SD**
Size	Length: 4.78 ± 0.5 mm
	Width: 1.41 ± 0.11 mm
	Thickness: 1.68 ± 0.38 mm
	Arithmetic mean diameter: 2.62 ± 0.33 mm
	Geometric mean diameter: 3.79 ± 0.007 mm
Sphericity	0.79 ± 0.01
Mass of 1000 seeds	4.32 ± 0.87 g
Bulk density	420. 71 ± 9.24 kg/m^3
True density	1130.3 ± 19.59 kg/m^3
Porosity	59.56 ± 2.15 %
Angle of repose	41.98 ± 0.43°
Coefficient of friction	0.89 ± 0.09

The mean length, width and thickness of dill seed was 4.78 ± 0.5 mm, 1.41 ± 0.11 mm and 1.68 ± 0.38 mm respectively at moisture of 12.22 %. The average arithmetic mean diameter and geometric mean diameter was 2.62 ± 0.33 mm and 3.79 ± 0.007 mm respectively. Geometric property is an important aspect during designing of separation and cleaning equipments for maximizing efficiency (Sunmonu *et al.,* 2015). The sphericity of the seeds found was 0.79 ± 0.01. The mass of thousand grains was 4.32 ± 0.87 g. Bulk density and the true density observed was 420. 71 ± 9.24 kg/m^3 and 1130.3 ± 19.59 kg/m^3 respectively. The porosity found was 59.56 ± 2.15 %. The angle of repose and coefficient of friction observed was 41.98 and 0.89° respectively. Frictional properties and angle of repose of the seed helps in handling the material to flow and in designing belt conveyors and screw conveyers etc. (Ukey *et al.,* 2010). The physical properties observed were similar to the observations reported by Singh *et al.* (2016) and Desai *et al.* (2013).

4.1.2 Chemical Properties of Dill Seed

Chemical constituents of dill seed viz. moisture, protein, crude fat, crude fiber, ash and essential oil were determined as per the method described in sections 3.2.2.1 to 3.2.2.7. The results obtained are tabulated in Table 4.2.

Table 4.2 Chemical properties of dill seed

Parameter	**Mean values (%) ± SD**
Moisture	12.22 ± 0.96
Protein	14.03 ± 0.20
Crude fiber	32.26 ± 0.20
Crude fat	7.30 ± 0.28
Ash	7.03 ± 0.48
Carbohydrate	27.14 ± 0.52
Essential oil	2.00 ± 0.08

The moisture content of dill seed found was 12.22 ± 0.96 %. Protein, crude fiber, crude fat and ash observed was 14.03 ± 0.2 %, 32.26 ± 0.2 %, 7.3 ± 0.28 % and 7.03 ± 0.48 % respectively. The carbohydrate content was 27.14 ± 0.52 %. The results observed for moisture, crude fiber and ash content were similar to the observations reported by Saleh-E-In and Roy

(2007). The protein and ash content observed was similar to the results reported by Meena *et al.* (2019). Crude fiber and ash content obtained were similar to the results reported by Desai *et al.* (2013).

4.1.3 Chemical Characterization of Dill Seed Oil

Dill seed oil was extracted as per the procedure described in section 3.2.3.1 to 3.2.3.7. The chemical characteristics of dill seed oil namely specific gravity, refractive index, acid value, iodine value, saponification value, peroxide value and induction period were carried out as per the procedures described in the previous chapter. The results observed are tabulated in Table 4.3.

Table 4.3 Chemical characterization of dill seed oil

Parameter	**Dill seed oil**	
	Oil extracted from petroleum ether	**Oil extracted from hexane**
Yield (%)	7.3 ± 0.28	7.14 ± 0.29
Specific gravity at 30 °C	0.93 ± 0.03	0.92 ± 0.02
Refractive index - 40 °C - 20 °C	1.46 ± 0.01 1.47 ± 0.01	1.46 ± 0.01 1.47 ± 0.01
Acid value (mg KOH/g)	1.23 ± 0.07	1.01 ± 0.05
Iodine value (g I/100 g)	213.70 ± 0.09	198 ± 0.04
Saponification value (mg KOH/g)	183.26 ± 0.04	179.3 ± 0.09
Peroxide value (meq/kg oil)	12.74 ± 0.07	10.41 ± 0.06
Induction period at 130 °C (h)	27.5	33.69

Dill seed oil yield was 7.3 ± 0.28 % and 7.14 ± 0.29 % in petroleum ether and hexane respectively. The yield was higher in petroleum ether compared to hexane. The specific gravity at 30 °C was 0.94 ± 0.03 and 0.92 ± 0.02 for the oil extracted by petroleum ether and hexane respectively which indicated that the oils contain lower molecular weight fatty acids. The refractive index measured at 40 °C and 20 °C was same for both the oils i.e. 1.46 ± 0.01 and 1.47 ± 0.01 The observations for both specific gravity and the refractive index of the oil extracted by petroleum ether were similar to the results reported by Saleh-E-In and Roy (2007).

The acid value, iodine value, saponification value and peroxide value obtained was 1.23 ± 0.07, 213.70 ± 0.09, 183.26 ± 0.04 and 12.74 ± 0.07 respectively for the oil extracted by petroleum ether whereas it was 1.01 ± 0.05, 198 ± 0.04, 179.3 ± 0.09 and 10.41 ± 0.06 respectively for the oil extracted by hexane. The saponification value and peroxide value obtained was similar to the results reported by Ying *et al.,* (2018) and Saleh-E-In and Roy (2007). The induction period was found to be higher for oil extracted with hexane i.e. 33.69 h at 130 °C (Figure 4.1) which indicates less free fatty acids and reactive products compared to the induction period of 27.5 h for the oil extracted with petroleum ether.

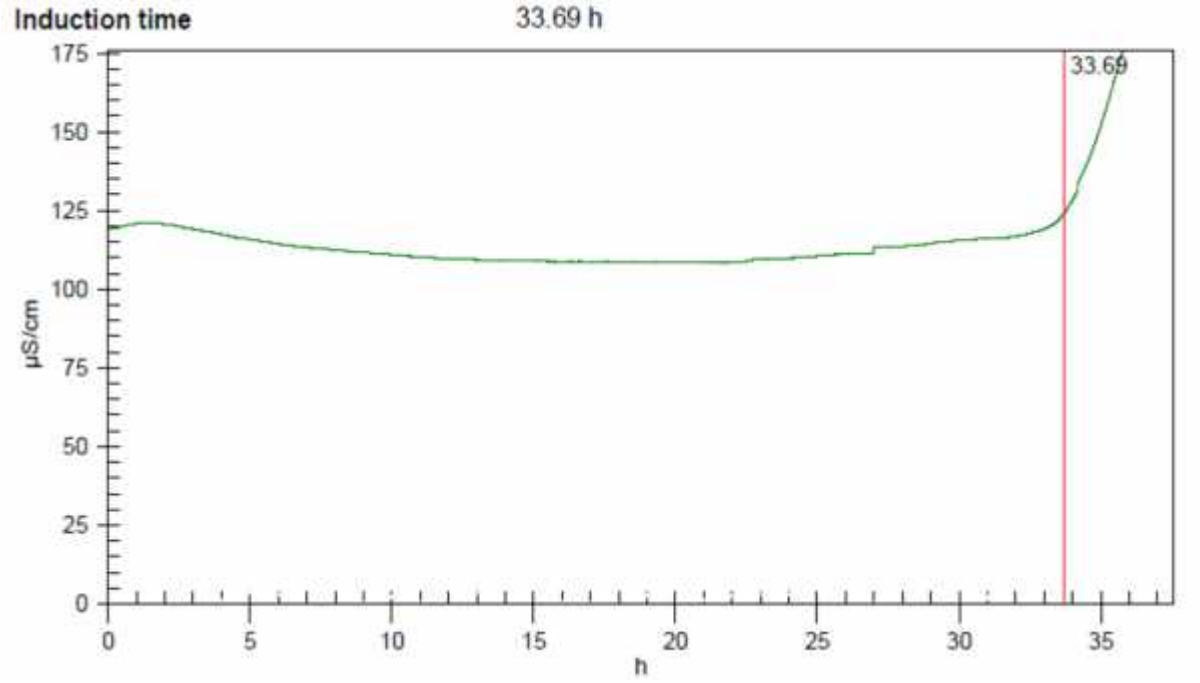


Figure 4.1 Induction period of dill seed oil extracted from hexane

4.2 CRYOGENIC GRINDING OF DILL SEED

Grinding time required for size reduction of dill seed varied with all three independent parameters viz. temperature, feed rate and sieve size. Cryogenic grinding requires less time when compared to ambient temperature grinding. This is due to the change in the structure of the seed at a lower temperature. The brittle structure of the material at low temperature gets ruptured easily during the grinding process which ultimately decreases the grinding time. The effect of individual independent parameters viz. temperature, feed rate and sieve size on the dependent variables viz. grinding time, liquid nitrogen consumption, energy consumption,

essential oil, moisture and the mean particle size are discussed and the results obtained are given in below tables and graphs.

4.2.1 Effect of Temperature, Feed Rate and Sieve Size on Grinding Time

ANOVA for the effect of temperature, feed rate and sieve size on grinding time of dill seed is presented in Tables 4.4 and 4.5. It was observed that the temperature and feed rate had a significant effect on grinding time ($p < 0.01$) at 1 % level whereas sieve size had a significant effect on grinding time at ($p < 0.05$) 5 % level.

Table 4.4 ANOVA for grinding time

Source	Sum of Squares	df	Mean Square	F-value	p-value	Test	SEm	CV (%)
Model	29.00	7	4.14	112.12	< 0.0001	*		3.18
A-Temperature (°C)	27.42	1	27.42	742.05	< 0.0001	*	0.0203	
B-Feed Rate (kg/h)	1.10	1	1.10	29.89	< 0.0001	*	0.0287	
C-Sieve Size (mm)	0.3133	1	0.3133	8.48	0.0046	*	0.0248	
AB	0.0314	1	0.0314	0.8489	0.3596	NS	0.0203	
AC	0.0378	1	0.0378	1.02	0.3145	NS	0.0351	
BC	0.0627	1	0.0627	1.70	0.1962	NS	0.0287	
ABC	0.0307	1	0.0307	0.8314	0.3645	NS	0.0248	
Error	0.7936	60	0.0132				0.0351	
Total	32.03	89						

(* = significant, NS = non-significant)

4.2.1.1 Effect of temperature and feed rate on grinding time

Figure 4.2 shows the effect of temperature and feed rate on grinding time. The two-way interaction of temperature and feed rate was found to be non-significant on the grinding time of the material. This was due to the higher feed rate which reduces the time taken to convey the material into the pin mill. The grinding time was found to be decreasing with a decrease in the temperature and with an increase in the feed rate. This is probabily due to the brittle structure of the seed at lower temperature. The decrease in the hardness and increase in the fracturability of the seed during grinding takes less time at lower temperature. Similar findings were reported by Sexena *et al.* (2015) for cryoground cumin seed.

