PHYTOCHEMICAL, ANTIOXIDANT AND SENSORY ANALYSIS OF BASIL SEED BEVERAGE INCORPORATED WITH BASIL SEED GUM AS A HYDROCOLLOID

by

Rabina B.K

Department of Food Technology

Central Campus of technology

Institute of Science and Technology

Tribhuvan University, Nepal

2024

Phytochemical, Antioxidant and Sensory Analysis of Basil Seed Beverage Supplemented with Basil Seed Gum as a Hydrocolloid

A dissertation submitted to the department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of B. Tech. Food Technology

by

Rabina B.K

Department of Food Technology

Central Campus of Technology, Dharan

Institute of Science and Technology

Tribhuvan University, Nepal

December, 2024

Tribhuvan University

Institute of Science and Technology

Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This ***dissertation*** entitled ***Phytochemical, Antioxidant and Sensory Analysis of Basil Seed Beverage Supplemented with Basil Seed Gum as a Hydrocolloid*** presented by **Rabina B. K** has been accepted as partial fulfillment of the requirement for the **B. Tech. Food Technology.**

Dissertation Committee

1. **Head of the Department** _____
   (Mr. Navin Gautam, Asst. Prof.)

2. **External Examiner** _____
   (Mr. Raj Kumar Rijal, Senior Food Scientist.)

3. **Supervisor** _____
   (Mrs. Babita Adhikari, Assoc. Prof.)

4. **Internal Examiner** _____
   (Mr. Navin Gautam, Asst. Prof.)

December 18, 2024

Acknowledgements

First and foremost, I would like to express my deep gratitude to my respected supervisor Assoc. Prof. Mrs. Babita Adhikari Dahal for her excellent guidance, constant support, encouragement and constructive recommendations throughout the work.

I am also grateful to Asst. Prof. Navin Gautam, HOD, Department of Food Technology, Assoc. Prof. Dr. Dil kumar limbu, Campus Chief, Central Campus of technology, Prof. Basanta kumar Rai, HOD, Central Department of Food Technology, for their kind support and valuable suggestions and also for making available all facilities to carry out the dissertation work. I am extremely thankful to all teaching faculties in the Central Campus of Technology for providing me the knowledge that made me competent enough to carry out and complete this work.

Sincere gratitude to Asst. prof. Damber Bahadur Khadka, for letting me use his lab equipment and chemicals during the work. I am highly indebted to Mr. Om Nath Khatiwada, Head of CCT Library whose expertise was of great aid for the dissertation. I am also grateful to Mr. Mahesh Shrestha, Mr. Sachin Basnet and all other lab personnels for their assistance during the work and all campus employees who never hesitated to help whenever required either during campus days or during dissertation work.

I am also grateful to my seniors Sunita karki, Saurav Pokharel, Prajwal Dulal, Rabu Thapa Magar, Nisha Marattha and my friends Kushum Thapa, Sandeep Khatri, Preeti Limbu, Biju Shrestha, Samyog Sangraula, Mohit Khadka, Rijan Rai, Merilung Limbu, Utsav Pokhrel, Pramisha Khatiwada, Bhawana Basyal, Lochan Mishra and Pooja Pokhrel for their immense support and help during the work.

Finally, words cannot adequately express the support, encouragement, love and inspiration that I have got from my parents, grandparents and all other family members. They have been my greatest supporter during the days of triumphs and disasters. Apologies to those whom I might have missed to acknowledge and sincere thankfulness to all the hands behind the work.

Date of submission: December 18, 2024

(Rabina B. K)

Abstract

This study was carried out with the objective to prepare Basil seed (*Ocimum basilicum*) beverage and analyze its phytochemical, antioxidant and sensory parameters. BSB samples A(control), B, C, D were prepared with addition of various proportion of BSG (0%, 0.1%, 0.2% and 0.3%, respectively). Basil seed, control and the selected BSB were subjected to organic solvent(methanol) for methanolic extraction. The procedure used for extraction was maceration (48 h for TPC and TFC and 24 h for antioxidant activity at 27 °C). major phytochemicals (phenol and flavonoid) content along with DPPH radical scavenging activity were determined for all prepared extracts. The sensory analysis was carried out for color, taste, appearance, mouthfeel and overall acceptability of four BSB samples using 9-point hedonic scale rating test. Analysis of variance (ANOVA) was done and Tukey's honesty test was performed using Genstat (Genstat Discovery Edition 12, 2009) to check the significant relationship between the mean values of the samples at 5% level of significance.

From ANOVA, sample B with 0.1% BSG was obtained as the best among the four samples. The moisture, protein, fat, ash, crude fiber and carbohydrate content of the basil seeds were found to be 5.50%, 21.86%, 28.05%, 4.58%, 37.95% and 7.56% consecutively. The BSB sample A (without BSG) and sample B (best sample with BSG, 0.1%) were found to have TSS, Ph, acidity, vitamin C and sedimentation height of 11Bx, 3.43, 0.286%, 6.067 mg/100g and 57.14% and 11 °Bx, 3.28, 0.273, 6.537 mg/100g and 96.49%, respectively. The WHC of BSG was 68.09 g/g BSG. From the methanolic extract of basil seed, TPC, TFC, and antioxidant activity as DPPH RSA were found to be 25.010 mg GAE/g seed sample (DM), 11.635 mg QE/g seed sample (DM) and 78.94%, respectively. Likewise, TPC, TFC and DPPH RSA of sample A and sample B was found to be 476.588 µg GAE/ml BSB, 73.409 µg QE/ml BSB and 45.64% and 568.892 µg GAE/ml BSB, 86.790 µg QE/ml BSB and 49.16%, consecutively. Results indicated that basil seeds are high in nutrition and biochemical compounds as claimed in the work of other researchers and BSB sample with 0.1% BSG showed significantly good sensory attributes and higher percentage of sedimentation height that confirms the hydrocolloid property of BSG. Furthermore, in comparison to sample A sample B with BSG (0.1%) depicted higher antioxidant activity, TPC and TFC.

Contents

Approval Letter…… ii

Acknowledgements…….. iii

Abstract…… iv

List of tables…….. ix

List of figures…… x

List of abbreviations…….... xi

1. Introduction……... 1-4

1.1 General introduction…… 1

1.2 Statement of the problem……. 2

1.3 Objective of the study…….. 3

1.3.1 General objective……... 3

1.3.2 Specific objectives…… 3

1.4 Significance of the work**……** 3

1.5 Limitations of the work……..... 4

2. Literature review…… 5-21

2.1 General introduction of basil and basil seed…… 5

2.1.1 Basil……... 5

2.1.2 Basil seeds……. 6

2.2 Benefits of basil seeds…… 7

2.3 Composition of basil seeds……. 8

2.4 Basil as functional food…… 11

2.4.1 Concept of functional food……. 11

2.4.2 Polyphenolic compounds in basil seed……... 11

2.4.3 Flavonoids in basil seed……...…….. 12

2.4.4 Antioxidant capacity in basil seed…….. 14

2.5 Factors affecting nutritional and phytochemical composition of basil seed….. 14

2.5.1 Cultivar effect…….. 15

2.5.2 Geographical and environment condition……... 15

2.5.4 Others…….. 15

2.6 Uses of basil seeds……. 16

2.7 Basil seed gum and its application…… 16

2.8 Basil seed beverage……... 18

2.8.1 Beverages……. 18

2.8.2 Basil seed beverage……. 18

2.9 9-Point hedonic scale reading…… 21

3. Materials and methods……. 22-30

3.1 Raw materials……... 22

3.1.1 Basil seed…… 22

3.1.2 Mangoes…….. 22

3.1.3 Packaging material…….. 22

3.1.4 Materials, equipment and chemicals…….. 22

3.2 Methods…… 22

3.2.1 Extraction of BSG…….. 22

3.2.2 Experimental design……... 23

3.2.3 Basil seed beverage preparation……. 25

3.2.4 Analysis of proximate of seeds…….. 26

3.2.4.1 Determination of moisture content...…… 26

3.2.4.2 Determination of crude fat…… 26

3.2.4.3 Determination of crude protein……. 26

3 .2.4.4 Determination of ash content……... 26

3.2.4.5 Determination of carbohydrate content……. 26

3.2.4.6 Determination of crude fiber……. 26

3.2.5 Analysis of BSG…….. 26

3.2.5.1 Water holding capacity of BSG…….. 26

3.2.6 Analysis of proximate of BSB…… 27

3.2.6.1 Determination of TSS…… 27

3.2.6.2 Determination of titratable acidity……... 27

3.2.6.3 Determination of pH……... 27

3.2.6.4 Determination of sugar……... 27

3.2.6.5 Determination of ascorbic acid…….. 27

3.2.6.6 Microbial analysis…….. 27

3.2.7 Preparation of extract for phytochemical analysis…….. 28

3.2.8 Analysis of phytochemicals……. 28

3.2.8.1 Analysis of Total Phenolic Content……... 28

3.2.8.2 Analysis of flavonoids……28

3.2.9 Determination of DPPH radical scavenging activity…… 29

3.2.10 Sensory analysis of BSB……. 30

3.2.11 Statistical analysis……... 30

4. Results and discussions……. 31-40

4.1 Sensory evaluation…… 31

4.1.1 Color……... 31

4.1.2 Taste…… 32

4.1.3 Mouthfeel…… 33

4.1.4 Appearance…….. 34

4.1.5 Overall acceptability……... 35

4.2 Proximate analysis……... 37

4.2.1 Proximate analysis of basil seed…… 37

4.2.2 Analysis of BSG……. 38

4.2.3 Analysis of BSB……. 38

4.3 Quantitative analysis of phytochemicals…….. 39

4.3.1 Total Phenolic Content (TPC)…… 39

4.3.2 Total Flavonoid Content (TFC)…….. 40

4.4 Antioxidant Activity**……..** 40

5. Conclusion and recommendations…… 42

6. Summary……. 43

References……... 44

Appendices…….. 52

List of Tables

Table No.	Title	Page No.
2.1	Taxonomic classification of sweet basil	6
2.2	Nutrition composition of basil seed	9
2.3	Mineral composition of basil seed	10
2.4	Polyphenols in basil seed	12
2.5	Flavonoid content of basil seed	13
3.1	Formulations for BSB	24
3.2	Amounts of reagents and extracts in cuvettes for DPPH assay	30
4.1	Sensory evaluation of BSB	31
4.2	Proximate composition of basil seed	37
4.3	Physio-chemical properties of BSB	39
4.4	TPC, TFC and antioxidant activity of basil seed and BSB	41
A.I	List of chemicals used	52
A.II	List of equipment used	53
C.I	One way ANOVA for color	55
C.II	One way ANOVA for taste	55
C.III	One way ANOVA for mouthfeel	56
C.IV	One way ANOVA for appearance	56
C.V	One way ANOVA for overall acceptability	57

List of Figures

Figure No.	Figure	Page No.
2.1	Preparation of BSB	20
3.1	Extraction of BSG	23
3.2	Preparation of BSB	25
4.1	Mean score for sensory evaluation of color	32
4.2	Mean score for sensory evaluation of taste	23
4.3	Mean score for sensory evaluation of mouthfeel	34
4.4	Mean score for sensory evaluation of appearance	35
4.5	Mean score for sensory evaluation of overall acceptability	36
D.I	Standard gallic acid curve	59
D.II	Standard quercetin curve	59

List of Abbreviations

Abbreviation	Full from
ABTS	2,2-Azino Bis-3-Ethylbezothiazoline-6-Sulfonic Acid
ANOVA	Analysis Of Variance
AOA	Antioxidant Activity
BSB	Basil Seed Beverage
BSG	Basil Seed Sum
BSM	Basil Seed Mucilage
CCT	Central Campus of Technology
CMC	Carboxy Methyl Cellulose
CUPRAC	Cupric Reducing Antioxidant Activity
Db	Dry basis
DM	Dry Matter
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
FOSHU	Food Safe for Health Use
FRAP	Ferric Ion Reducing Ability of Plasma
GAE	Gallic Acid Equivalent
GT	Gum Tragacanth
HOD	Head Of the Department
LDL	Low- Density Lipoprotein
OHC	Oil Holding Capacity
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
QE	Quercetin Equivalent

RCS	Reactive Chloride Species
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RSA	Radical Scavenging Activity
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
WHC	Water Holding Capacity

Part I

Introduction

1.1 General introduction

Basil (*Ocimum basilicum*) is one of the member of genus Ocimum comprising of 50 and 150 species of herbs and shrubs and it is native to tropical regions like Asia, Africa, Mediterranean regions, America, etc. (Nadeem *et al.*, 2020; Paton *et al.*, 1999; Simon *et al.*, 1999). The plant is widely used in in food, pharmaceuticals, cosmetics and perfume industries all around the world(Purushothaman *et al.*, 2018). It's leaves are popular in Italian cuisine and Southeast cuisines(Snoussi *et al.*, 2016)and are used in both fresh and dried forms. Both flowers and the whole plant are used for the production of essential oil (Pushpangadan and George, 2012) and the roots also has high value in medicine(Chopra and Nayar, 1956). The seeds are steeped in water and eaten or added to beverages and ice cream or in other food products, milled or whole, like in bakery products.(Hajmohammadi *et al.*, 2016; Pushpangadan and George, 2012; Rezapour *et al.*, 2016).

The seeds exhibit good nutritional and biochemical profile which makes it noteworthy source of nutrition. Along with good nutritional value, it has additional properties such as antidiabetic, anti-inflammatory, antioxidant, antimicrobial and so on (Bilal *et al.*, 2012; Mabood *et al.*, 2017; Nadeem *et al.*, 2020). The excellent nutritional composition, good functional properties and high dietary fiber content make it a great choice for enrichment of beverages and dairy and confectionary products(Munir *et al.*, 2017; Naji-Tabasi and Razavi, 2017a).

The enrichment of food products is the need of modern world in order to fulfill the nutritional requirement of every individual. Busy lifestyles in recent years have led to a preference for readily available foods, contributing to numerous health issues due to low dietary fiber intake and nutritional deficiencies(Capra, 2006). Scientists and food technologist all around the globe are striving for the enrichment and supplementation of readily available foods in order to help people lead a successful and healthy life(Alqahtani *et al.*, 2014).

Beverages are great medium for incorporation of nutrient rich material as they are in liquid state and popular among all age groups and basil seeds are excellent product to enrich foods as it is highly nutritious, cost-effective and easily available(Munir *et al.*, 2017). Basil seed beverage is a mixture of calculated proportion of soaked basil seeds with any kind of beverage, but preference to fruit juices makes it healthier and affordable. Basil seed incorporated beverages are gaining global recognition under various brand names. The drink is a healthier option to the plain juice brands with high sugar content and less nutritious components.

Despite being highly nutritious, the sedimentation of seeds upon storage decreases its sensory characteristics. Appearance is as important as flavor and texture for the quality of the product (Jaros *et al.*, 2000) and stability of basil seeds in the suspension of basil seed beverage(BSB) is a factor affecting its appearance. Hydrocolloids are required to stabilize the seeds in the suspension and nowadays, Carboxymethyl cellulose(CMC) is one of the popular hydrocolloid used as stabilizer in many beverages by food industries(Hajmohammadi *et al.*, 2016). However, research on use of several other hydrocolloids can be found, for the quest of the best. Likewise, in this study a new approach has been made by the use of gum extracted from basil seed itself that is, Basil seed gum (BSG). BSG acts as hydrocolloid to stabilize the seeds in the suspension in the BSB.

1.2 Statement of the problem

Increase in the number of health concerned people and their busy life have led to flourishing of the beverage industry and its international market especially, that of juices and smoothies. But most of these products are high in sugar content and lack proper nutrients, dietary fiber, minerals, and other bioactive compounds such as polyphenols, flavonoids and so on, which our body require for its proper regulation. There are few products in the market with proper nutrient but they are either unaffordable or inaccessible to the people of developing countries like Nepal.

Although basil seed beverage is the nutrient rich product with proper amount of macro and micronutrients and phytochemical content which is thriving in western and some eastern countries, in Nepal its availability and accessibility is low for which proper knowledge of its nutritional quality and good marketing is must. Basil is one of the underutilized plants in Nepal as per . As per papers and reviews around the global scientific community, the climate

of Nepal is favorable for basil cultivation but due lack of knowledge of its power packed nutrients, they have been underutilized.

Furthermore, the commercialization of BSB is flourishing in international market and for good marketing, industries use different stabilizers like CMC, GT, etc., for stability of seeds in the suspension to increase its sensory features. As technical communities involved in innovations related to food products, search for the best suited stabilizer (hydrocolloid) through various research is responsibility of all concerned personnels. In this paper, the similar effort is being tried to make.

1.3 Objective of the study

1.3.1 General objective

The general objective of this study is phytochemical, antioxidant and sensory evaluation of basil seed beverage supplemented with BSG as a hydrocolloid.

1.3.2 Specific objectives

1. To perform proximate analysis of basil (*Ocimum basilicum*) seeds.
2. To extract basil seed gum from basil seeds.
3. To prepare basil seed beverage for specific formulations with and without addition of BSG.
4. To perform sensory evaluation of the prepared basil seed beverages.
5. To perform proximate analysis of basil seed beverage.
6. To analyze WHC of BSG.
7. To perform phytochemical analysis of seeds and BSB.
8. To determine DPPH radical scavenging activity of basil seeds and basil seed beverage

1.4 Significance of the work

Interest of industries, health sciences, cosmetics, pharmaceuticals, etc., in medicinal and aromatic plants and plant materials is increasing due to their functional properties and benefits to human health. Basil seed is one of the kinds which has been in use in different regions of world for a long time and has been a subject of research, more of interest these days, in order to discover more benefits and application.

It is the duty of food technologists and scientists to assure nutrient rich products are available and accessible to the people. The product which can compete in international market both in terms of nutritional benefits and sensory characteristics to sustain in the market and benefit the consumers. Studies have strongly suggested that consumption of certain plant materials such as seeds, leaves, fruits and roots may reduce the risk of chronic diseases related to oxidative stress on account of their antioxidant activity and promote health benefits(Ramarathnam *et al.*, 1995). Basil seed beverage is one of the food products which contains basil seed that is highly nutritious and beneficial for various health aspect. The present study not only focus on a functional drink but also on its sensory attributes, majorly on its appearance which is affected by the stability of the seeds on the suspension. For the stability, BSG as a hydrocolloid extracted from the basil seed itself is used in the current work.

1.5 Limitations of the work

- Shelf life of the product was not estimated.
- Only one variety of basil seed (*Ocimum basilicum*) was used.
- Only one organic solvent was used for the extraction of phytochemicals.

Part II

Literature review

2.1 General introduction of basil and basil seed

2.1.1 Basil

Basil (*Ocimum basilicum)* is an annual aromatic herb of lamiaceae family. According to Bilal *et al.* (2012), it is known as basil, common basil or sweet basil in English whereas in Hindi and Bengali, its called Babui Tulsi. The plant is known as Badrooj, Hebak or Rihan in Arabic; as Tohrakhurasani and Okimon in Persian and Unani languages. The plant is referred as Barbari by Bhava misra, a famous Ayurvedic Acharya. It is also mentioned in some classical Ayurvedic texts like Susruta Samhita, Charak shamhita, Ashtangahrdayam, etc(Purushothaman *et al.*, 2018).

It is a cosmopolitan plant which flourishes well in fairly to high rainfall, humid condition and high or moderate temperature which implies tropical and sub- tropical climate are perfect for basil cultivation. The plant prefers warm climate like that of Iran, India, Africa but are also commercially cultivated in France, Greece, other southern European countries as well as in north and south America. Cultivation of the plant is majorly done for the production of seeds and aromatic leaves(Prakasa Rao *et al.*, 2011).

The plant can thrive in variety of soil type, for an instance, rich loam to poor laterite and saline or alkaline to moderately acidic. Basil is glabrous herb with white or pale purple flowers but number of subspecies and varieties are flourishing due to polymorphism and cross pollination. The plantation of basil can be carried out by nursery sowing, direct sowing or through transplantation (Pushpangadan and George, 2012). The botanical classification of sweet basil (Ocimum basilicum L.) is shown in the **Table 2.1**.

As per the study of Calderón Bravo *et al.* (2021), this herb has been in use from ancient times, the leaves can be used to add distinctive flavor and aroma to foods, beverages, liqueurs, cheese, etc. The essential oil from the flowers and leaves can be used in pharmaceuticals, cosmetics and perfume industries, the stem and bark can be used in the production of essential oils to be used in different products and the flowers and fresh leaves

are used in different cuisines for decoration purpose. The seeds have been used in ancient medicine and as food in south Asian regions.

Table 2.1 Taxonomic classification of sweet basil

Taxonomic classification	
Kingdom:	Plantae—plants
Sub-kingdom:	Tracheobionta
Super-division:	Spermatophyta
Division:	Magnoliophyte
Class:	Magnoliopsida
Sub-class:	Ateridae
Order:	Lamiales
Family:	Lamiaceae
Genus:	Ocimum L.
Species:	Basilicum
Binomial name:	Ocimum basilicum (sweet basil)

Source: Pushpangadan and George (2012)

2.1.2 Basil seeds

Basil (*Ocimum basilicum*) seeds are tiny, ellipsoid and black seeds which are rich in fiber, protein, minerals, antioxidants and polyphenolic compounds(Calderón Bravo *et al.*, 2021). The seeds vary in size depending on the region of origin. Kišgeci *et al.* (2011) reported significant difference in terms of length, thickness and width in the basil seeds planted in different regions of Serbia. Calderón Bravo *et al.* (2021) suggests the length, width and thickness of the seeds ranges between (2.31 - 3.11) mm, (1.3 - 1.82) mm and (0.99 - 1.34) mm respectively. Choi *et al.* (2020) found out there was increase in moisture content with increased size of the basil seeds. The geographic origin impacts the nutritional composition

too, as variation in composition is observed in many studies among the seeds from different region of origin.