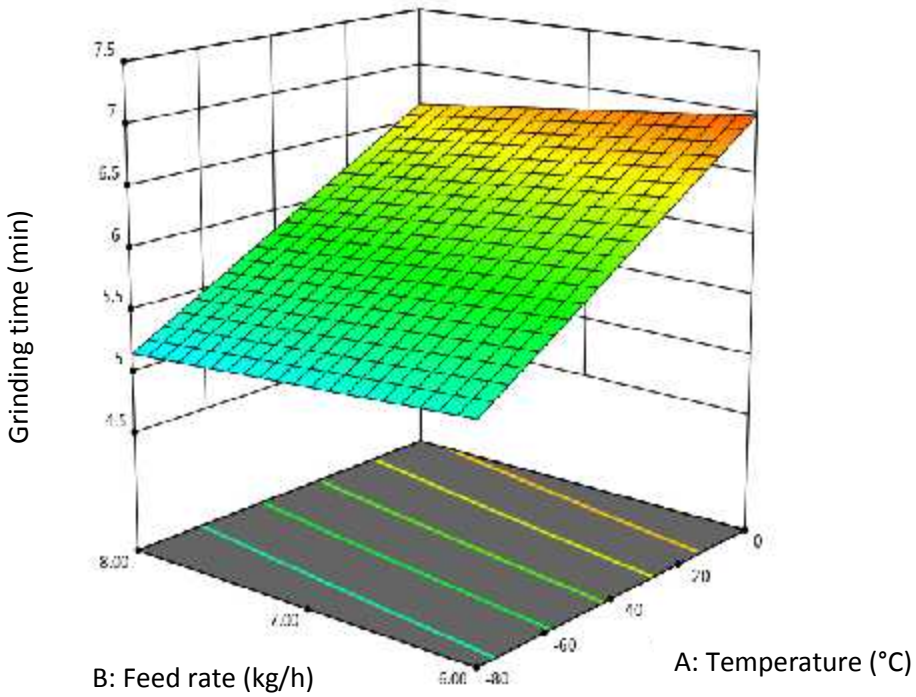


Figure 4.2 Effect of temperature and feed rate on grinding time

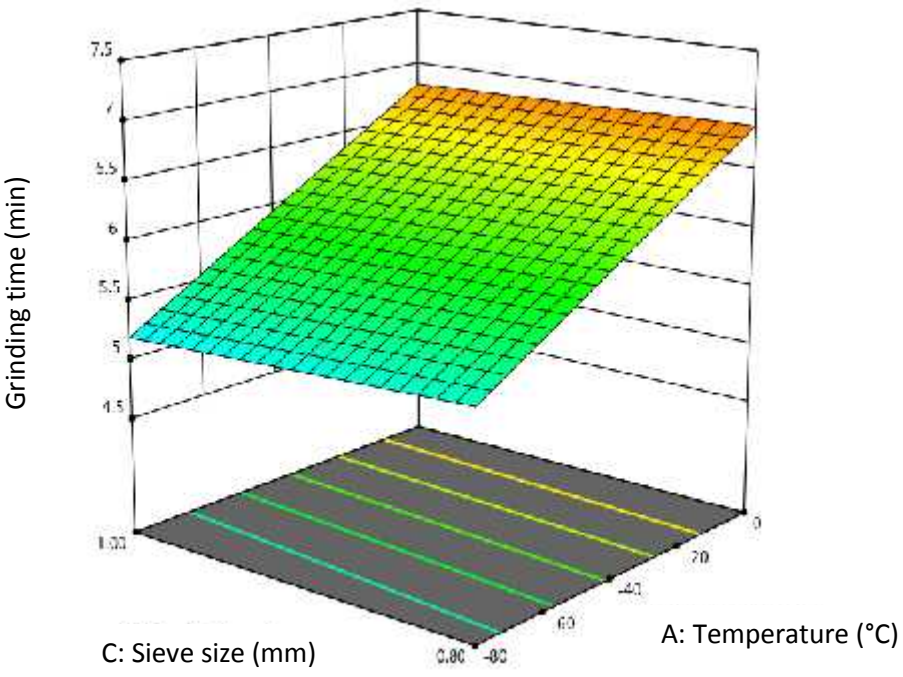


Figure 4.3 Effect of temperature and sieve size on grinding time

Table 4.5 Effect of temperature, feed rate and sieve size on grinding time

Temperature (°C)	Feed Rate (Kg/h)	Sieve Size (mm)	Grinding time (min)
0 °C	6	0.8	7.17
	6	1.0	7.07
	7	0.8	6.72
	7	1.0	6.55
	8	0.8	6.55
	8	1.0	6.42
-20 °C	6	0.8	6.37
	6	1.0	6.42
	7	0.8	6.41
	7	1.0	6.38
	8	0.8	6.40
	8	1.0	6.31
-40 °C	6	0.8	6.34
	6	1.0	6.24
	7	0.8	6.30
	7	1.0	6.20
	8	0.8	6.31
	8	1.0	6.19
-60 °C	6	0.8	5.72
	6	1.0	5.73
	7	0.8	5.68
	7	1.0	5.52
	8	0.8	5.50
	8	1.0	5.40
-80 °C	6	0.8	5.38
	6	1.0	5.26
	7	0.8	5.37
	7	1.0	5.23
	8	0.8	5.20
	8	1.0	4.72
25 °C	6	0.8	7.17
	6	1.0	7.07
	7	0.8	6.72
	7	1.0	6.55
	8	0.8	6.55
	8	1.0	6.42

4.2.1.2 Effect of temperature and sieve size on grinding time

Figure 4.3 shows the effect of sieve size and temperature on grinding time. The two-way interaction of sieve size and temperature showed a non-significant effect on grinding time of dill seed. It was observed that the time taken for grinding the material increases with an increase in the temperature at different levels and decreases with the increase in sieve size. This is probably because the grinding requires less time to produce coarse particles when compared to that of fine particles with smaller sieve size. The grinding time of the material decreased from 7.17 min to 4.72 min as the temperature decreased from 0 ° to -80 °C. Similar findings were reported by Sexena *et al.* (2017) and Sexena *et al.* (2015) for cryoground cumin seed.

4.2.2 Effect of Temperature, Feed Rate and Sieve Size on Liquid Nitrogen Consumption

The ANOVA in Tables 4.6 and 4.7 shows that the effect of temperature, feed rate and sieve size on liquid nitrogen consumption is significant at ($p < 0.1$) at 1 % level. The independent variables viz. temperature and sieve size had a significant effect on liquid nitrogen consumption at ($p < 0.1$) at 1 % level. The feed rate had a non-significant effect on liquid nitrogen consumption.

4.2.2.1 Effect of temperature and feed rate on liquid nitrogen consumption

The two-way interaction of temperature and feed rate on liquid nitrogen consumption is depicted in Figure 4.4 and was found to be non-significant. The liquid nitrogen consumption increased from 1.71 to 3.29 kg as the temperature decreased from 0 ° to -80 °C. This is because the liquid nitrogen required for griniding increases with a decrease in temperature. The present investigation findings are similar to Singh and Goswami (2000) who reported similar effect of temperature and feed rate on liquid nitrogen consumption of cryoground cloves.

4.2.2.2. Effect of temperature and sieve size on liquid nitrogen consumption

Figure 4.5 shows the effect of temperature and sieve size on liquid nitrogen consumption. The two-way interaction of temperature and sieve size on liquid nitrogen consumption was found to be significant ($p < 0.1$) at 1 % level. Liquid nitrogen consumption decreased with increasing feed rate from 6 kg/h to 8 kg/h and also with decreasing temperature

from 0 ° to -80 °C. Singh and Goswami (2000) also reported similar effect of temperature on cryoground cloves.

Table 4.6 ANOVA for liquid nitrogen consumption

Source	Sum of Squares	df	Mean Square	F-value	p-value	Test	SEm	CV (%)
Model	18.30	7	2.61	271.51	< 0.0001	*		4.20
A-Temperature (°C)	17.75	1	17.75	1844.30	< 0.0001	*	0.0103	
B-Feed Rate (kg/h)	0.0091	1	0.0091	0.9481	0.3331	NS	0.0146	
C-Sieve Size (mm)	0.2035	1	0.2035	21.14	< 0.0001	*	0.0127	
AB	0.0011	1	0.0011	0.1122	0.7385	NS	0.0103	
AC	0.3200	1	0.3200	33.25	< 0.0001	*	0.0179	
BC	0.0042	1	0.0042	0.4328	0.5124	NS	0.0146	
ABC	0.0039	1	0.0039	0.4003	0.5287	NS	0.0127	
Error	0.0656	60	0.0011				0.0179	
Total	19.08	89						

4.2.3 Effect of Temperature, Feed Rate and Sieve Size on Energy Consumption

The effect of the independent variables on energy consumption was as shown in Tables 4.8 and 4.9 and it was found to be significant ($p < 0.1$) at 1 % level. The temperature and sieve size had a significant effect on energy consumption at ($p < 0.1$) at 1 % level whereas the feed rate was found to be non-significant. The energy consumption for the grinding of the material varied from 0.30 kWh to 0.23 kWh as the temperature decreased from 0 ° to -80 °C.

4.2.3.1 Effect of temperature and feed rate on energy consumption

Figure 4.6 shows the two-way interaction of temperature and feed rate and it was found to be non-significant on energy consumption. The energy consumption decreases with decrease in grinding temperature from 0 ° to -80 °C and also with increasing feed rate from 6 kg/h to 8 kg/h. This is because the low temperature makes the material brittle which requires less energy for size reduction. As the feed rate increases the material enters into the pin mill earlier when compared to the seed of lower feed rate that ultimately reduces the energy required for grinding. Similar findings were reported by Saxena *et al.* (2017) for cryoground cumin seed.

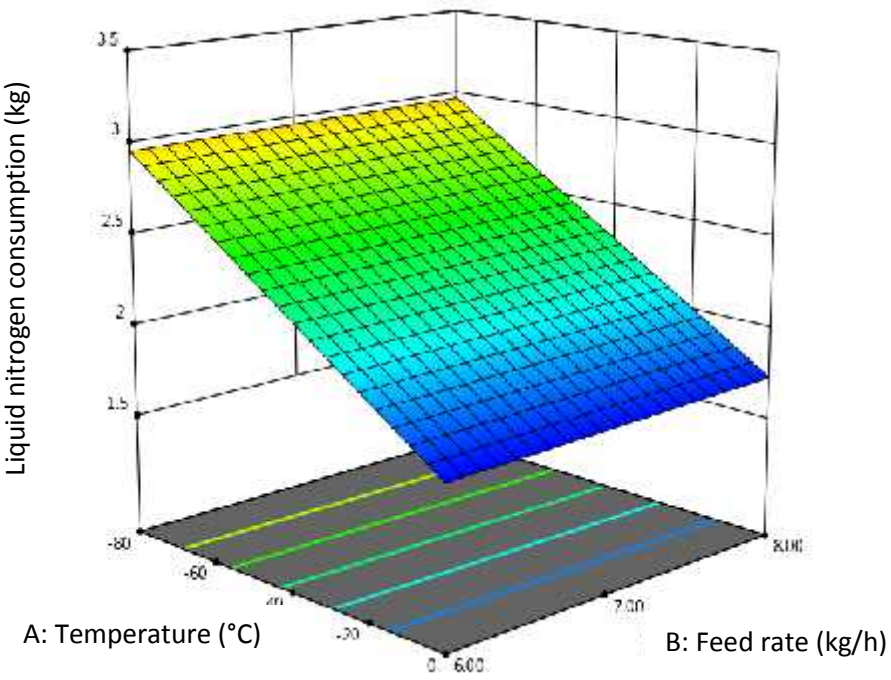


Figure 4.4 Effect of temperature and feed rate on liquid nitrogen consumption

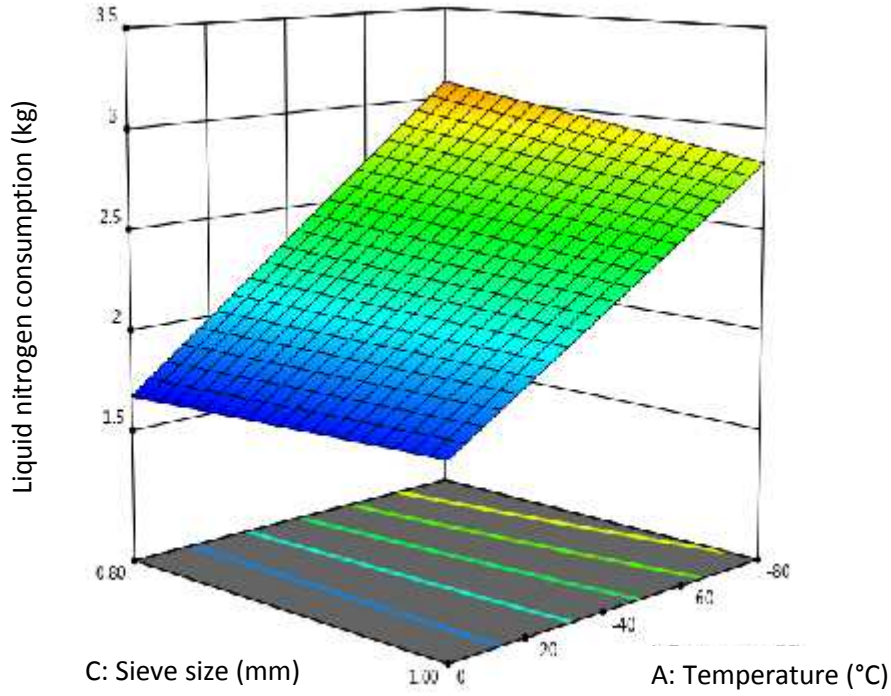


Figure 4.5 Effect of temperature and sieve size on liquid nitrogen consumption

Table 4.7 Effect of temperature, feed rate and sieve size on liquid nitrogen consumption

Temperature (°C)	Feed Rate (Kg/h)	Sieve Size (mm)	Liquid nitrogen consumption (kg)
0 °C	6	0.8	1.79
	6	1.0	1.75
	7	0.8	1.79
	7	1.0	1.73
	8	0.8	1.74
	8	1.0	1.71
-20 °C	6	0.8	2.17
	6	1.0	2.08
	7	0.8	2.12
	7	1.0	2.08
	8	0.8	2.02
	8	1.0	2.01
-40 °C	6	0.8	2.33
	6	1.0	2.27
	7	0.8	2.28
	7	1.0	2.22
	8	0.8	2.21
	8	1.0	2.18
-60 °C	6	0.8	2.58
	6	1.0	2.55
	7	0.8	2.62
	7	1.0	2.54
	8	0.8	2.62
	8	1.0	2.50
-80 °C	6	0.8	3.29
	6	1.0	2.90
	7	0.8	3.26
	7	1.0	2.89
	8	0.8	3.29
	8	1.0	2.85