The seeds are porous as described by Uematsu *et al.* (2020)through SEM in Thai basil seeds. When the seeds are soaked in water, they swell up and produce gelatinous mass because of the poly saccharide layer present on the outer epidermis wall of the seeds(Azuma *et al.*, 2003). The polysaccharide when extracted from the seeds, which also can be called as basil seed gum(BSG),has high values in food, pharmaceutical and other industries as they offer great potential for use as gelling, binding, thickening, foaming and stabilizing agent(Naji-Tabasi and Razavi, 2017a) and its various other uses are being studied by researchers all around the globe. The seeds are packed with nutrient rich compounds like protein, lipids (especially ALA i.e. Alpha Linoleic Acid), polyphenols, flavonoids, dietary fiber etc.

2.2 Benefits of basil seeds

The consumption of basil seeds stands out not only for its nutritious value but also for its significant health benefits, such as antidiabetic, antimicrobial, antioxidant, anticancer activities etc., due to presence of significant amount of phytochemicals(Cherian, 2019; Gajendiran *et al.*, 2016).

- The seeds have been used in traditional medicine to treat colic ulcer, dyspepsia, diarrhea and inflammations.
- High fiber content of the seed makes it a non -conventional source of fiber (Mathews *et al.*, 1993)and its incorporation in food with low fiber content such as plain juice, bakery and desserts can make these food more healthier and nutritious.
- Phenolic compounds with strong antioxidant properties have been identified in the seed's extracts. The seed consists of different biological compounds known for their biological activity which act as antioxidants and can be included in food products to prevent oxidative deterioration or incorporated to enrich the foods functional properties (Javanmardi *et al.*, 2003; Mabood *et al.*, 2017; Sarfraz *et al.*, 2011).
- Gajendiran *et al.* (2016) demonstrated antimicrobial properties of basil seed against nine clinical pathogens (*Staphylococcus aureus, Enterococcus spp., Proteus mirabilis, Shigella dysenteriae, Salmonella spp., Klebsiella pneumoniae, Serratia marcescens, and Pseudomonas aeruginosa*) where different level of activity was

observed against all the pathogens except for *Bacillus subtilis* and *Micrococcus luteus*.

- The antimicrobial properties of basil seed, it's gum in meat as coating or its essential oil were studied by several researchers like Singh and Majumdar (1995),Majdinasab *et al.* (2020), Adamu *et al.* (2005) etc.
- In the study by Idris *et al.* (2020), it was found that the seed is rich in essential fatty acid such as linoleic acid (LA) and α-linolenic acid (ALA).The seed is economic and potential source of omega-3 fatty acid i.e. ALA among the plant source.
- There is presence of alkaloids, flavonoids, terpinoids, steroids, etc. in the petroleum extract of the basil seed which are reported to have anti-inflammatory, analgesic, antipyretic properties and hypoglycemic activities (Bever, 1980; Manach *et al.*, 1996).
- The anticancer property of the seeds was confirmed by evaluation of cytotoxic effect on human osteosarcoma cell lines (Gajendiran *et al.*, 2016).

2.3 Composition of basil seeds

There have been numerous papers published about the composition of basil seeds, including those of Razavi *et al.* (2009), Nazir *et al.* (2021) , Bilal *et al.* (2012), Mathews *et al.* (1993),Gajendiran *et al.* (2016). Basil seed is considered to possess generous percentage of carbohydrate, lipid, protein, minerals and to be magnificent source of fiber and ALA which comes from high level of omega – 3 fatty acid(Gajendiran *et al.*, 2016). Data for the proximate composition is shown in **Table 2.2** as per Nazir *et al.* (2021) and that for the mineral composition is shown in **Table 2.3** as per Khursheed *et al.* (2023).

Table 2.2 Nutritional composition of basil seed (% wet basis)

Parameter	Percentage
Moisture	8.90 ± 0.11
Protein	9.40 ± 0.08
Fat	33.01 ± 0.61
Ash	5.20 ± 0.02
Carbohydrate	43.50 ± 0.12
Crude fiber	36.30 ± 0.90

Source: Nazir *et al.* (2021)

Review of literatures shows that the composition of basil seeds is variable depending on different factors like, cultivar, geographical region, environment condition, soil type and use of fertilizers and manures, harvesting conditions, post-harvest storage, etc.(Calderón Bravo *et al.*, 2021; Pushpangadan and George, 2012). For instance, according to Razavi *et al.* (2009), the chemical composition of basil seeds from India was found to be 9.63% moisture, 14.76% protein, 13.8% fat, 7.7%ash and 63.8% carbohydrate while the compositions of the seeds from different region of Iran were found to be 5-6.5% moisture, 17-20% protein, 21-23% fat, 4-5.7% and 47-50% carbohydrate. The mineral composition of seeds has been reported only by few researchers. The data recorded by Munir *et al.* (2017) is found to be similar with the mineral composition as reported by Khursheed *et al.* (2023) as shown in **Table 2.3**. It is also evident that the seeds are good source of vitamins with the presence of thiamine, riboflavin, niacin, vitamin C, vitamin A, tocopherol and others (Khursheed *et al.*, 2023).

Table 2.3 Mineral composition of basil seed (mg/100g)

Mineral	Content(mg/100g)
Ca	636± 0.17
Fe	2.27± 0.03
Mg	31.55± 0.29
P	19.05± 0.07
K	481± 0.24
Zn	1.58± 0.01
Mn	1.01±0.05
Cu	1.21± 0.07

Source: Khursheed *et al.* (2023)

The fatty acid profile and amino acid composition of basil seed are quite considerable. The amino acid components of the seeds are aspartic acid, glutamic acid, glycine, serine, arginine, alanine, histidine, threonine, tyrosine, proline , valine, leucine, cysteic acid, isoleucine, lysine and others; glutamic acid and arginine being major amino acids while serine being the limiting one and tryptophan and cysteic acid were found to be deficient in basil seeds(Calderón Bravo *et al.*, 2021; Khursheed *et al.*, 2023). As for the fatty acid profile, basil seeds are rich in unsaturated fatty acids followed by monounsaturated fatty acid and saturated fatty acid. Omega-3 fatty acids i.e. alpha- linolenic acid is one the major PUFA (polyunsaturated fatty acid) present in basil seed which is considered to be crucial for normal brain functioning. Other major PUFAs present in basil seeds are linoleic acid and arachidic acid and for saturated fatty acid palmitic acid and stearic acid are the major ones.

2.4 Basil as functional food

2.4.1 Concept of functional food

The term functional food was first coined in 1984, in Japan where functional food has a formal legislative food category called FOSHU (food for specific health use). Food needs to satisfy three requirements to qualify for FOSHU, viz., (1) effectiveness in clinical studies (2) safety in clinical and non- clinical studies, and (3) presence of active/effective components.

All foods are generally functional as they provide nutrients and energy to grow and support cellular activities and there is no universal definition of functional food (Roberfroid, 2000). Functional food, however, is generally considered to go beyond the provision of basic nutrients to potentially provide additional benefits such as reducing risk of disease and/or furnishing optimal health to the consumer(Hasler, 2002). Few terms comparable to functional food has been introduced lately, viz., nutraceuticals, pharmafoods, vitafoods, dietary supplements, fortified foods and designer foods(Martirosyan *et al.*, 2015). Among all, nutraceutical is often used interchangeably with functional food in scientific papers while medical foods are used in treatment of disease or metabolic problems. Lastly, dietary supplements are meant to provide extra health benefits beyond basic nutrition. And all these confusing nutritional vocabulary has created complication in consumers understanding(Roberfroid, 2000).

Basil seeds have the recognition of being a functional food because of its components with health promoting and medicinal value such as polyphenols, ALA, mucilage, antioxidants etc. (Calderón Bravo *et al.*, 2021; Cherian, 2019; Nazir *et al.*, 2021). In addition, the mucilage from the basil seeds i.e. BSG is considered to have prebiotic properties as per Shahmoradi *et al.* (2023)

2.4.2 Polyphenolic compounds in basil seed

Polyphenols are the widely groups of natural products in the plant kingdom, the secondary metabolites, that plant produce to protect themselves from other organisms (Veberič, 2010). They are the type of chemical compounds with a hydroxyl group (-OH) that is directly linked to an aromatic hydrocarbon group (Altıok, 2010). Plant phenolics are the major groups of compounds acting as antioxidants or free radical scavenging agents(Wang *et al.*,

1996). These compounds has antioxidant, anti-inflammatory, antiulcer, anticancer, antispasmodic, antibiotic and several other properties(Ghasemzadeh *et al.*, 2010).

As per Khursheed *et al.* (2023),the major phenolic compounds found in basil seeds are chlorogenic acid, caffeic acid and gallic acid along with the presence of rosamaric acid, ferulic acid, p-coumaric acid and others. Chlorogenic acid protects against free radicals and inhibit the peroxidation of fats making it a great antioxidant and caffeic acid is believed to inhibit low density lipoprotein (LDL) oxidation protecting against cardiovascular diseases.s

According to Khursheed *et al.* (2023) the polyphenols in basil seeds are as follows:

Table 2.4 Polyphenols in basil seeds

Major polyphenols	Composition (µg GAE/g)
Chlorogenic acid	2275.37
Caffeic acid	4750.01
Gallic acid	2330.52
Rosameric acid	353
Minor polyphenols	
Ferulic acid	40.12
P-coumaric acid	112.1
Epicatechin	9.5

2.4.3 Flavonoids in basil seed

Flavonoids are group of hydroxylated polyphenolic compounds having benzo-pyrone structure and are secondary metabolites which are ubiquitously present in plants(Kumar and

Pandey, 2013). They are subgroup of polyphenols and can be most commonly classified into the classes as; flavanones, flavones, flavanols, catechins(flavan-3-ols), isoflavones and anthocyanidins. Some of the important examples of flavonoids are quercetin, rutin, catechins and anthocyanins. Anthocyanins are the most widely distributed pigment groups in the plant kingdom which are responsible for colors in plant(Schwartz *et al.*, 2017). The main sources of flavonoids are tea, berries, red wine, apples, etc. Flavonoids has different beneficial properties and some of them are;

- Antioxidant activity
- Ability to chelate metals such as Fe, Cu etc.
- Ability to scavenge ROS (reactive oxygen species)
- Ability to inhibit nitrosation.
- Ability to scavenge electrophiles.
- Capability to modulate certain cellular enzyme activities.

The protective effects of flavonoids in biological systems are ascribed to their capacity to scavenge ROS, chelate metal catalyst, activate antioxidant enzymes, inhibit oxidases, etc.(Heim *et al.*, 2002). ROS causes oxidative stress against which flavonoids acts either directly with their antioxidant activity or indirectly by stimulating the cell to up- regulate its endogenous antioxidant activity(Domínguez-Ávila *et al.*, 2017).

Papers regarding the flavonoids content of basil seeds are few. According to Khursheed *et al.* (2023), the flavonoids present in basil seeds are quercetin, kaempferol (rosaminiric acid) and rutin, the quantity of which is shown in **Table 2.5**. Cherian (2019) suggests the presence of vicenin, orietin and beta carotene in seeds.

Table 2.5 Flavonoid content of basil seed

Flavonoids	Composition (µg QE/g)
Quercetin	0.55
Kaempferol	0.34
Rutin	0.398

Source: Khursheed *et al.* (2023)

2.4.4 Antioxidant capacity in basil seed

Antioxidants are any substance that when present at low concentrations compared to those of an oxidizable substrate delays or prevents oxidation of that substrate. Antioxidants help body to protect itself against damage caused by reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive chlorine species (RCS) associated with degenerative diseases. The mechanism by which they work may vary depending on the compositional characteristics of the food(substrate), including its minor constituents(Shahidi and Naczk, 2003). The mechanism can include; removal of O_2, scavenging reactive oxygen/nitrogen species or inhibiting ROS/RNS formation, binding metal ions needed for catalysis of ROS generation, and up regulation of endogenous antioxidant defenses(Halliwell, 1996).

The antioxidant properties of basil seeds are mainly due to its phenolic and flavonoid content followed by other antioxidant metabolites like, carotenoids, volatile oils, and others(Gajendiran *et al.*, 2016). Several papers support the fact that higher amount of phenolic compounds and flavonoids results in higher antioxidant capacity as it is seen in green tea, black tea, apple, blueberries, etc. Intriguing findings were reported by Zamani Ghaleshahi *et al.* (2020), concerning tocopherol, which suggested that basil seeds contained significantly higher concentrations of α, β, γ-tocopherol when compared with flax and perilla seeds.

The antioxidant activity (AOA) or capacity of basil seeds around the globe differs depending on several factors like varieties, region of origin, environmental condition and others, and are found in the range of 34.2-968.49% as per several researchers(Akshatha *et al.*, 2019; Calderón Bravo *et al.*, 2021; Khursheed *et al.*, 2023; Sarfraz *et al.*, 2011). The property is mostly reported in terms of radical scavenging activity by DPPH assay. The *Ocimum basilicum* L. mostly shows AOA ranging between 30–85%(Gajendiran *et al.*, 2016; Sarfraz *et al.*, 2011).

2.5 Factors affecting nutritional and phytochemical composition of basil seed

There are various factors that affects the nutritional and phytochemical composition of basil seeds. As the plant is cosmopolitan in nature the composition will vary according to the geographical region of cultivation, the variety of the seed, the environmental condition and many more. Some of them era discussed below:

2.5.1 Cultivar effect

Different varieties of basil are cultivated around the globe according to the required traits to be retained when propagated. As per Darrah (1974),naturally there are seven different varieties of basil in addition to which different other species and varieties have been introduced through cross breeding, genetic modification etc. as for their culinary, ornamental and other purposes and as potential source of new aromas. For instance, a short duration crop with higher essential oil yield, CIM-Saumya, has been developed and likewise CIM-Snigda for distinct leaf morphology and CIM-Surabhi for unique chemical composition and high oil yield were developed too.

2.5.2 Geographical and environment condition

Variation in nutritional composition and bioactive components in basil seeds is seen according to the agronomic management (if any fertilizers are used or fortification is done during sowing), environmental condition (if the climate is extreme or moderate with high or low humidity), geographic location (if the location of cultivation is tropical, temperate region or other regions), altitude, soil properties, degree of water absorption by the plant and other various other environmental factors(Choi *et al.*, 2020; Munir *et al.*, 2017; Naji-Tabasi and Razavi, 2017a; Pushpangadan and George, 2012).

2.5.4 Others

There are several other factors and influencers that has effect on the composition of basil seeds which are illustrated as;

- The storage condition of the seeds such as the humidity, temperature of the storeroom and condition of packaging.
- The methods used for the analysis, as some methods might not give accurate result for a certain for some commodities but will do for others.
- As per the review by Calderón Bravo *et al.* (2021),several researchers reported that the solvent used for extraction also has high impact on analysis of biochemical compounds for an instance presence of more polar than nonpolar compounds gives higher yields in the result with methanol than with n-hexane.

2.6 Uses of basil seeds

Traditionally, basil seeds have been commonly used in medicine for their cooling and digestive properties which also regulate blood sugar, aid in weight loss efforts, relieves stress, lowers blood pressure, improve vision, lower cholesterol and reduce inflammation (Cherian, 2019). Its culinary use is seen in Asian, Middle eastern and Mediterranean cuisines. In many parts of Asia basil seeds are used in the preparation of traditional beverages and ice desserts like Falooda (Hosseini-Parvar *et al.*, 2010).

Recently the seeds are used as source of dietary fiber in beverages, bakery products and other food products by researchers like Munir *et al.* (2017), Hajmohammadi *et al.* (2016), Rezapour *et al.* (2016), Akshatha *et al.* (2019) and many other researchers. The seeds are used to extract essential oil as it is rich in ALA and for its pharmaceutical importance too. In Arabic countries the seeds are mostly being researched for its polysaccharide i.e. BSG which has wide range of uses as hydrocolloid, foaming agent, fat replacer, encapsulating agent etc. which are discussed in detail in the next section i.e. 2.7. There are research papers where incorporation of basil seed powder in different food products such as in ice -cream, baguette, and others (James, 2020; Rezapour *et al.*, 2016) in order to increase the food value which open s the new doors for proper utilization and commercialization of basil seeds and products with its incorporation.

2.7 Basil seed gum and its application

Basil seed gum is a plant derived, high molecular weight, surface-active hydrocolloid that imparts pseudo-plastic and viscous behavior (Naji-Tabasi and Razavi, 2017b). When soaked in water, the seed swells up producing mucilaginous mass due to presence of the polysaccharide layer on the outer epidermis of the wall of seed. It is considered an anionic hetero-polysaccharide and the gum extracted from cold water extraction and alcohol extraction have been reported to consist two major fraction: an acid-stable fraction glucomannan with glucose and mannose in the ratio 10:2 and other fraction is a(1-4)- linked xylan having acidic side chains at C-2 and C-3 of the xylosol residues in acid-soluble portion and a minor fragment of glucan (Anjaneyalu and Gowda, 1979). The percentage of different fraction such as glucomannan is 43% and that of (1-4) linked is 24.29% along with some minor fractions of glucan (2.31%).

BSG can be a valuable product to be used in food and pharmaceutical systems due to its various functional properties such as thickening, gelling, stabilizing, emulsifying, foaming and foam stabilizing, fat replacing and others (Hosseini-Parvar *et al.*, 2010; Naji-Tabasi and Razavi, 2017a; Razavi and Naji-Tabasi, 2023). It demonstrates significant physical and chemical properties, for instance high water holding capacity, oil holding capacity and so on. It not only contains carbohydrates (polysaccharides) but also non polysaccharide such as fat, minerals, protein and also certain phytochemical such as polyphenols and antioxidants (HUSSAIN *et al.*, 2019; S. Y. Kim *et al.*, 2020). At the same time the hydrocolloids from seeds like basil seed can be extremely useful in food formulations because of their appropriate price, easy availability and proper functionality (Salehi *et al.*, 2015).

In recent times, the utilization of basil seeds for food products are being researched all over the globe. Food industries, nowadays are focusing on the uses of new plant-based food hydrocolloid as gelling agents in product development with unique sensory characteristics. The major reason for the use of hydrocolloid in food is due to its ability to modify the rheology of the food systems by the long polymer chains of polysaccharides and proteins which has a property to develop gel-like characteristic when soaked in water (Salehi *et al.*, 2015).

The use of BSG along with other gums such as xanthan, k-carrageenan and gaur as fat replacer in some products was considered in few research (Biglarian *et al.*, 2021; Hesarinejad *et al.*, 2021). BSG is one of the hydrocolloids which is gaining huge popularity in the scientific community for its wide range of application in fields of frozen food, bakery, edible coating, pharmaceuticals, nanofibers, adhesive for animal feed, drilling fluids and others. The gums are used as hydrocolloid in juices in various research work (Genovese and Lozano, 2001; Hajmohammadi *et al.*, 2016; Lv *et al.*, 2017; Thanushree Prabhuswamy *et al.*, 2019).

2.8 Basil seed beverage

2.8.1 Beverages

Beverages, at the most basic level, are defined as the potable drinks other than water that humans consume for energy, hydration or to satisfy thirst (Arora *et al.*, 2019).

Generally, there are two major group of beverages which are;

- Alcoholic beverages
- Non-alcoholic beverages

Here in this paper, non-alcoholic beverage is of our concern which as per Arora *et al.* (2019), is further divided into carbonated and non- carbonated beverage. Concerns about health has made the global market for non-alcoholic beverage huge and with the exposure of people to knowledge about healthy foods, mostly through internet and social media, the market will only grow in coming days which seems to imply the great potential for functional beverage to gain proper attention in the market globally. There are many functional beverages such as caffeinated, dairy and fruit juice with added raw pieces of fruits and with seeds like chia seeds and basil seeds etc., in the international market, some of which are already ruling the market like green tea and few are still on the way of progress. And with the proper research and information, the market for all the functional beverage only seems to flourish.

2.8.2 Basil seed beverage

Basil seed beverage are popular in many parts of world, which is mainly the infusion of soaked basil seeds with fruit juices, water or milk with or without sweeteners, flavorings or fruit pieces (Hajmohammadi *et al.*, 2016; Munir *et al.*, 2017). BSB is a non-carbonated beverage which can be considered functional beverage as it consists of the basil seeds which has bioactive compounds with functional values. It is mostly consumed in southeast Asian regions but it is being commercialized in various parts of the world now due to its beneficial properties.

Some of the popular brands thriving in the international market are:

- Rudolf Wild GmbH &Co. KG – It's a brand based in Germany, known for flavored BSB.
- Chaokoh, Kato, Pandan, COCO, Chabaa – These are some famous brands from Thailand.
- Sun Tropics and Healthy Food Brands – Both are USA based brands
- Patanjali, Kalya, Sofit, Organic India – These are BSB brands from India that offers range of beverages with basil seeds.