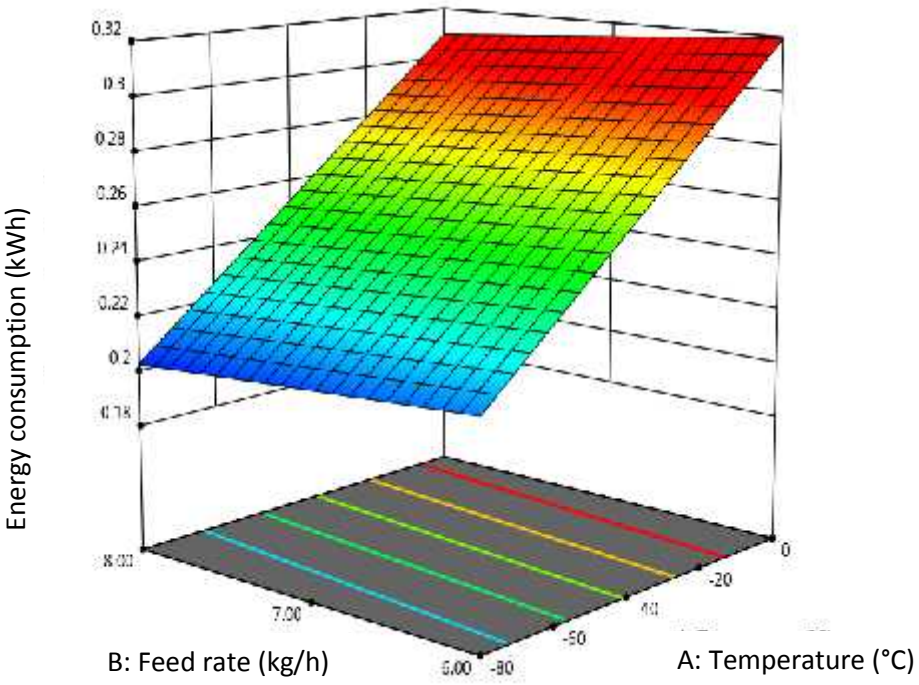


Figure 4.6 Effect of temperature and feed rate on energy consumption

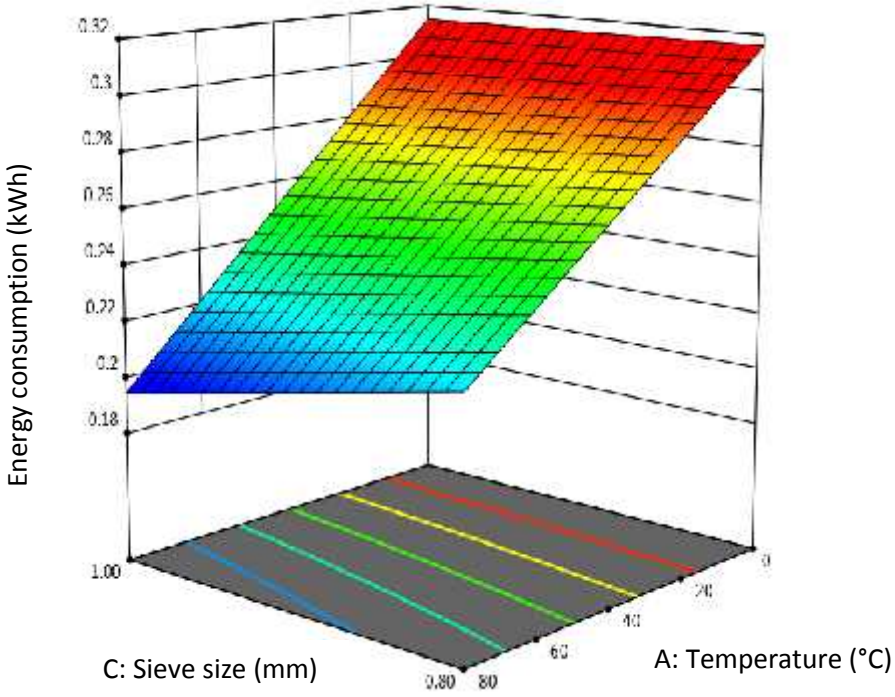


Figure 4.7 Effect of temperature and sieve size on energy consumption

Table 4.8 Effect of temperature, feed rate and sieve size on energy consumption

Temperature (°C)	Feed Rate (Kg/h)	Sieve Size (mm)	Energy consumption (kWh)
0 °C	6	0.8	0.30
	6	1.0	0.30
	7	0.8	0.30
	7	1.0	0.30
	8	0.8	0.30
	8	1.0	0.30
-20 °C	6	0.8	0.30
	6	1.0	0.30
	7	0.8	0.30
	7	1.0	0.30
	8	0.8	0.30
	8	1.0	0.30
-40 °C	6	0.8	0.30
	6	1.0	0.30
	7	0.8	0.30
	7	1.0	0.27
	8	0.8	0.27
	8	1.0	0.23
-60 °C	6	0.8	0.27
	6	1.0	0.20
	7	0.8	0.27
	7	1.0	0.20
	8	0.8	0.20
	8	1.0	0.20
-80 °C	6	0.8	0.23
	6	1.0	0.20
	7	0.8	0.20
	7	1.0	0.20
	8	0.8	0.20
	8	1.0	0.20
25 °C	6	0.8	0.40
	6	1.0	0.40
	7	0.8	0.40
	7	1.0	0.40
	8	0.8	0.40
	8	1.0	0.33

Table 4.9 ANOVA for energy consumption

Source	Sum of Squares	df	Mean Square	F-value	p-value	Test	SEm	CV (%)
Model	0.1371	7	0.0196	20.92	< 0.0001	*		11.72
A-Temperature (°C)	0.1280	1	0.1280	136.71	< 0.0001	*	0.0032	
B-Feed Rate (kg/h)	0.0015	1	0.0015	1.60	0.2092	NS	0.0046	
C-Sieve Size (mm)	0.0054	1	0.0054	5.81	0.0181	*	0.0040	
AB	0.0000	1	0.0000	0.0000	1.0000	NS	0.0032	
AC	0.0020	1	0.0020	2.14	0.1477	NS	0.0056	
BC	0.0002	1	0.0002	0.1780	0.6742	NS	0.0046	
ABC	0.0000	1	0.0000	0.0000	1.0000	NS	0.0040	
Error	0.0400	60	0.0007				0.0056	
Total	0.2139	89						

4.2.3.2 Effect of temperature and sieve size on energy consumption

The effect of temperature and sieve size on energy consumption is shown in Figure 4.7. It was observed that energy consumption decreased with increase in sieve size. A two-way interaction of temperature and sieve size on energy consumption was found to be non-significant. This is due to the fact that the energy required for grinding of the material is higher for the sieve of the smaller mesh size. Similar findings were reported by Saxena *et al.* (2017) for cryoground cumin seed.

4.2.4 Effect of Temperature, Feed Rate and Sieve Size on Essential Oil

Tables 4.10 and 4.11 shows the effect of temperature, feed rate and sieve size on the yield of essential oil. The essential oil content is the indicator for the production of superior quality spice powder which is highly affected during conventional grinding. All three independent variables had a significant effect on the essential oil content at ($p < 0.1$) at 1 % level. The essential oil content increased from 1.27 % to 2.33 % as the temperature decreased from 0 ° to -80 °C. This is due to the lower heat generation during the grinding of the material that preserves the loss of essential oil.

4.2.4.1 Effect of temperature and feed rate on essential oil

The effect of temperature and feed rate on the essential oil is shown in Figure 4.8. The two-way interaction of temperature and feed rate was found to be non-significant on the essential oil. The essential oil content was found to be increasing as the temperature decreases from 0 ° to -80 °C and decreasing as the feed rate increases from 6 kg/h to 8 kg/h. This may be due to the fact increase in the feed rate results in more heat generation in the pin mill during grinding. Wolf and Pahl (1990) reported that cryoground caraway seed yield increased 32 % more than that of ambient grinding temperature.

Table 4.10 ANOVA for essential oil

Source	Sum of Squares	df	Mean Square	F-value	p-value	Test	SEm	CV (%)
Model	8.28	7	1.18	70.66	< 0.0001	*		7.71
A-Temperature (°C)	7.44	1	7.44	444.42	< 0.0001	*	0.0136	
B-Feed Rate (kg/h)	0.3840	1	0.3840	22.93	< 0.0001	*	0.0193	
C-Sieve Size (mm)	0.2351	1	0.2351	14.04	0.0003	*	0.0167	
AB	0.0270	1	0.0270	1.61	0.2077	NS	0.0136	
AC	0.0500	1	0.0500	2.99	0.0878	NS	0.0236	
BC	0.0240	1	0.0240	1.43	0.2347	NS	0.0193	
ABC	0.1203	1	0.1203	7.19	0.0089	*	0.0167	
Error	0.7200	60	0.0120				0.0236	
Total	9.66	89						

4.2.4.2 Effect of temperature and sieve size on essential oil

Figure 4.9 shows the effect of temperature and sieve size on the essential oil. The two-way interaction of temperature and sieve size on essential oil content was found to be non-significant. This is probably due to the fact that the essential oil increases with decreasing temperature and sieve size. Similar results were observed by Sexena *et al*. (2017) for grinding of cumin seed.

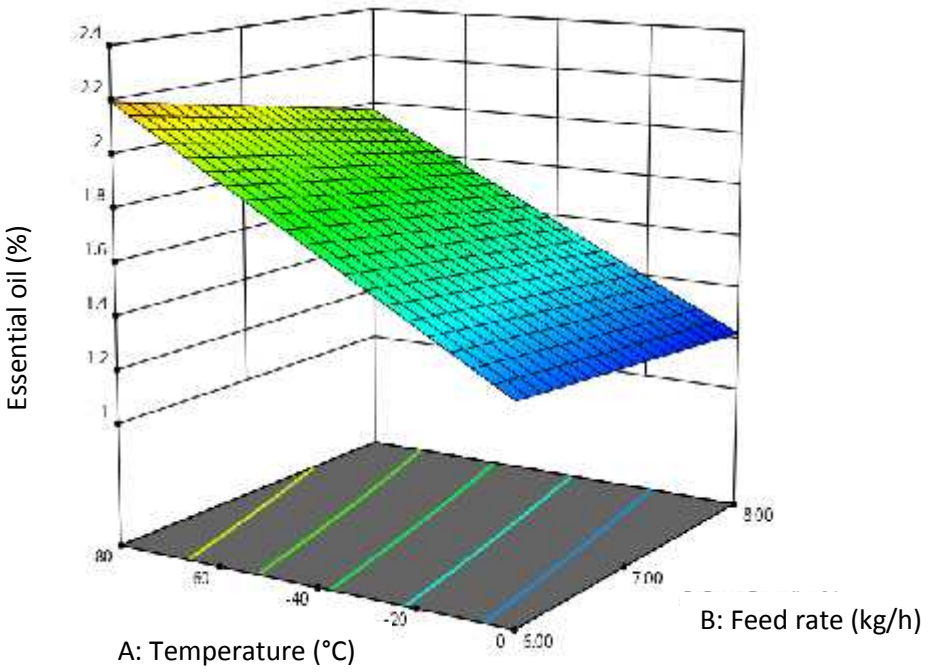


Figure 4.8 Effect of temperature and feed rate on essential oil

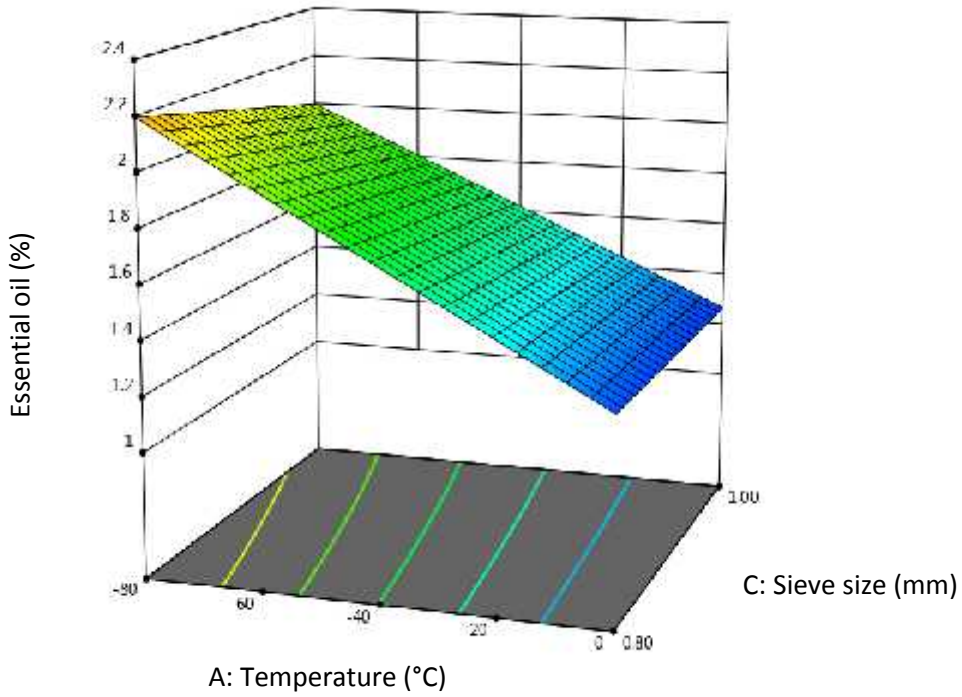


Figure 4.9 Effect of temperature and sieve size on essential oil

Table 4.11 Effect of temperature, feed rate and sieve size on essential oil

Temperature (°C)	Feed Rate (kg/h)	Sieve Size (mm)	Essential oil (%)
0 °C	6	0.8	1.27
	6	1.0	1.27
	7	0.8	1.33
	7	1.0	1.27
	8	0.8	1.33
	8	1.0	1.20
-20 °C	6	0.8	1.60
	6	1.0	1.53
	7	0.8	1.53
	7	1.0	1.33
	8	0.8	1.47
	8	1.0	1.33
-40 °C	6	0.8	1.73
	6	1.0	1.80
	7	0.8	1.67
	7	1.0	1.73
	8	0.8	1.53
	8	1.0	1.47
-60 °C	6	0.8	1.93
	6	1.0	2.00
	7	0.8	1.87
	7	1.0	1.93
	8	0.8	1.73
	8	1.0	1.87
-80 °C	6	0.8	2.33
	6	1.0	2.07
	7	0.8	2.27
	7	1.0	2.07
	8	0.8	2.07
	8	1.0	2.00
25 °C	6	0.8	0.77
	6	1.0	0.63
	7	0.8	0.77
	7	1.0	0.53
	8	0.8	0.63
	8	1.0	0.57