There are not many papers on properly optimized process for preparation of basil seed beverage except for some. The work on basil seed beverage has been done by Munir *et al.* (2017), Hajmohammadi *et al.* (2016) and Thanushree Prabhuswamy *et al.* (2019). Figure 2.1 depicts the process of preparation of basil seed beverage as per Hajmohammadi *et al.* (2016).For detailed understanding of the process further information on procedure is described below.

As per the process reported by him, hydrocolloids (CMC and GT) were first prepared. GT was in flakes, so it was first grinded and both were separately dissolved in water at 70 °C for 2 h under continuous stirring by the use of a magnetic hot plate. After hydration, the hydrocolloid solutions were kept at 41°C for complete hydration.

To prepare basil seed beverage, the ingredients were added as per the specification for various formulations where TSS was set to 12.5°Bx, mango puree 30%, various proportions of CMC and GT and water. Furthermore, ratio of swollen basil seed added was 10, 30 and 50:100 on the basis of weight of basil to total amount of beverage. Lastly, the mixture was pasteurized at 80°C for 1 min in a microwave oven and hot filled in 250 ml bottles.

The flowchart showcases the preparation of basil seed beverage:

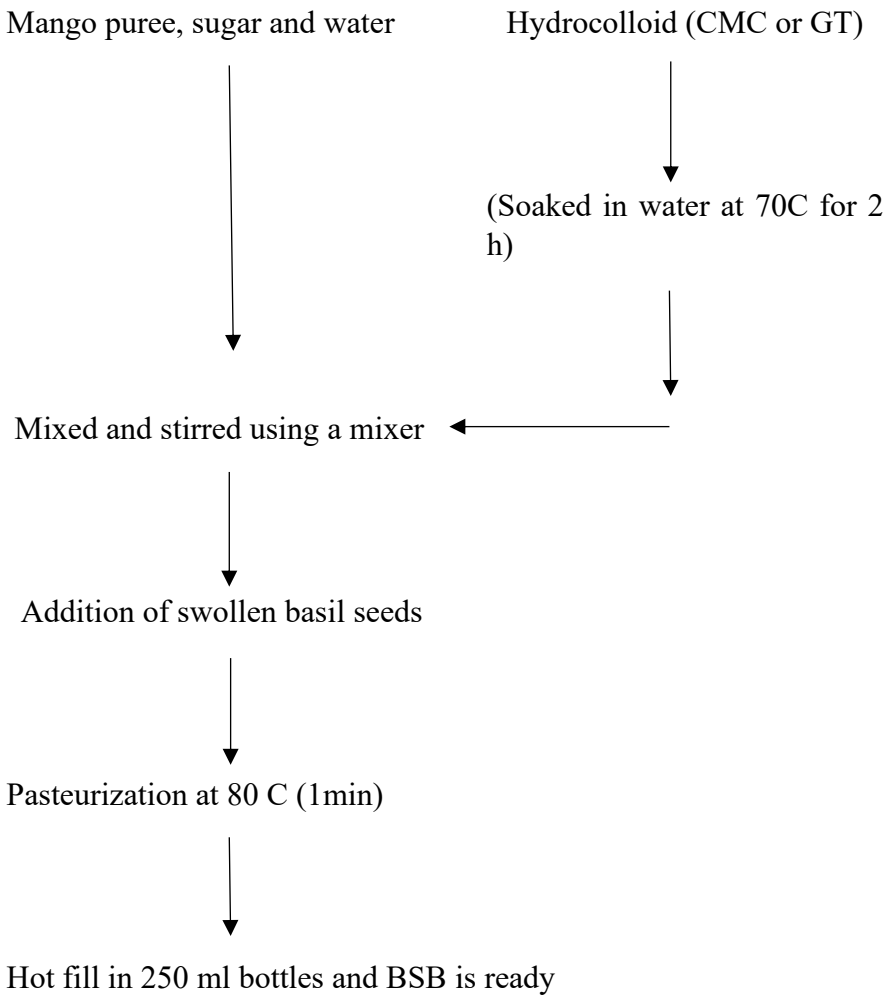

Fig 2.1 Preparation of Basil seed beverage

Source: Hajmohammadi *et al.* (2016)

2.9 9-Point hedonic scale reading

The 9-point hedonic scale has been use in food science for a long time now and it as originally developed by U.S army for menu planning for their canteens, consisting nine verbal categories representing degrees of likelihood from ‘dislike extremely’ to ‘like extremely’ (Nicolas *et al.*, 2010). Since its development, it has been mostly used as scale for testing preference and acceptability of food products (Lim and preference, 2011). The author also reported that, the 9-point hedonic scale is considered as a balanced bipolar scale with four positive and four negative categories on each side of a neutral center. For statistical analysis, the verbal categories are converted into numerical values: ‘like extremely’ as ‘9’ and ‘dislike extremely’ as ‘1’.

The primary reason for the wide acceptance of the 9-oint hedonic scale is that in comparison to other scaling methods (e.g., magnitude estimation), its categorical nature and limited choices makes it easier for both researchers and participants to use and also no extensive training is required for its use. Therefore, it is considered as a simple and effective scale for prediction of acceptance of food and other consumer products(Lim and preference, 2011).

Part III

Materials and methods

3.1 Raw materials

3.1.1 Basil seed

The seeds were purchased from Mother Spices and Herb Centre, Banepa-7, Kavrepalanchok, Bagmati and identified by the botanist of central campus of technology, Dharan.

3.1.2 Mangoes

The mango was collected from the market of Dharan and identified by the botanist of central campus of technology.

3.1.3 Packaging material

The packaging material i.e. glass bottle used were taken from the campus dairy lab.

3.1.4 Materials, equipment and chemicals

All materials, equipment, and chemicals required were used from the laboratory of the Central Campus of Technology, Dharan. All the materials, equipment, and chemicals used in the research purpose are listed in **Appendix A.**

3.2 Methods

3.2.1 Extraction of BSG

The extraction of BSG was carried out as suggested in the report of Razavi *et al.* (2009) with some modification which is shown below in Figure 3.1.

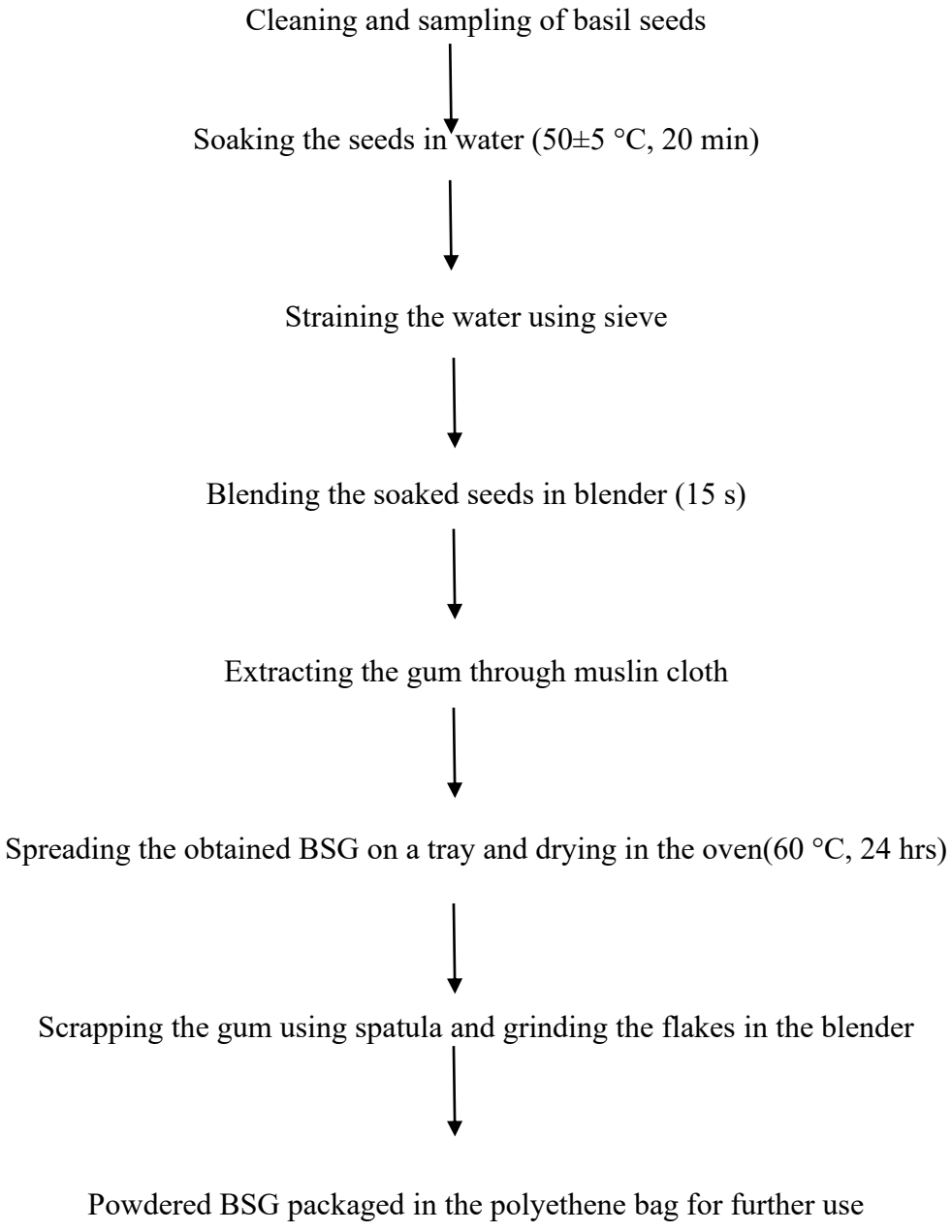

Fig3.1 Extraction of BSG

3.2.2 Experimental design

BSG in different concentrations was added to BSB and its effect on the sensory features, physicochemical as well as phytochemical and antioxidant capacity were studied. For incorporation of basil seeds, in his study, Munir *et al.* (2017) suggested 2 gm dry basil seeds to have good sensory characteristics while Hajmohammadi *et al.* (2016) did not suggested the exact proportion however, reported decrease in sensory features on increasing basil seed

incorporation. In this work, a preliminary trial was performed where swollen basil seeds were added to the base formulation (mango juice with TSS 12 Bx, acidity 0.3%, mango pulp 10%) in the ratio 10, 20, 30, 40 ,50: 100 (swollen basil seeds: base formulation). The formulation having ratio of 30:100 (swollen basil seeds: base formulation) was concluded to be best for which the same ratio was used for all BSB samples.

In context of BSG, previous studies on hydrocolloid have reported 0.3% hydrocolloid to be acceptable in beverage products. As there were no studies on use of BSG in beverages, preliminary trial was performed by prepari· BSB sample containing 0.1%, 0.2%, 0.3%, 0.4% and 0.5% BSG. BSB with 0.4% and ʊ.ɔ% BSG were too thick in consistency while 0.3% was somehow acceptable. Thus, an upper threshold value of 0.3% was set for powdered BSG in the BSB.

A total of four different formulations were prepared for BSB with the base formulation (mango juice) of TSS 12 °bx, acidity 0.3%, mango pulp 10% with the variation in the percentage of BSG as shown in Table 3.1.

Table 3.1 Formulations for BSB

Sample	A	B	C	D
Fruit pulp (%)	10	10	10	10
TSS (%)	12	12	12	12
Acidity (%)	0.3	0.3	0.3	0.3
Water (%)	77.7	77.6	77.5	77.4
Basil seed gum (%)	0	0.1	0.2	0.3

0.1%, 0.2% and 0.3% of BSG were added to sample B, C and D and no BSG (0%) was added to sample A. Basil seeds were swollen as demonstrated in preparation process (**Fig.3.2**) and BSG was extracted and powdered as shown in **Fig.3.1** before incorporation in BSB.

3.2.3 Basil seed beverage preparation

The preparation of basil seed was carried out by the combination of processes reported by Hajmohammadi *et al.* (2016), Munir *et al.* (2017), Thanushree Prabhuswamy *et al.* (2019) with some modifications which is shown below in Fig.3.2.

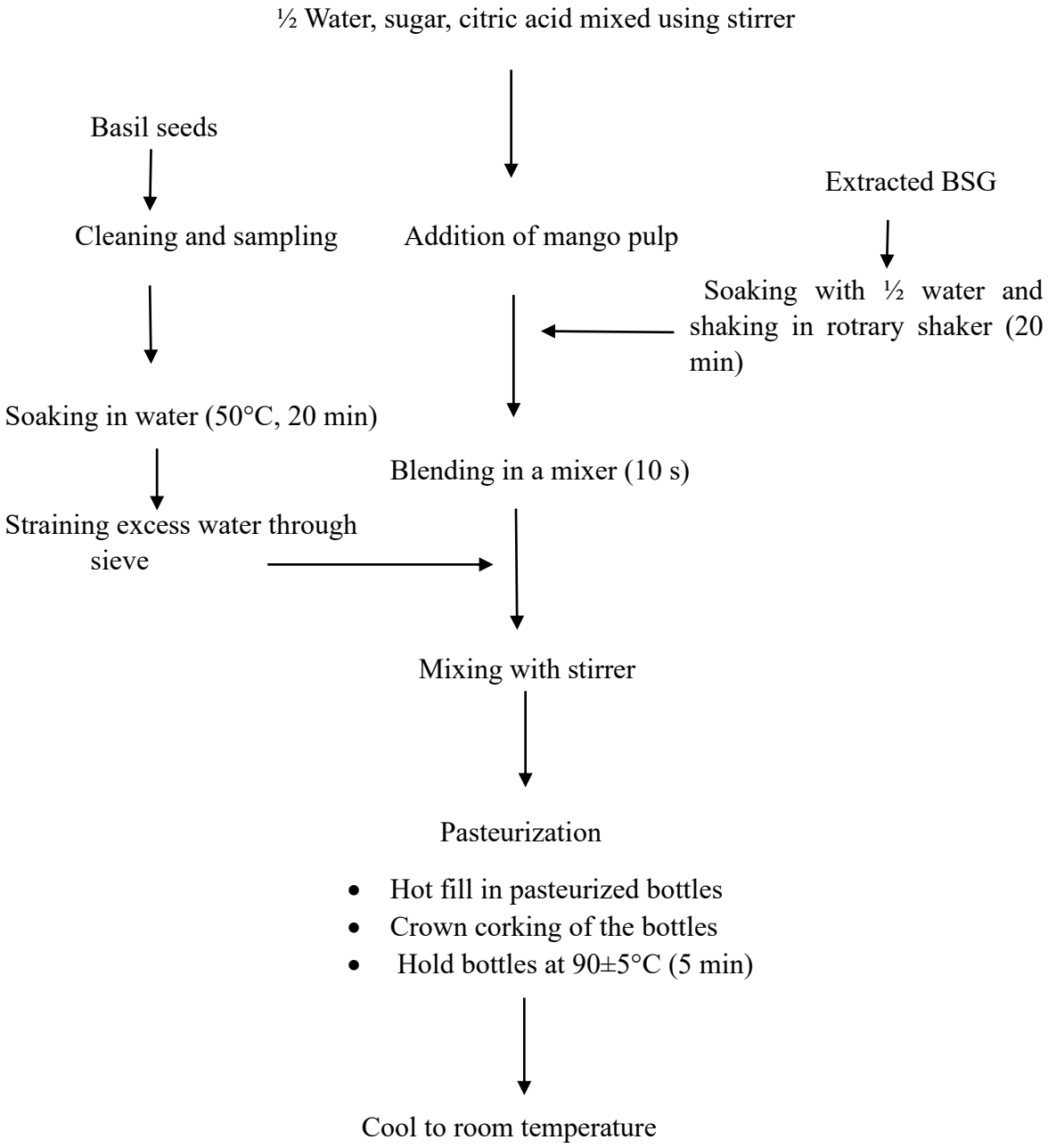

Fig 3.2 Preparation of BSB

3.2.4 Analysis of proximate of seeds

The seed sample was powdered using mortar and pestle for all of the proximate analysis.

3.2.4.1 Determination of moisture content

Moisture content of the sample were determined by heating the powdered sample in the hot air oven at 100 ±5 °C as described in Kc and Rai (2007).

3.2.4.2 Determination of crude fat

The crude fat of the samples were determined by Soxhlet extraction as per the method described in Kc and Rai (2007).

3.2.4.3 Determination of crude protein

The analytical procedure used to determine crude protein of the samples was Kjeldahl Nitrogen method as described in Ranganna (1986). Micro Kjeldahl method was used and factor 6.25 was used to convert the nitrogen content into crude protein content.

3.2.4.4 Determination of ash content

Dry-ashing was done in the muffle furnace at 520± 5 °C in order to determine the total ash content of the seeds in accordance to Ranganna (1986).

3.2.4.5 Determination of carbohydrate content

The carbohydrate content of the seeds were evaluated by difference method as mentioned in Ranganna (1986).

3.2.4.6 Determination of crude fiber

The determination of crude fiber of the defatted samples were done with chemical digestion following the process as illustrated in Kc and Rai (2007). The digestion was carried out in 1.25 %H_2SO_4 and 1.25% NaOH for 30 minutes each.

3.2.5 Analysis of BSG

3.2.5.1 Water holding capacity of BSG

The water holding capacity of the BSG was determined as per the process used by Javed et al. (2022). 1 gm sample was mixed with 15 ml of distilled water in a 50 ml centrifuge tube. The gum was soaked overnight in room temperature then centrifuged at 15000×g for 20

min. The free was discarded and absorbed water was weighed. WHC was expressed in gram of water per gram of sample (dry matter).

3.2.6 Analysis of proximate of BSB

The BSB samples were grinded in the food processor for grinding of seeds and its equal distribution in the bottle of beverage.

3.2.6.1 Determination of TSS

The total soluble solid of the beverage was directly determined by the use of a portable refractometer.

3.2.6.2 Determination of titratable acidity

Titratable acidity was evaluated by titrating clear juice with standard N/10 NaOH and the result was expressed as percentage citric acid.

3.2.6.3 Determination of pH

It was directly measured using a calibrated pH meter which was calibrated using buffer solution of pH 4.0 and 7.0 before use.

3.2.6.4 Determination of sugar

Total sugar and reducing sugar were determined using Lane and Enyon method(Ranganna, 1986). And non-reducing sugar was calculated by the difference of total sugar and reducing sugar.

3.2.6.5 Determination of ascorbic acid

The ascorbic acid of the BSB was calculated using 2,6-dichlorophenol visual titration method(Ranganna, 1986).

3.2.6.6 Microbial analysis

Total plate count and yeast mold count was performed for microbial analysis of pasteurized BSB. Both total plate count and yeast and mold count was done using spread plate technique. For total plate count, plate count agar (PCA) medium was used and the prepared plates were incubated at 30+1 for 48 h in inverted position. The colonies were then counted using colony counting equipment. For yeast and molds, potato dextrose agar (PDA) medium

was used and the prepared plates were incubated at 25-27 C for 48-72 h (Horwitz and Latimer, 2005).

3.2.7 Preparation of extract for phytochemical analysis

The extract was prepared by maceration of 2 gm powdered sample of basil seed in 100 ml 80% methanol for 24 hours and as for BSB 10 ml sample was dissolved in the 100 ml 80% methanol as well and maceration was done for 24 hours. The extract was then, filtered using filter paper and the extract stored in cold to be used for determination of the phytochemicals.

3.2.8 Analysis of phytochemicals

3.2.8.1 Analysis of Total Phenolic Content

Total phenolic content (TPC) in the methanolic extract of seeds and BSB were determined spectrophotometrically using Folin-Ciocalteu colorimetric method as described by Singleton *et al.* (1999) with some modifications. The method has been used by several researchers who worked with basil varieties such as H.-J. Kim *et al.* (2006); Munir *et al.* (2017); Pedro *et al.* (2016) and others.

For the standard curve, different concentrations of gallic acid solutions were prepared in methanol (50, 100, 150, 200 and 250). In a 20 ml test tube, 1ml gallic acid each concentration was added and 5ml of 10%FC reagent was added too and incubated in dark for 5 min. Next, 4ml of 7% Na2CO3 was added to get total volume of 10 ml. The blue color was formed in the mixture which was shaken well and incubated in dark for 30 min then absorbance was measured at a wavelength of 756 nm using a UV-Vis spectrophotometer.

For the extracts, various concentrations were prepared (10, 50, 100) as it was prepared for gallic acid. The abovementioned procedure was followed for the sample as it was for gallic acid standard curve, and absorbance for each concentration were measured and recorded. Total phenolic contents of the extracts were expressed as mg gallic acid equivalent (GAE) per gm of sample in dry weight.

3.2.8.2 Analysis of flavonoids

Total flavonoid content of basil seeds and BSB was determined using modified aluminum chloride assay method as described by Phuyal *et al.* (2019). Several researchers such as Gudej and Tomczyk (2004); Hendrawan *et al.* (2019); Pękal and Pyrzynska (2014); Shraim

et al. (2021) have modified and optimized the aluminum chloride assay method for determination of flavonoid contents which was first proposed by Christ and Müller (1960).

At first for the standard curve, 1ml solution of each concentration was taken in the test tube and 2ml distilled water was added. Afterwards, 0.3 ml of 5% NaNO2 was added to the test tube and after 5 min, 0.3 ml of 10% AlCl3 was added too. After 6 min, addition of 2ml of 1M NaOH was done and the volume was made up to 10 ml with the addition of distilled water. After the absorbance of each concentration were taken using a spectrophotometer at 520nm and standard curve was plotted.