4.2.5 Effect of Temperature, Feed Rate and Sieve Size on Moisture

Tables 4.12 and 4.13 shows the ANOVA for the effect of temperature, feed rate and sieve size on moisture and a significant effect was observed for all the three independent variables ($p < 0.1$) at 1 % level. As the temperature decreased from 0 ° to -80 °C the moisture of the material increased from 7.76 % to 9.86 %.

Table 4.12 ANOVA for moisture

Source	Sum of Squares	df	Mean Square	F-value	p-value	Test	SEm	CV (%)
Model	46.05	7	6.58	321.93	< 0.0001	*		1.63
A-Temperature (°C)	44.71	1	44.71	2187.85	< 0.0001	*	0.0151	
B-Feed Rate (kg/h)	1.19	1	1.19	58.37	< 0.0001	*	0.0213	
C-Sieve Size (mm)	0.0683	1	0.0683	3.34	0.0711	NS	0.0185	
AB	0.0267	1	0.0267	1.31	0.2563	NS	0.0151	
AC	0.0117	1	0.0117	0.5716	0.4518	NS	0.0261	
BC	0.0346	1	0.0346	1.69	0.1971	NS	0.0213	
ABC	0.0069	1	0.0069	0.3377	0.5628	NS	0.0185	
Error	1.27	60	0.0212				0.0261	
Total	47.73	89						

4.2.5.1 Effect of temperature and feed rate on moisture

The effect of temperature and feed rate on moisture is depicted in Figure 4.10. The two-way interaction of temperature and feed rate was found to be non-significant. This is because of the less heat generation in the pin mill during the grinding of the material at a lower temperature which ultimately increases the moisture. Similar effect of temperature on moisture was reported by Barai *et al.* (2017) for cryoground cumin seeds.

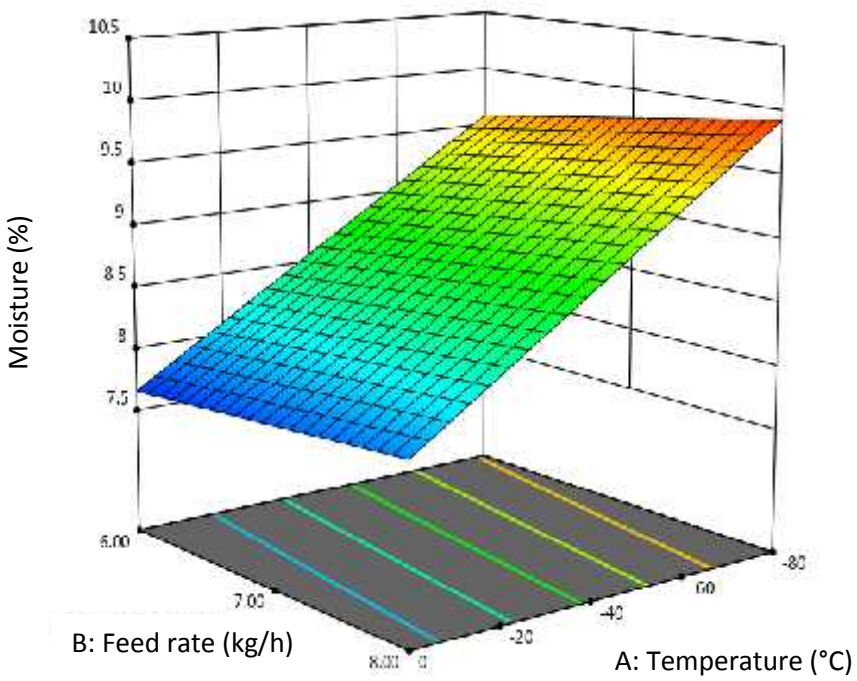


Figure 4.10 Effect of temperature and feed rate on moisture

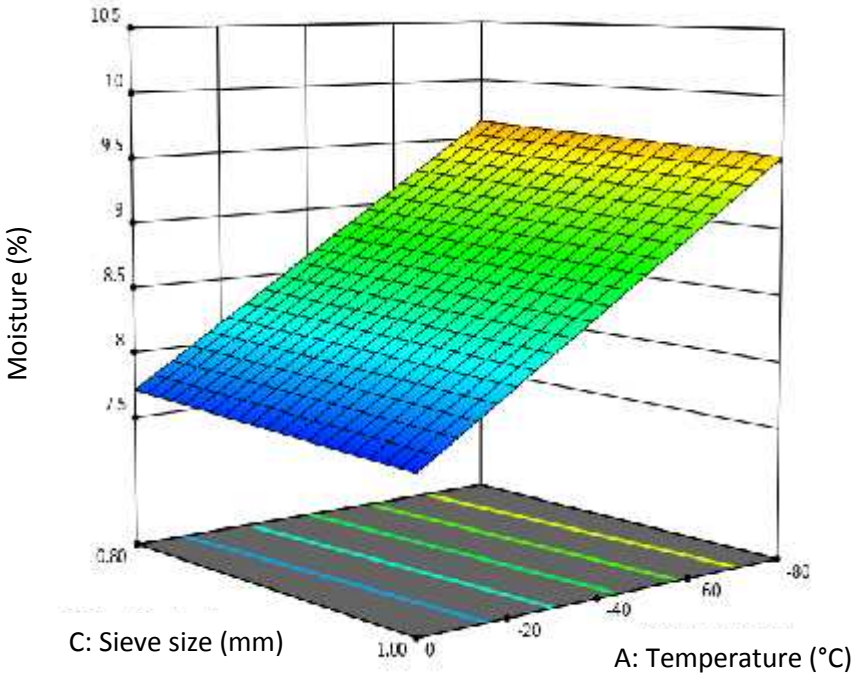


Figure 4.11 Effect of temperature and sieve size on moisture

Table 4.13 Effect of temperature, feed rate and sieve size on moisture

Temperature (°C)	Feed Rate (kg/h)	Sieve Size (mm)	Moisture (%)
0 °C	6	0.8	7.76
	6	1.0	7.72
	7	0.8	7.88
	7	1.0	7.83
	8	0.8	7.82
	8	1.0	7.93
-20 °C	6	0.8	8.09
	6	1.0	7.98
	7	0.8	8.25
	7	1.0	8.20
	8	0.8	8.43
	8	1.0	8.36
-40 °C	6	0.8	8.65
	6	1.0	8.53
	7	0.8	8.76
	7	1.0	8.70
	8	0.8	8.82
	8	1.0	8.89
-60 °C	6	0.8	9.13
	6	1.0	8.94
	7	0.8	9.43
	7	1.0	9.26
	8	0.8	9.41
	8	1.0	9.39
-80 °C	6	0.8	9.64
	6	1.0	9.61
	7	0.8	9.86
	7	1.0	9.85
	8	0.8	9.95
	8	1.0	9.86
25 °C	6	0.8	7.43
	6	1.0	7.44
	7	0.8	7.42
	7	1.0	7.42
	8	0.8	7.65
	8	1.0	7.51

4.2.5.2 Effect of temperature and sieve size on moisture

The effect of temperature and sieve size on moisture is depicted in Figure 4.11. The two-way interaction of temperature and sieve size on the moisture was found to be non-significant. The effect of two independent parameters on the moisture was negligible. The moisture was found to be increasing with an increase in the sieve size. This is probably because as the sieve size increases from 0.8 to 1 mm, the time required to grind the material decreases. This decrease in the time decreases the moisture evaporation in the pin mill during grinding. Similar effect was reported by Rajendra (2018) for cryoground ajwain seed.

4.2.6 Effect of Temperature, Feed Rate and Sieve Size on Mean Particle Size

Tables 4.14 and 4.15 represents the effect of temperature, feed rate and sieve size on the mean particle size. All three independent variables showed a significant effect on the particle size ($p < 0.1$) at 1 % level. The mean particle size of the material was found to be slightly increasing with a decrease in the temperature i.e. 0.229 to 0.306 mm from 0 ° to -80 °C.

Table 4.14 ANOVA for particle size

Source	Sum of Squares	df	Mean Square	F-value	p-value	Test	SEm	CV (%)
Model	0.0585	7	0.0084	266.07	< 0.0001	*	0.0006	2.13
A-Temperature (°C)	0.0420	1	0.0420	1339.31	< 0.0001	*	0.0008	
B-Feed Rate (kg/h)	0.0107	1	0.0107	340.89	< 0.0001	*	0.0007	
C-Sieve Size (mm)	0.0030	1	0.0030	97.13	< 0.0001	*	0.0006	
AB	0.0021	1	0.0021	68.13	< 0.0001	*	0.0010	
AC	0.0004	1	0.0004	13.11	0.0005	*	0.0008	
BC	0.0001	1	0.0001	3.87	0.0527	NS	0.0007	
ABC	7.820	1	7.820	0.0249	0.8750	NS	0.0010	
Error	0.0001	60	9.350					
Total	0.0610	89						

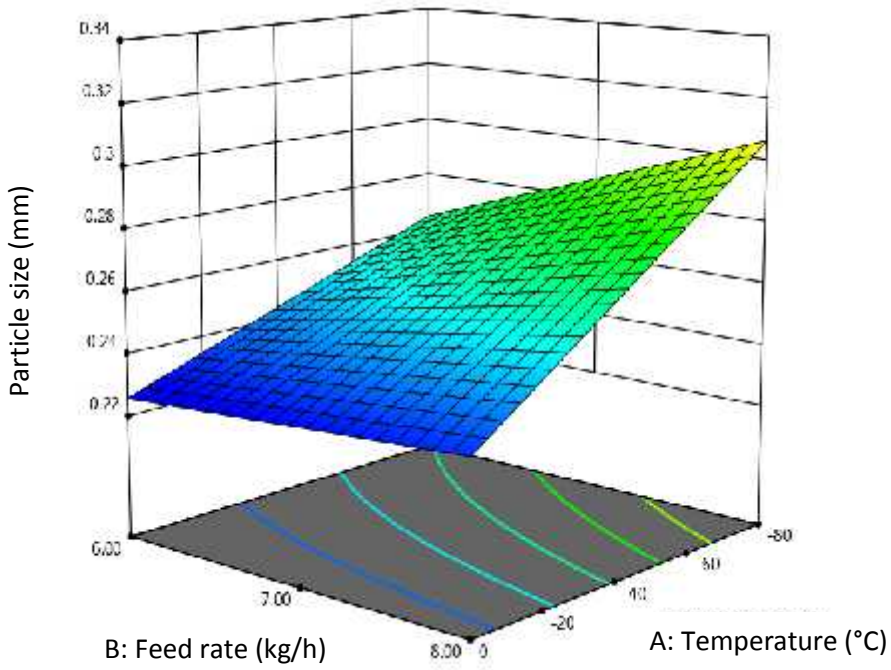


Figure 4.12 Effect of temperature and feed rate on mean particle size

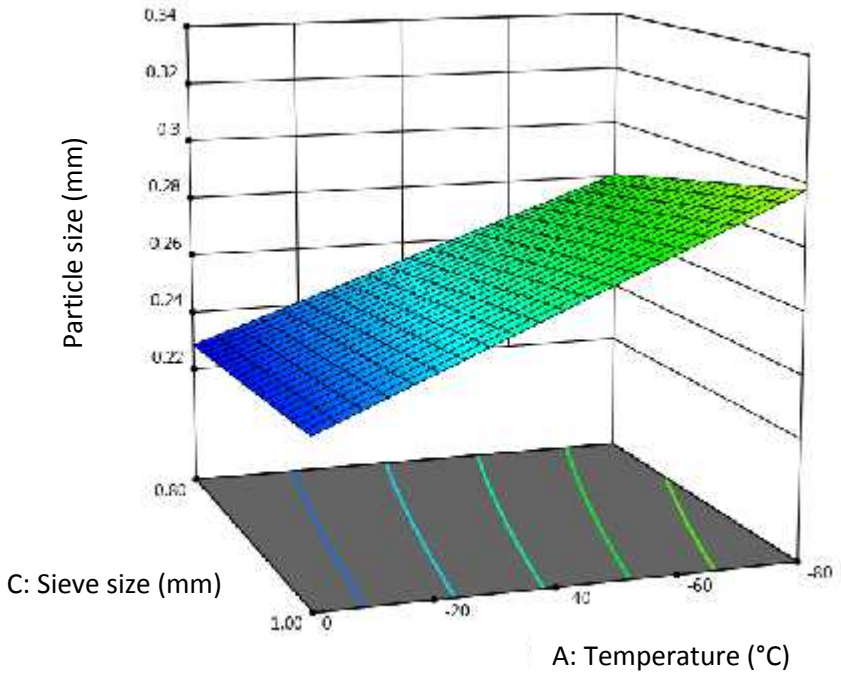


Figure 4.13 Effect of temperature and sieve size on mean particle size

Table 4.15 Effect of temperature, feed rate and sieve size on particle size

Temperature (°C)	Feed Rate (Kg/h)	Sieve Size (mm)	Particle size (mm)
0 °C	6	0.8	0.229
	6	1.0	0.233
	7	0.8	0.235
	7	1.0	0.241
	8	0.8	0.240
	8	1.0	0.248
-20 °C	6	0.8	0.234
	6	1.0	0.240
	7	0.8	0.240
	7	1.0	0.248
	8	0.8	0.246
	8	1.0	0.260
-40 °C	6	0.8	0.241
	6	1.0	0.248
	7	0.8	0.249
	7	1.0	0.259
	8	0.8	0.262
	8	1.0	0.273
-60 °C	6	0.8	0.250
	6	1.0	0.264
	7	0.8	0.267
	7	1.0	0.284
	8	0.8	0.284
	8	1.0	0.306
-80 °C	6	0.8	0.269
	6	1.0	0.284
	7	0.8	0.292
	7	1.0	0.306
	8	0.8	0.310
	8	1.0	0.331
25 °C	6	0.8	0.224
	6	1.0	0.227
	7	0.8	0.225
	7	1.0	0.231
	8	0.8	0.228
	8	1.0	0.235

4.2.6.1 Effect of temperature and feed rate on mean particle size

The effect of temperature and feed rate on mean particle size is presented in Figure 4.12. The two-way interaction of temperature and feed rate on mean particle size was significant ($p < 0.1$) at 1 % level. The mean particle size of the material increased with decreasing temperature from 0 ° to 80 °C and with increasing feed rate from 6 kg/h to 8 kg/h. Similar findings were reported by Wolf and Pahl (1990) for ground caraway powder.

4.2.6.2 Effect of temperature and sieve size on mean particle size

The effect of temperature and sieve size on mean particle size is presented in Figure 4.13. The two-way interaction was found to be significant ($p < 0.1$) at 1 % level. The mean particle size of the material increased with an increase in sieve size and a decrease in temperature from 0 ° to 80 °C. Similar findings were reported by Wolf and Pahl (1990) for ground caraway powder and by Saxena *et al.* (2017) for cryoground cumin seed.

4.3 OPTIMIZATION OF PROCESSING PARAMETERS OF CRYOGENIC GRINDING OF DILL SEED POWDER

The optimized parameters obtained from the statistical analysis using factorial CRD in the form of ramps are presented in Figure 4.14

The optimized parameters are:

Temperature : -60 °C

Feed rate : 6 kg/h

Sieve size : 0.8 mm

4.3.1 Gas Chromatographic- Mass Spectroscopic Analysis of Essential Oil

The hydrodistillated essential oil of the optimized cryoground parameters was analysed for its marker compounds i.e. carvone and limonene by GC-MS. The chromatogram obtained for standard and the sample analysed is presented in Figure 4.15 and Figure 4.16 respectively. The essential oil contained 27.63 % carvone and 7.21 % limonene.

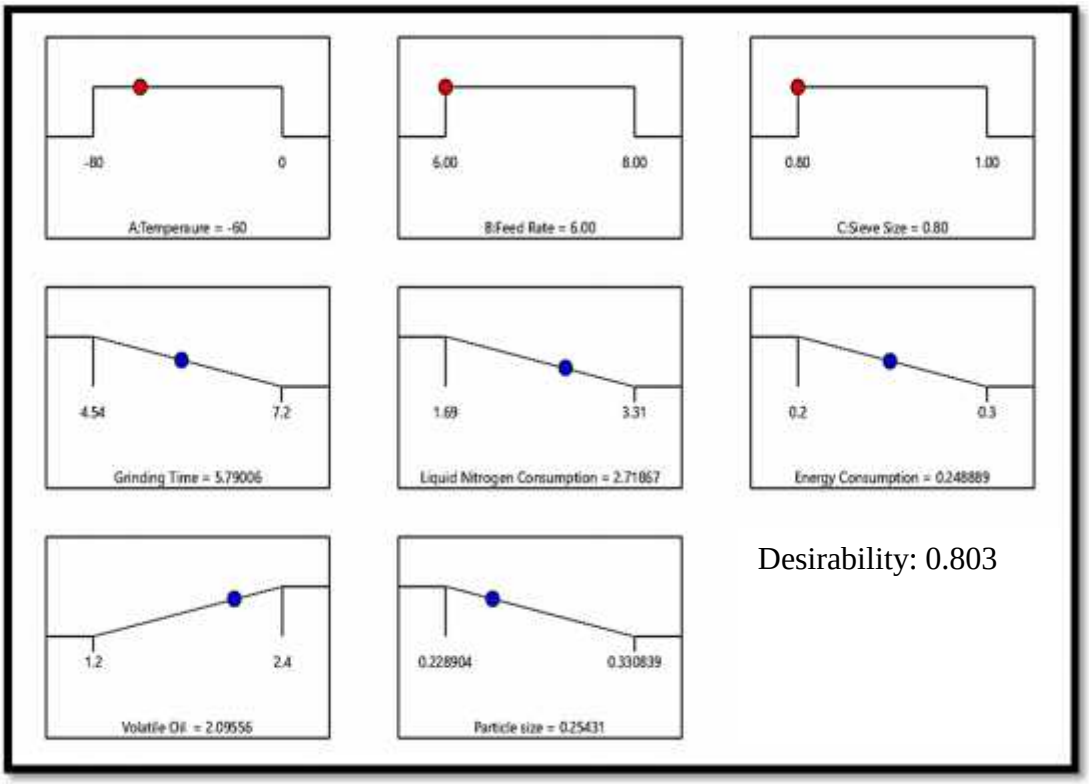


Figure 4.14 Optimized parameters for production of cryoground dill seed powder

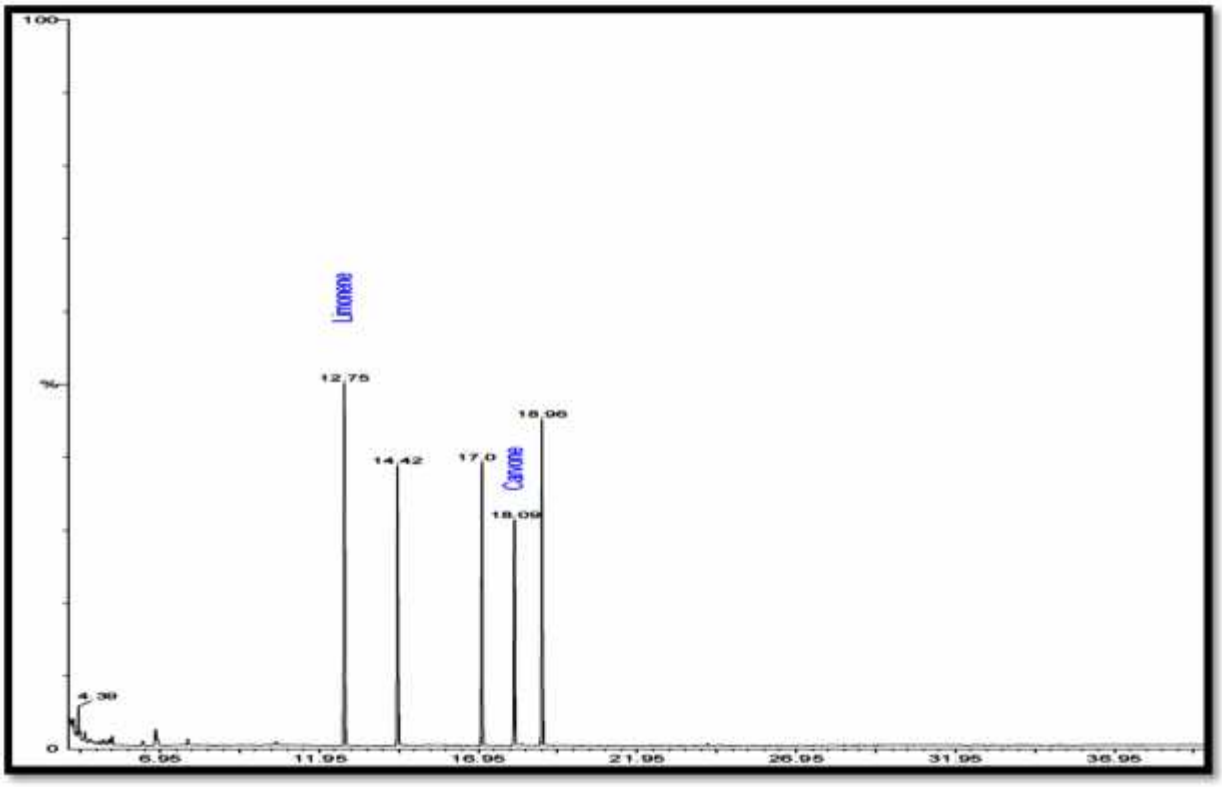


Figure 4.15 GC-MS chromatogram of standards

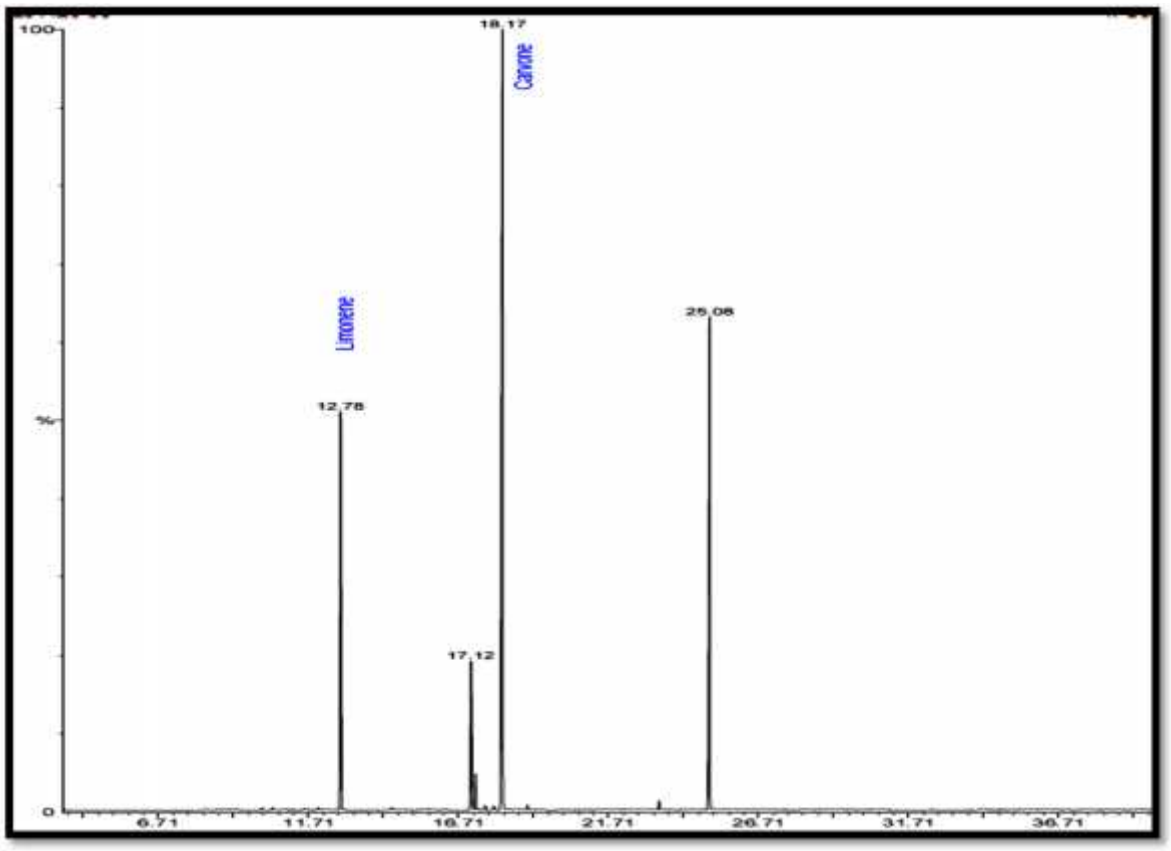


Figure 4.16 GC-MS chromatogram of dill seed essential oil

4.4 ENACPAULATION OF ESSENTIAL OIL

Encapsulation of dill seed essential oil using alginate-polymer composite blends with different carrier materials was carried out as per the procedure described in sections 3.4.1 and 3.4.2. In the present investigation, the carrier materials viz. gum tragacanth, maltodextrin, agar agar and gelatine at concentrations of 0.50 %, 1 % and 1.5 % were used as independent variables. The statistical analysis carried out and the ANOVA for the polymeric blends and the effect of independent variables on moisture, encapsulation efficiency, colour value and particle size are tabulated in Table 4.16.