Similar procedure as described for the standard curve of quercetin was followed for the sample and after the absorbance were measured at 510nm, the flavonoid content was expressed as mg quercetin equivalent per gram dried sample (mg QE/g) using the linear equation obtained from the standard calibration curve.

3.2.9 Determination of DPPH radical scavenging activity

The DPPH radical scavenging capacity of basil seeds and BSB samples were determined using the method described by (Blois, 1958; Brand-Williams *et al.*, 1995) with some modifications. The method was used by several other researchers with some modifications, such as by Akshatha *et al.* (2019); Gajendiran *et al.* (2016); Khursheed *et al.* (2023); Nazir *et al.* (2021); Sarfraz *et al.* (2011) and others for determining antioxidant capacity of basil seeds.

For the determination of DPPH radical scavenging activity, 2 ml of the methanolic extract (or the standard) was mixed with 2 ml of DPPH (0.004% in methanol) in a cuvette and incubated for 20 minutes, at 37°C in dark (wrapped with aluminum foil), before spectrophotometric analysis. After setting the absorbance to zero for the blank, absorbance was measured at 517 nm for sample as well as the standard.

Table 3.2 Amounts of reagents and extracts in cuvettes for DPPH assay

Reagents	Control	Blank	Extracts			
DPPH, ml	2.0	0	2.0	2.0	2.0	2.0	2.0
Extract, ml	0	2.0	0.4	0.8	1.2	1.6	2.0
Methanol, ml	2.0	2.0	1.6	1.2	0.8	0.4	0
Total, ml	4.0	4.0	4.0	4.0	4.0	4.0	4.0

3.2.10 Sensory analysis of BSB

9-point hedonic scale was used to establish preference rating for BSB in accordance with its color, taste, mouthfeel, appearance and overall acceptability. As per Ranganna (1986), the method can be used to measure the consumer acceptability of the food products either with trained panelists or with untrained ones. The beverage samples were scored by the 8 semi-trained panelists of Central Campus of Technology, Hattisar, Dharan.

3.2.11 Statistical method

The data obtained were expressed as the mean SD of three triplicates. Analysis of variance was done through one way ANOVA along with Tukey's honesty test which were performed by Genstat (Genstat Discovery Edition 12, 2009), checking significant relationship among the mean values of the samples at 5% level of significance. MS- Excel (2016) was employed for general calculations, graph and diagram construction.

Part IV

Results and discussions

4.1 Sensory evaluation

Sensory evaluation was carried out for: color, taste, mouthfeel, appearance and overall acceptability of the BSB by 9 semi-trained panelists using 9-point hedonic scale. The statistical analysis was done through one way ANOVA using Genstat (Genstat Discovery Edition 12, 2009) and MS- Excel (2016) was used to generate the bar graph.

Table 4.1 Sensory evaluation of BSB

Sample	Color	Taste	Mouthfeel	Appearance	Overall
A	6.625 ± 0.743^{a}	6.250 ± 0.744^{a}	6.500 ± 0.531^{a}	6.125 ± 0.641^{a}	6.250 ± 0.857^{a}
B	7.125 ± 0.834^{a}	7.375 ± 0.744^{b}	7.500 ± 0.531^{b}	7.250 ± 0.886^{b}	7.375 ± 0.696^{b}
C	7.000 ± 0.640^{a}	6.875 ± 0.354^{ab}	6.875 ± 0.835^{ab}	7.125 ± 0.781^{b}	6.625 ± 0.484^{ab}
D	6.875 ± 0.734^{a}	6.250 ± 0.886^{a}	6.250 ± 0.886^{a}	7.375 ± 0.781^{b}	6.375 ± 0.875^{a}

*Values are the means of triplicates ± standard deviation of the triplicates. Values in the column having different superscripts are significantly different at 5% level of significance.

4.1.1 Color

The mean sensory score of the color of the samples A, B, C and D was found to be 6.625, 7.125, 7.000 and 6.875 respectively. The highest score was obtained for sample B and the least was for sample A. There was no significant difference among the samples ($p<0.05$) in terms of color as shown in the **Fig 4.1**.

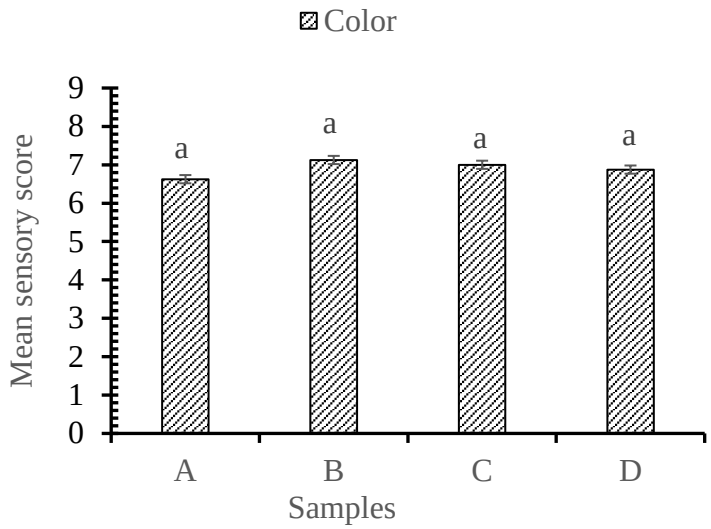

Fig 4.1: Mean sensory score for color

The yellowish orange color was imparted by the mango pulp and the textured pattern was given by the presence of basil seed which made the bottle of BSB appealing. Since the seeds in the sample A sedimented to the bottom of the bottle, the color for the upper part was slightly different which might be the reason for its comparatively lower score. The results were to some extent in alignment with the sensory score obtained for color of basil seed beverage as reported by Thanushree Prabhuswamy *et al.* (2019). He also reported no significance difference in the color of the beverages.

4.1.2 Taste

The mean sensory scores for the taste of the beverage samples A, B, C and D were 6.250, 7.375, 6.875 and 6.250 respectively. The highest score was observed for sample B and least was for sample D. In terms of taste, sample B was significantly different($p>0.05$) from D and A whereas no significant difference is evident among sample B and C as can be seen in the **Fig 4.2**.

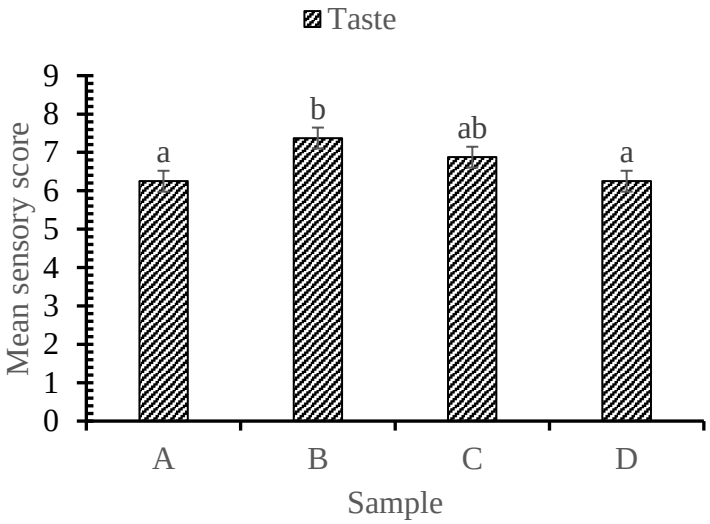

Fig 4.2: Mean sensory score for taste

The score for D is lowest for which the reason for which, as per the comments from panelists, seemed to be the thick consistency which was claimed to be interfering with the actual taste of the beverage. All other sample were likable to the panelists with citrus flavor and properly balanced sweetness. The score for the taste of the beverage increases when viscosity is increased up to certain level but exceeding the level showcases the decrease in the score(Hajmohammadi *et al.*, 2016; Thanushree Prabhuswamy *et al.*, 2019).

4.1.3 Mouthfeel

The mean sensory scores for the mouthfeel of BSB were 6.500, 7.500, 6.875 and 6.250 for A, B, C and D respectively. Sample B with the highest sensory score is the most preferred beverage sample while sample D with lowest score of 6.250 is the least preferred one in terms of mouthfeel. As it can be seen in the **Fig 4.3**, the sample B has no significant difference from sample C and A whereas it is significantly different from sample D.

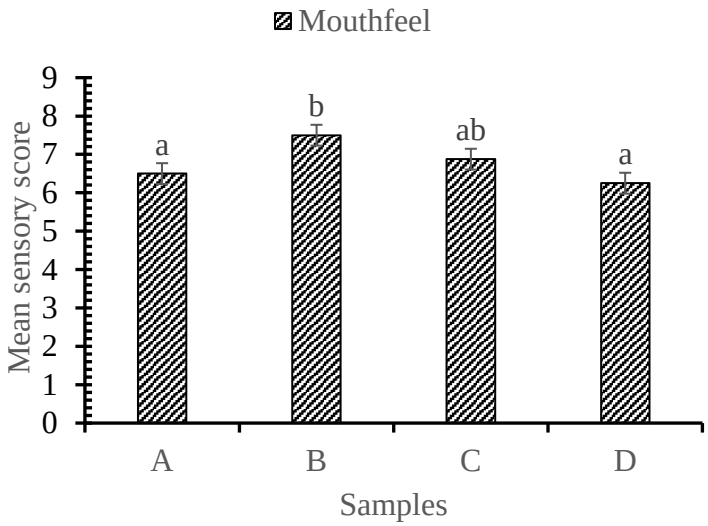

Fig 4.3: Mean sensory score for mouthfeel

From figure 4.1 it can be observed that sample D has the lowest score for mouthfeel which is due to its highly viscous consistency. The bar graph also suggests the mouthfeel is best for slightly viscous drink than for the drink with either too thick consistency or too thin one. The similar results were evident in the reports of Hajmohammadi *et al.* (2016); Thanushree Prabhuswamy *et al.* (2019)

4.1.4 Appearance

The mean sensory scores for appearance of the beverage were 6.125, 7.250, 7.125 and 7.375 for samples A, B, C and D, consecutively. The highest sensory score was observed for sample D while lowest was observed for sample A. There seemed no significance difference in the appearance of sample B, C and D while sample A was significantly different from B, C and D.