4.4.1 Effect of Polymeric Blends on Moisture of Encapsulated Powder

The moisture of the encapsulated powder from different polymeric blends varied from 2.01 % to 3.94 %. Lower moisture was observed in alginate-tragacanth blend whereas higher moisture was observed in alginate-gelatine blend (Figure 4.17). This may be due to the fact

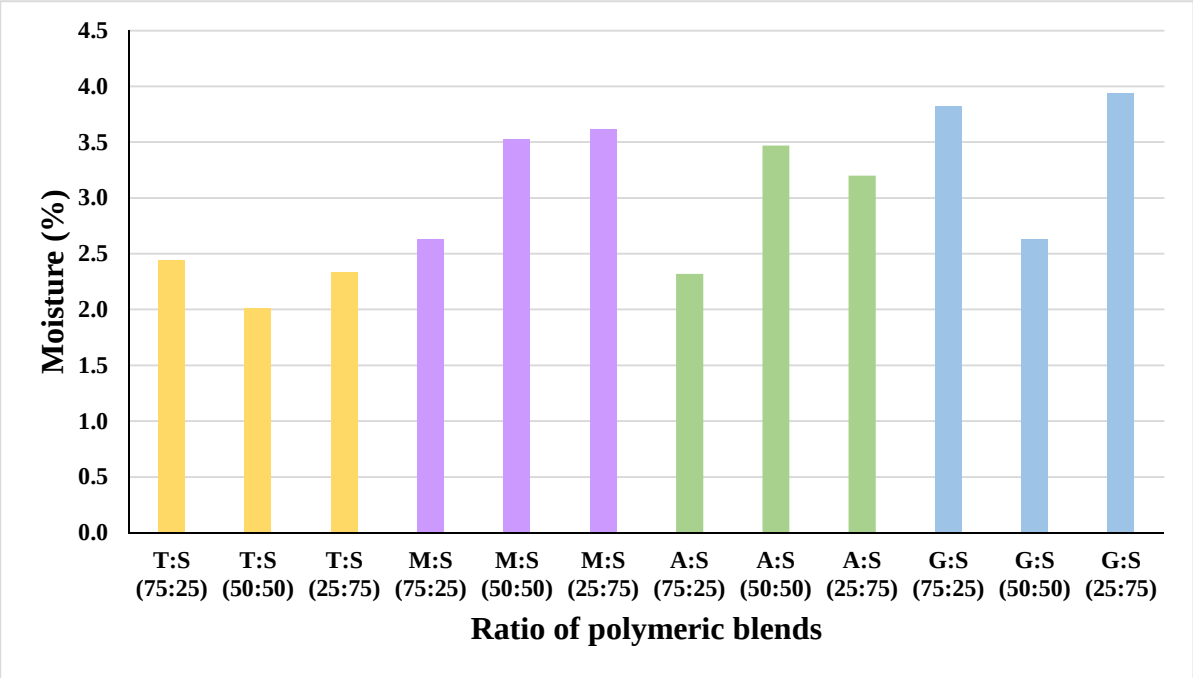
Table 4.16 Effect of polymeric blends on characteristics of encapsulated powder

Polymer	Polymer: Sodium alginate	Moisture (%)	Encapsulation efficiency (%)	L*	a*	b*	Particle size (µm)
Control	0.00:1.00	2.95	78.63	57.12	-0.96	13.25	40.65
Tragacanth	0.75:0.25	2.44	80.42	51.95	4.36	12.65	36.63
Tragacanth	0.50:0.50	2.01	80.00	82.41	3.25	14.42	44.07
Tragacanth	0.25:0.75	2.34	79.40	48.35	1.19	9.80	38.50
Maltodextrin	0.75:0.25	2.63	78.47	64.75	0.30	9.45	36.64
Maltodextrin	0.50:0.50	3.53	78.03	49.28	-0.75	7.48	43.76
Maltodextrin	0.25:0.75	3.62	77.92	48.37	0.13	11.42	40.51
Agar agar	0.75:0.25	2.32	80.02	49.31	-0.08	7.08	35.57
Agar agar	0.50:0.50	3.47	74.10	22.58	4.68	35.46	41.71
Agar agar	0.25:0.75	3.20	79.40	78.59	-0.10	10.25	35.01
Gelatine	0.75:0.25	3.82	75.93	61.32	0.17	15.90	44.07
Gelatine	0.50:0.50	2.63	78.53	62.57	-0.97	10.32	42.44
Gelatine	0.25:0.75	3.94	76.42	48.53	-0.83	17.70	38.57
F-value		1901.69	2.57	3091.79	1071.42	2578.7	239.9
p-value		<0.0001	0.0259	<0.0001	<0.0001	<0.0001	<0.0001
SEm		0.0219	2.63	0.4021	0.0869	0.2124	0.2996
CV %		0.896	4.08	0.8847	11.25	1.93	0.9257

that both sodium alginate and gelatine absorb large amount of water to form gelation with high viscous polymeric solution. Sodium alginate-tragacanth blend of equal ratio recorded lowest moisture of 2.01 % among all combinations. The effect of all polymeric blends on moisture was found highly significant ($p < 0.1$) at 1 % level. The results observed are similar to the findings reported by Fernandes *et al.* (2014) where they reported the moisture of different polymeric blends ranging from 2.25 to 3.27 %.

4.4.2 Effect of Polymeric Blends on Encapsulation Efficiency of Encapsulated Powder

The encapsulation efficiency of different polymeric blends varied from 80.42 % to 74.10 % (Figure 4.18). Higher efficiency was observed in alginate-tragacanth blend 0.25:0.75



(T:S = Tragacanth:Sodium alginate, M:S = Maltodextrin:Sodium alginate, A:S = Agar agar:Sodium alginate, G:S = Gelatine:Sodium alginate)

Figure 4.17 Effect of polymeric blends on moisture of encapsulated powder

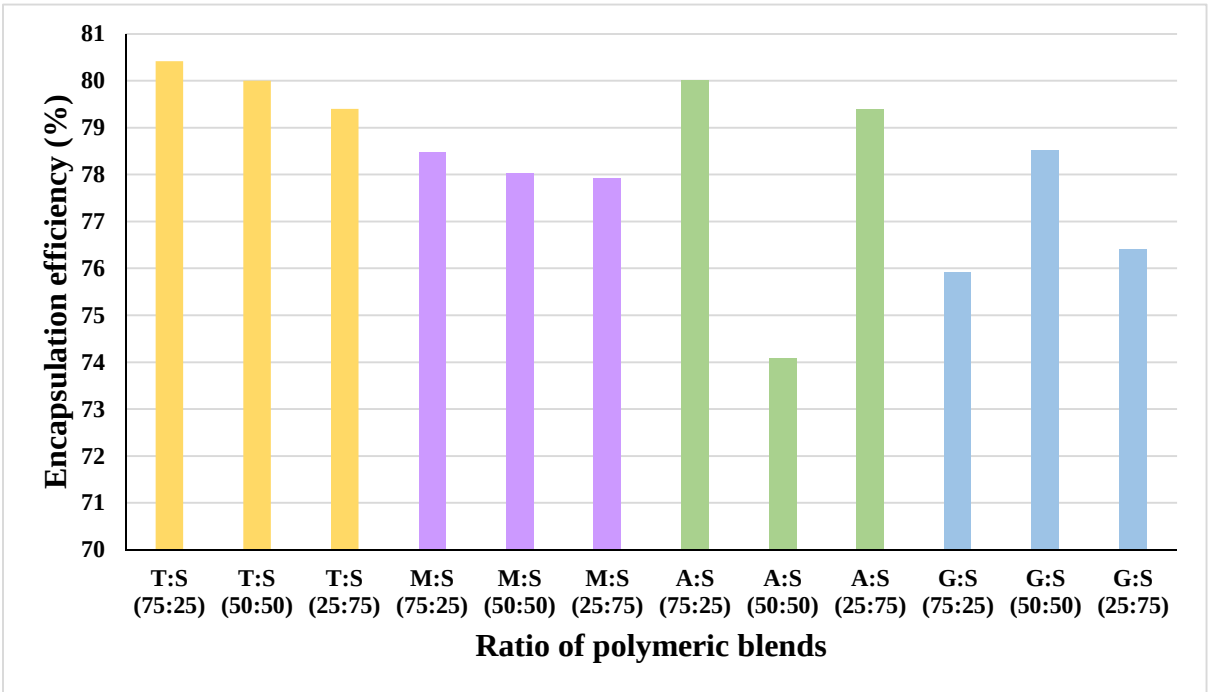


Figure 4.18 Effect of polymeric blends on encapsulation efficiency of encapsulated powder

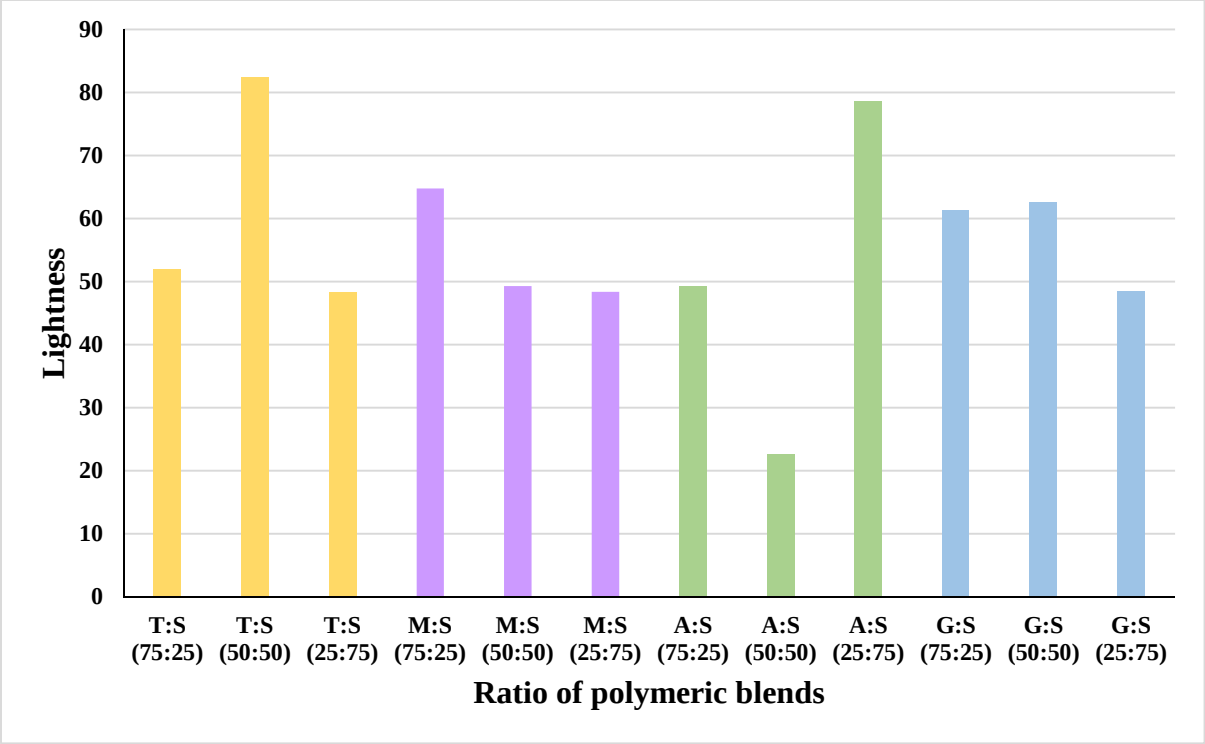


Figure 4.19 Effect of polymeric blends on L* value of encapsulated powder

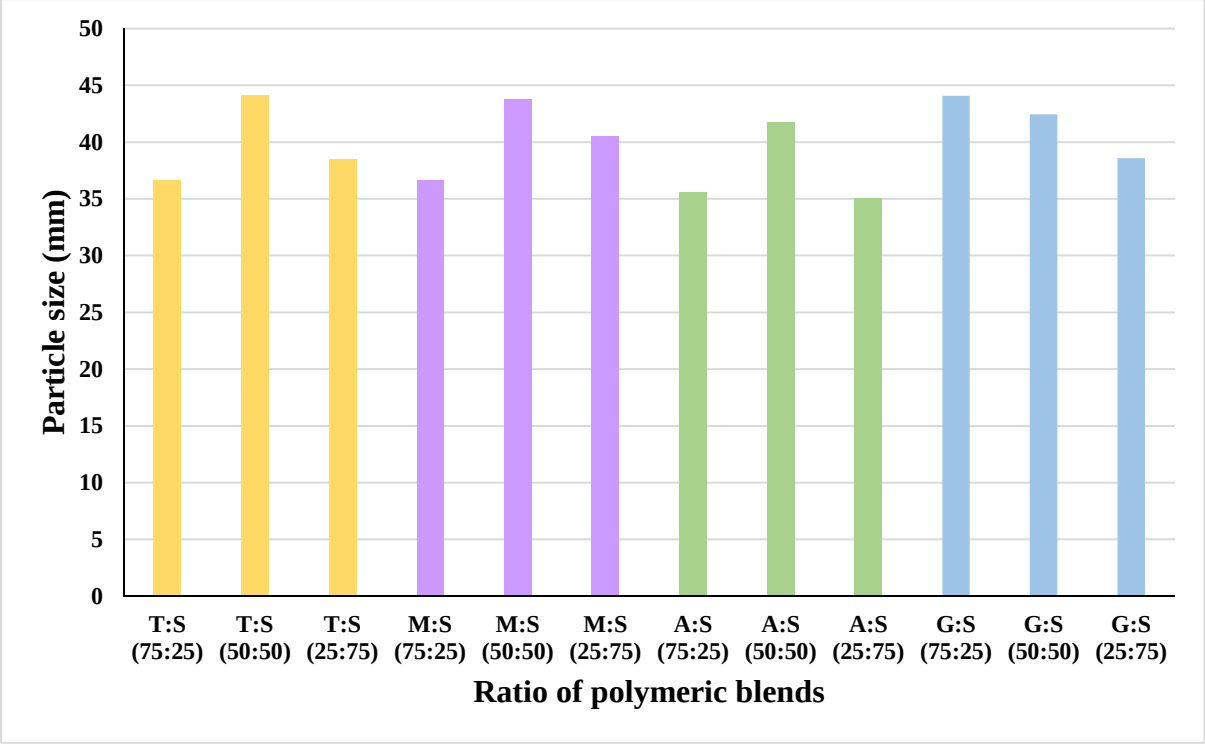


Figure 4.20 Effect of polymeric blends on particle size of encapsulated powder

among all combinations. The effect of polymeric blends of all the combinations studied on encapsulation efficiency were found to be significant ($p < 0.5$) at 5 % level.

4.4.3 Effect of Polymeric Blends on Colour Value of Encapsulated Powder

The colour value of the encapsulated powder prepared from polymeric blends is presented in Table 4.10. The degree of lightness (L*) of the encapsulated powders varied from 22.58 to 82.41 (Figure 4.19). The a* values for most of the polymeric blends were found positive indicating degree of redness in the encapsulated powder. The b* values for all polymeric blends were found positive indicating degree of yellowness in the encapsulated powder. The effect of all polymeric blends on L*, a* and b* values were found highly significant ($p < 0.1$) at 1 % level.

4.4.4 Effect of Polymeric Blends on Particle Size of Encapsulated Powder

The average particle size of the encapsulated powder prepared from polymeric blends varied from 35.01 µm to 44.07 µm (Figure 4.20). The effect of all the polymeric blends on particle size was found highly significant ($p < 0.1$) at 1 % level. The particle size of the encapsulated powder was similar to the findings reported by Jafari *et al.* (2008) where in they reported 38 to 63 µm for fish oil encapsulated powder.

4.4.5 Optimization of Polymeric Blend for Encapsulated Powder

The optimized polymeric blend obtained from statistical analysis using factorial CRD in the form of ramps are presented in Figure 4.21. The optimized polymeric blend obtained was sodium alginate-agar agar of ratio 75:25, with encapsulation efficiency of 79.40 % which was higher than control (78.63 %).

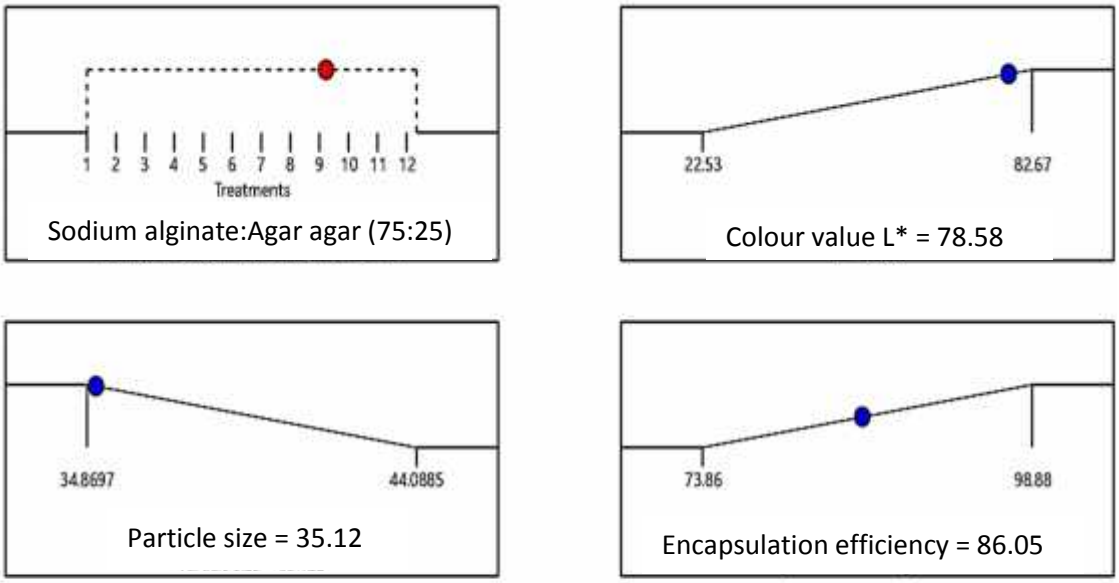


Figure 4.21 Optimized polymeric blend

CHAPTER V

SUMMARY AND CONCLUSIONS

Spices are an important part of the human diet used for seasoning and as a preservative. Apart from improving flavour and taste of food, spices are also recognised for their therapeutic properties because of their bioactive compounds. Importance of spices and their bioactive compounds are now slowly re-emerging as an area of interest for improving human health which not only provides nutrition but also helps in preventing and curing of diseases and disorders. Essential oils and oleoresins of spices contain volatile chemical compounds extracted from different parts i.e. leaves, roots, bark, seeds, flowers etc. These bioactive compounds mainly contain hydrocarbons, terpenes, ketones, esters, aldehydes, acids etc. The secondary metabolites of these compounds exhibit antimicrobial, antiviral, antioxidant, anticancer, hyperlipidaemic, antiulcer properties. *Anethum graveolens*, commonly known as dill is an annual aromatic herb which belongs to the family Umbelliferae (Apiaceae). The Indian dill plant is cultivated throughout India, chiefly in Punjab, Uttar Pradesh, Gujarat, Maharashtra, Assam and West Bengal, which is known as “sowa". Both seeds and leaves of dill are valued as spices. Its essential oil is rich in terpenes like carvone and limonene which has got wide applications in food, flavour, pharmaceutical, detergent and soap, cosmetics and pesticide industries. Its oil is used for aromatherapy in traditional medicinal treatment.

Thermal damage is one of the main disadvantages in conventional grinding of spices that cause significant loss in their essential oil, flavour compounds and natural colour that results in production of inferior quality product. These hurdles of the conventional grinding can be overwhelmed by cryogenic grinding that produces high-quality spice powder with uniform particle size distribution. The low temperature maintained by the cryogens will captivate the heat generated in the pin mill during the grinding of the spices and retains essential compounds.

Encapsulation is a technique in which tiny droplets (solid, liquid or gas) are coated or entrapped within another homogeneous or heterogeneous polymeric continuous phase system. Encapsulation creates a physical barrier to the core material and protects its sensitive components. Food grade biopolymers like proteins and polysaccharides are used to create a suitable system for encapsulating, protecting and delivering lipophilic functional components. Encapsulation is of great importance and relevance in food and

flavour industries due to the virtue of its ability to protect sensitive food components against degradation reactions and loss of volatiles.

Keeping above importance and views in mind, a scientific study was undertaken with the following objectives:

Objectives

1. Physio-Chemical characterization of dill seed and solvent extracted dill seed oil
2. Optimization of cryogenic grinding parameters for the maximum retention of essential oil from dill seed
3. Optimization of encapsulation parameters for the extracted essential oil

Fresh, mature and uniform quality dill seed cultivar of variety Gujarat Suva-2 was procured. Physical (size, sphericity, bulk density, true density, angle of repose, porosity, coefficient of friction) and chemical properties (moisture, protein, crude fiber, crude fat, ash and carbohydrate) of dill seed were analysed. Dill seed oil was extracted using a Soxhlet apparatus and was analysed for its chemical properties. Cryogenic grinding of dill seed was carried out using a lab-scale cryogenic grinding system. Independent parameters viz. temperature (0 ° to -80 °C), feed rate (6, 7 and 8 kg/h) and sieve size (0.8 and 1.0 mm) were evaluated for various dependent parameters viz. grinding time, liquid nitrogen consumption, energy consumption, essential oil, moisture content and particle size. The essential oil of optimized dill seed powder was quantified for its marker compounds (carvone and limonene) using GC-MS. The essential oil obtained from the optimized dill seed powder was encapsulated using different ratios of polymeric materials. The encapsulated powder obtained was characterized for moisture, encapsulation efficiency, colour value and particle size.

Following conclusions were drawn from the present investigation:

1. The mean length, width and thickness of dill seed were found to be 4.78 ± 0.5, 1.41 ± 0.11 and 1.68 ± 0.38 mm, respectively. The average arithmetic mean diameter and geometric mean diameter observed were 2.62 ± 0.33 and 3.79 ± 0.00 mm, respectively. Other physical parameters observed were sphericity (0.79 ± 0.01), bulk density (420.71 ± 9.24 kg/m^3), true density (1130.3 ± 19.59 kg/m^3), porosity (59.56 ± 0.15 %), angle of repose (41.98 ± 0.43 °) and coefficient of friction (0.89 ± 0.09).

2. The dill seed were found to have moisture 12.22 ± 0.96 %, protein 14.03 ± 0.2 %, crude fat 7.3 ± 0.28 %, crude fibre 32.26 ± 0.2 %, ash 7.03 ± 0.48, carbohydrate 27.14 ± 0.52 % and essential oil 2 ± 0.08 %.

3. Dill seed oil extracted with petroleum ether yielded 7.3 ± 0.28 %. The chemical properties were found to be specific gravity (0.93 ± 0.03 at 30°C), refractive index (1.47 ± 0.01 at 20°C), acid value (1.23 ± 0.07 mg KOH/g), iodine value (213.70 ± 0.09), saponification value (183.26 ± 0.04 mg KOH/g), peroxide value (12.74 ± 0.07 meq/kg) and induction period 27.5 h at 130°C.

4. Dill seed oil extracted with hexane yielded 7.14 ± 0.29 %. The chemical properties were found to be specific gravity (0.92 ± 0.02 at 30°C), refractive index (1.47 ± 0.01 at 20°C), acid value (1.01 ± 0.057 mg KOH/g), iodine value (198 ± 0.04), saponification value (179.3 ± 0.09 mg KOH/g), peroxide value (10.41 ± 0.06 meq/kg) and induction period 33.69 h at 130°C.

5. Following conclusions were made from cryogenic grinding of dill seed

    a. Grinding time decreased from 7.1 to 4.72 min when the temperature decreased from 0 ° to -80 °C.

    b. Liquid nitrogen consumption increased from 1.71 to 3.29 kg as the temperature decreased from 0 ° to -80 °C.

    c. Energy consumption decreased from 0.30 to 0.23 kWh with decrease in grinding temperature from 0 ° to -80 °C.

    d. With the decrease in temperature of grinding from 0 ° to -80 °C, the essential oil content increased from 1.27 to 2.40 %.

    e. The moisture of dill seed powder increased from 7.76 to 9.86 % when grinding temperature decreased from 0 ° to -80 °C.

    f. The particle size of the dill seed powder increased from 0.22 to 0.30 mm as the grinding temperature decreased from 0 ° to -80 °C.

6. The optimized parameters obtained from the statistical analysis for production of essential oil-rich dill seed powder were -60 °C (grinding

temperature), 6 kg/h (feed rate) and 0.8 mm (particle size). All three independent variables showed high significance for grinding time, essential oil, moisture and particle size. However, the feed rate showed no significance for liquid nitrogen consumption, energy consumption. The sieve size showed no significance for the moisture of cryoground powder.

7. Dill seed essential oil extracted from optimized cryogenic parameters contained 27.63 % carvone and 7.21 % limonene.
8. Following conclusions were made for the encapsulated powder of dill seed essential oil.
    a. Five polymeric materials (sodium alginate, gum tragacanth, maltodextrin, agar agar and gelatine) at three different ratios (0.5 %, 1 % and 1.5 %) were evaluated for encapsulation of essential oil and the moisture, encapsulation efficiency, L*, a*, b* and particle size of encapsulated powder was found to vary from 2.01 to 3.94 %, 80.42 to 74.10 %, 22.58 to 82.41, -010 to 4.68, 7.08 to 35.46 and 35.01 to 44.07 µm.
    b. The optimized blend of polymeric ratio was found to be sodium alginate:agar agar at 75:25 ratio. All independent polymeric blends showed high significance for moisture, colour values and particle size.

Food processing industries are using encapsulated oils as one of the effective methods for preservation and ease of use of essential oil of spices. Due to the enormous health benefits of spice essential oils, the encapsulated powders can be added in supplementary, functional and speciality foods. Potential prospects of encapsulation include the use of low-temperature drying, effective spraying techniques for improving encapsulation efficiency and development of continuous encapsulation process system in order to avoid the loss of volatile compounds in spices.

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Appendix – I

Observation table for cryogenic grinding of dill seed at 25 °C

Replication	Feed rate (kg/h)	Sieve size (mm)	Grinding time (min)	Energy consumption (kWh)	Essential oil (%)	Moisture (%)	Particle size (mm)
R_1	6.00	0.80	8.09	0.40	0.80	7.63	0.224
R_2	6.00	0.80	8.12	0.40	0.80	7.13	0.224
R_3	6.00	0.80	8.20	0.40	0.70	7.54	0.223
R_1	7.00	0.80	7.59	0.40	0.80	7.30	0.225
R_2	7.00	0.80	6.00	0.40	0.80	7.46	0.226
R_3	7.00	0.80	7.56	0.40	0.70	7.51	0.226
R_1	8.00	0.80	7.57	0.40	0.60	7.90	0.229
R_2	8.00	0.80	7.54	0.40	0.70	7.49	0.228
R_3	8.00	0.80	7.50	0.40	0.60	7.56	0.228
R_1	6.00	1.00	7.56	0.40	0.60	7.24	0.226
R_2	6.00	1.00	7.51	0.40	0.60	7.62	0.228
R_3	6.00	1.00	8.03	0.40	0.70	7.45	0.228
R_1	7.00	1.00	7.57	0.40	0.60	7.44	0.231
R_2	7.00	1.00	7.53	0.40	0.50	7.60	0.232
R_3	7.00	1.00	7.46	0.40	0.50	7.21	0.232
R_1	8.00	1.00	7.41	0.30	0.50	7.38	0.235
R_2	8.00	1.00	7.46	0.30	0.60	7.52	0.236
R_3	8.00	1.00	7.51	0.40	0.60	7.64	0.235

Appendix – II

Observation table for cryogenic grinding of dill seed at 0 °C

Replication	Feed rate (kg/h)	Sieve size (mm)	Grinding time (min)	Liquid nitrogen consumption (kg)	Energy consumption (kWh)	Essential oil (%)	Moisture (%)	Particle size (mm)
R_1	6.00	0.80	7.20	1.79	0.30	1.40	7.83	0.23
R_2	6.00	0.80	7.15	1.78	0.30	1.20	7.55	0.23
R_3	6.00	0.80	7.17	1.79	0.30	1.20	7.90	0.23
R_1	7.00	0.80	6.59	1.79	0.30	1.40	8.01	0.24
R_2	7.00	0.80	6.55	1.78	0.30	1.40	7.70	0.23
R_3	7.00	0.80	7.02	1.79	0.30	1.20	7.92	0.23
R_1	8.00	0.80	6.55	1.74	0.30	1.40	7.98	0.24
R_2	8.00	0.80	6.57	1.74	0.30	1.40	7.65	0.24
R_3	8.00	0.80	6.54	1.75	0.30	1.20	7.84	0.24
R_1	6.00	1.00	7.12	1.76	0.30	1.20	7.96	0.23
R_2	6.00	1.00	7.09	1.75	0.30	1.40	7.66	0.23
R_3	6.00	1.00	7.01	1.75	0.30	1.20	7.54	0.23
R_1	7.00	1.00	6.55	1.73	0.30	1.20	7.87	0.24
R_2	7.00	1.00	6.52	1.74	0.30	1.40	7.69	0.24
R_3	7.00	1.00	6.59	1.73	0.30	1.20	7.92	0.24
R_1	8.00	1.00	6.49	1.71	0.30	1.20	7.81	0.25
R_2	8.00	1.00	6.38	1.71	0.30	1.20	8.03	0.25
R_3	8.00	1.00	6.40	1.70	0.30	1.20	7.96	0.25

Appendix – III

Observation table for cryogenic grinding of dill seed at -20 °C

Replication	Feed rate (kg/h)	Sieve size (mm)	Grinding time (min)	Liquid nitrogen consumption (kg)	Energy consumption (kWh)	Essential oil (%)	Moisture (%)	Particle size (mm)
R_1	6.00	0.80	6.33	2.18	0.30	1.60	8.32	0.23
R_2	6.00	0.80	6.42	2.17	0.30	1.60	8.12	0.23
R_3	6.00	0.80	6.37	2.17	0.30	1.60	7.83	0.23
R_1	7.00	0.80	6.41	2.13	0.30	1.60	8.43	0.24
R_2	7.00	0.80	6.38	2.13	0.30	1.60	8.18	0.24
R_3	7.00	0.80	6.43	2.11	0.30	1.40	8.15	0.24
R_1	8.00	0.80	6.42	1.99	0.30	1.40	8.43	0.25
R_2	8.00	0.80	6.40	1.99	0.30	1.60	8.63	0.25
R_3	8.00	0.80	6.37	2.05	0.30	1.40	8.22	0.25
R_1	6.00	1.00	6.45	2.15	0.30	1.40	7.96	0.24
R_2	6.00	1.00	6.40	2.08	0.30	1.60	8.09	0.24
R_3	6.00	1.00	6.41	2.01	0.30	1.60	7.90	0.24
R_1	7.00	1.00	6.39	2.12	0.30	1.40	8.11	0.25
R_2	7.00	1.00	6.35	2.03	0.30	1.40	8.26	0.25
R_3	7.00	1.00	6.40	2.07	0.30	1.20	8.24	0.25
R_1	8.00	1.00	6.31	2.01	0.30	1.40	8.43	0.26
R_2	8.00	1.00	6.34	1.98	0.30	1.20	8.37	0.26
R_3	8.00	1.00	6.28	2.05	0.30	1.40	8.29	0.26

Appendix – IV

Observation table for cryogenic grinding of dill seed at -40 °C

Replication	Feed rate (kg/h)	Sieve size (mm)	Grinding time (min)	Liquid nitrogen consumption (kg)	Energy consumption (kWh)	Essential oil (%)	Moisture (%)	Particle size (mm)
R_1	6.00	0.80	6.30	2.17	0.30	1.80	8.66	0.24
R_2	6.00	0.80	6.42	2.21	0.30	1.60	8.49	0.24
R_3	6.00	0.80	6.31	2.25	0.30	1.80	8.79	0.24
R_1	7.00	0.80	6.22	2.26	0.30	1.60	8.58	0.25
R_2	7.00	0.80	6.32	2.29	0.30	1.80	8.73	0.25
R_3	7.00	0.80	6.35	2.30	0.30	1.60	8.98	0.25
R_1	8.00	0.80	6.33	2.35	0.20	1.60	8.94	0.26
R_2	8.00	0.80	6.28	2.31	0.30	1.60	8.72	0.26
R_3	8.00	0.80	6.31	2.32	0.30	1.40	8.79	0.26
R_1	6.00	1.00	6.25	2.28	0.30	1.80	8.47	0.25
R_2	6.00	1.00	6.28	2.24	0.30	1.80	8.61	0.25
R_3	6.00	1.00	6.19	2.29	0.30	1.80	8.52	0.25
R_1	7.00	1.00	6.21	2.23	0.30	1.60	8.74	0.26
R_2	7.00	1.00	6.15	2.21	0.20	1.80	8.60	0.26
R_3	7.00	1.00	6.25	2.23	0.30	1.80	8.76	0.26
R_1	8.00	1.00	6.20	2.22	0.30	1.60	8.87	0.27
R_2	8.00	1.00	6.19	2.16	0.20	1.40	8.85	0.27
R_3	8.00	1.00	6.17	2.15	0.20	1.40	8.94	0.27

Appendix – V

Observation table for cryogenic grinding of dill seed at -60 °C

Replication	Feed rate (kg/h)	Sieve size (mm)	Grinding time (min)	Liquid nitrogen consumption (kg)	Energy consumption (kWh)	Essential oil (%)	Moisture (%)	Particle size (mm)
R_1	6.00	0.80	5.57	2.56	0.20	2.40	8.98	0.25
R_2	6.00	0.80	5.59	2.60	0.30	2.20	9.17	0.25
R_3	6.00	0.80	6.01	2.59	0.30	2.20	9.23	0.25
R_1	7.00	0.80	6.00	2.63	0.20	2.00	9.33	0.27
R_2	7.00	0.80	5.54	2.63	0.20	1.80	9.56	0.27
R_3	7.00	0.80	5.51	2.60	0.20	1.80	9.40	0.26
R_1	8.00	0.80	5.47	2.61	0.20	1.80	9.35	0.28
R_2	8.00	0.80	5.52	2.65	0.30	1.80	9.58	0.28
R_3	8.00	0.80	5.50	2.59	0.30	1.60	9.31	0.28
R_1	6.00	1.00	6.05	2.54	0.20	1.80	8.86	0.26
R_2	6.00	1.00	5.55	2.52	0.20	2.00	8.93	0.26
R_3	6.00	1.00	5.59	2.60	0.20	1.80	9.03	0.26
R_1	7.00	1.00	5.56	2.54	0.20	2.00	9.02	0.28
R_2	7.00	1.00	5.49	2.60	0.20	1.80	9.49	0.29
R_3	7.00	1.00	5.51	2.49	0.20	2.00	9.27	0.29
R_1	8.00	1.00	5.44	2.48	0.20	1.80	9.20	0.31
R_2	8.00	1.00	5.41	2.50	0.20	2.00	9.55	0.31
R_3	8.00	1.00	5.36	2.51	0.20	1.80	9.43	0.31

Appendix – VI

Observation table for cryogenic grinding of dill seed at -80 °C

Replication	Feed rate (kg/h)	Sieve size (mm)	Grinding time (min)	Liquid nitrogen consumption (kg)	Energy consumption (kWh)	Essential oil (%)	Moisture (%)	Particle size (mm)
R_1	6.00	0.80	5.33	3.26	0.20	2.40	9.57	0.27
R_2	6.00	0.80	5.39	3.26	0.20	2.20	9.86	0.27
R_3	6.00	0.80	5.41	3.25	0.20	2.20	9.49	0.27
R_1	7.00	0.80	5.37	3.22	0.20	2.40	9.93	0.29
R_2	7.00	0.80	5.40	3.24	0.30	2.20	9.95	0.29
R_3	7.00	0.80	5.33	3.19	0.20	2.20	9.71	0.29
R_1	8.00	0.80	5.25	3.31	0.20	2.20	9.96	0.31
R_2	8.00	0.80	5.14	3.27	0.20	2.00	10.05	0.31
R_3	8.00	0.80	5.22	3.28	0.20	2.00	9.85	0.31
R_1	6.00	1.00	5.27	2.87	0.20	1.80	9.75	0.29
R_2	6.00	1.00	5.21	2.85	0.20	2.00	9.60	0.28
R_3	6.00	1.00	5.30	2.83	0.20	1.80	9.47	0.28
R_1	7.00	1.00	5.19	2.88	0.20	2.00	9.79	0.31
R_2	7.00	1.00	5.24	2.89	0.20	2.00	9.84	0.31
R_3	7.00	1.00	5.25	2.92	0.20	2.20	9.92	0.31
R_1	8.00	1.00	5.03	2.85	0.20	2.00	9.83	0.33
R_2	8.00	1.00	4.59	2.95	0.20	1.80	9.90	0.33
R_3	8.00	1.00	4.54	2.87	0.20	1.80	9.84	0.33