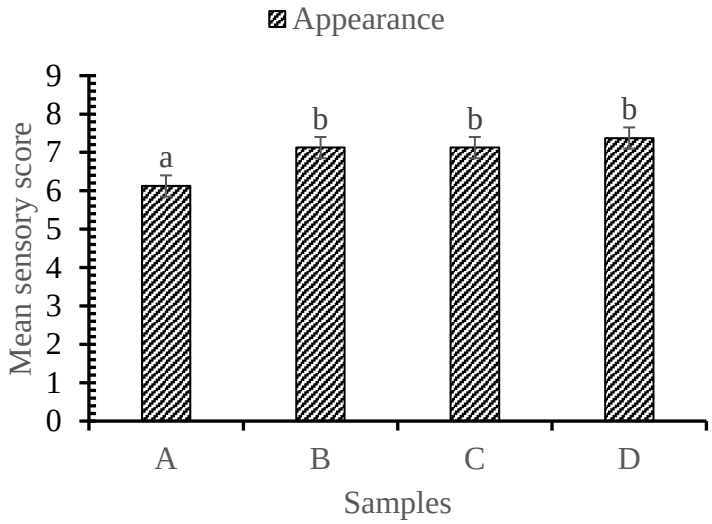

Fig 4.4: Mean sensory score for appearance

Due to sedimentation of seeds at the bottom sample A had lowest score as it did not seem appealing whereas in B, C and D the seeds were properly dispersed in the colloidal solution providing an appealing patterned appearance to the final product. With increasing gum concentration, the score for appearance was increasing which aligned with the report of Hajmohammadi *et al.* (2016) as he suggested the similar results in his work. With increasing percentage of gum, the distribution of seeds was more uniform and stable which contributed to similar increment in the score for appearance.

4.1.5 Overall acceptability

For the overall acceptability of the beverage, the sensory score for sample A, B, C and D are 6.250, 7.375, 6.675 and 6.375 respectively. The highest score is obtained for sample B and least score for sample A. There seemed significant difference between sample A and sample B and C. However, there was no significant difference between sample B and C.

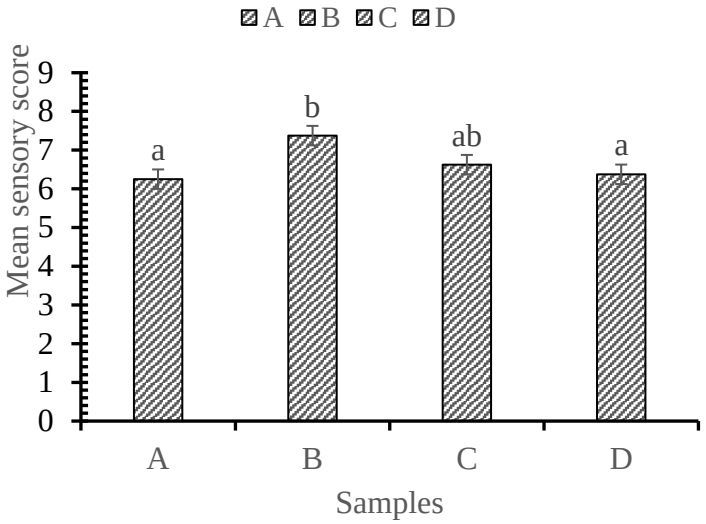

Fig 4.5: Mean sensory score for overall acceptability

Overall, sample D was of too thick consistency and in sample A due to sedimentation of seeds, its appearance was not satisfactory. These might be the reasons for sample A and D to have overall mean sensory score lesser than B and C. Among sample C and B, score for sample B is quite higher in terms of taste and mouthfeel. The reason behind it as per the comments of panelists was the sample C being little bit thicker in consistency. Most of them considered sample B to have balanced consistency that eventually led it to have higher mean score in terms of taste and mouthfeel. Hence sample B was considered best sample and further analysis were done on it in comparison to sample A(control).

4.2 Proximate analysis

4.2.1 Proximate analysis of basil seed

The proximate composition of basil seeds is shown in Table 4.2.

Table 4.2 Proximate composition of basil seed

Proximate composition	Content (%)
Moisture	5.50 ±0.10
Fat (db)	28.05 ±0.15
Protein (db)	21.86 ±0.50
Ash (db)	4.58 ±0.11
Crude fiber (db)	37.95 ±0.670
Carbohydrate (db)	7.56 ±0.72

*Values are the means of triplicates ± standard deviation of the triplicates.

The moisture content in the basil (*Ocimum basilicum*) seeds were found to be 5.50 %. The range for moisture content in basil seeds is 5%- 9.6%(Calderón Bravo *et al.*, 2021), which might be due to several factors such as cultivar effect, geographical variation, storage condition and so on. Razavi *et al.* (2009) in his study found that the moisture contents of the basil seeds from Iran were recorded in the range of 5%- 66.23% and that of the Indian basil seed was found to be 9.63%.

The fat content of the seeds was 28.05%. This suggests that basil seeds are good source of lipid. Khaliq *et al.* (2017) reported 29% lipid in the seeds and Nazir *et al.* (2021) found 33.01% in his research work. The range for fat in lipid as suggested by various reports was found to be 9.5%-33.01% (Akshatha *et al.*, 2019; Choi *et al.*, 2020; Khaliq *et al.*, 2017; Khursheed *et al.*, 2023; Razavi *et al.*, 2009; Rezapour *et al.*, 2016; Sarfraz *et al.*, 2011) .

The protein content in the seeds was recorded to be 21.86 %. It shows that basil seeds contain good amount of protein. The result was nearly in alignment with the result suggested by Hajmohammadi *et al.* (2016); Munir *et al.* (2017); Razavi *et al.* (2009). The range of the

protein content was found to be 9.4%-22.5% (James, 2020; Khaliq *et al.*, 2017; Munir *et al.*, 2017; Razavi *et al.*, 2009).

Ash content of the seeds was 4.58 %. High ash content suggests the seeds are good source of minerals as Khursheed *et al.* (2023); Munir *et al.* (2017) reported basil seeds to be the high in magnesium along with presence of calcium, zinc, iron and manganese. The proportion of crude fiber was 37.95% which suggests basil seeds are good source of fiber as reported by Hajmohammadi *et al.* (2016); Mathews *et al.* (1993). The carbohydrate content of the basil seeds was found to be 7.56 % by difference method.

4.2.2 Analysis of BSG

4.2.2.1 Water holding capacity

The water holding capacity was found to be 68.09±0.91g/g BSG. The result indicates that BSG have good water binding property that makes it hydrophilic in nature. Hence, it can be utilized in hydrogels, as suspending agents and for other several purposes where hydrophilic nature of gum is required. Javed *et al.* (2022) and Avlani *et al.* (2019) reported WHC of 30.94±0.95 g/g BSG and 97.50±2.4 g/g BSG, respectively.

4.2.3 Analysis of BSB

The physio-chemical properties of the sample without BSG(A) and the best sample among the sample with BSG (B) as per sensory analysis are determined and shown in the Table 4.3.

TSS and acidity of BSB were slightly lesser than the specified TSS and acidity of the base formulation which might due to water content of swollen basil seed. On microbial analysis of the BSB, total plate count was zero and there was no growth of any types of yeast or mold. It indicates that the BSB was properly pasteurized. The sedimentation height of the sample A (without BSG) is significantly lower than that of the sample B (with 0.1% BSG). The obtained result provides the evidence for BSG to be a good kind of hydrocolloid.

Table 4.3 Physio-chemical properties of BSB

Parameters	A	B
TSS (°BX)	11±0	11±0
Acidity (%)	0.286±0.0047	0.273±0.0039
PH	3.43±0.041	3.28±0.056
Sugar		
Reducing sugar (%)	4.67±0.18	5.26±0.081
Non reducing sugar (%)	12.08±0.087	11.59±0.127
Total sugar (%)	17.75±0.093	16.85±0.098
Ascorbic acid (mg/100gm)	6.067 ±0.129	6.537±0.118
Microbial count		
Yeast / Mold	Nil	Nil
Total plate count	Nil	Nil
Sedimentation height (%)	57.14±0.657	96.49± 0.581

*Values are the means of triplicates ± standard deviation of triplicates.

4.3 Quantitative analysis of phytochemicals

4.3.1 Total Phenolic Content (TPC)

The total phenolic content of seeds, sample A(BSB) and sample B (BSB) were found to be 25.020 mg GAE/g seeds(dm), 476.858µg GAE/ml sample and 568.892 µg GAE/ml sample, consecutively as it can be observed in the **Table 4.3**. Khursheed *et al.* (2023) reported TPC of the seeds to be 17.66 mg GAE/g dry sample whereas TPC of 63.780mg GAE/g of sample was reported by Munir *et al.* (2017). The difference in the values might be due to variation in extraction solvents, extraction time, variation in geographical origin of the seeds and so on.

The TPC of sample B is higher than that of sample A which indicates that with addition of gum, TPC increases. S. Y. Kim *et al.* (2020) also claimed that higher TPC was found in yoghurt supplemented with BSG.

4.3.2 Total Flavonoid Content (TFC)

The total flavonoid content (TFC) for basil seeds was found to be 11.635 mg QE/gm seeds (dm). It implies that basil seeds can be good source of flavonoids. This value does not align with the values reported in the previous reports , for instance with that provided by Akshatha *et al.* (2019); Khursheed *et al.* (2023). They reported 0.30 mg QE/g sample and 0.57 mg QE/ 100 gm sample, respectively. The huge difference in the values might be due to the process of determination of TFC, geographical origin of seeds, storage condition of seeds, extraction procedure and so on.

The TFC for BSB sample A and B were 73.409 µg QE /ml BSB and 86.79 µg QE /ml BSB, consecutively. As it was observed in TPC, higher TFC was evident in sample B than sample A. It suggests that BSG might contain certain amounts of flavonoids too. Higher TFC was found in the yoghurt supplemented with BSG in comparison to their control (S. Y. Kim *et al.*, 2020).

4.4 Antioxidant Activity

The antioxidant activity of basil seeds in terms of DPPH radical scavenging activity is 78.74%. Several research papers suggest several antioxidant activity (DPPH RSA) of the basil seeds (*Ocimum basilicum*). For instance, Khursheed *et al.* (2023) recorded 47.01%, Nazir *et al.* (2021) reported 30.30% and Hajmohammadi *et al.* (2016) and Akshatha *et al.* (2019) reported 14.66% and 22.27% respectively. The DPPH RSA reported in this paper showed relevance to some extent with the value determined by Sarfraz *et al.* (2011) which was 84.39% for methanolic extract of the seeds.

Table 4.4 TPC, TFC and antioxidant activity of basil seeds and BSB (sample A and B)

Samples	TPC	TFC	AOA (DPPH RSA)
Seeds	25.010±0.056 mg GAE/g seeds	11.635±0.038 mg QE/g seeds	78.94%±0.456
A	476.858± 0.0385µg GAE/ml BSB	73.409±0.0293µg QE/ml BSB	45.64%±0.451
B	568.892±0.0416µg GAE/ml BSB	86.790±0.0371µg QE/ml BSB	49.16%±0.379

*Values are triplicates of mean ± standard deviation of the triplicates.

The antioxidant activity of the BSB sample A and B were 45.64% and 49.16% in terms of DPPH radical scavenging activity. As it was observed in TPC and TFC, the BSB with BSG added to it showed higher antioxidant activity. Enhanced antioxidant activity was also reported in the yoghurt sample with added BSG (S. Y. Kim *et al.*, 2020). Several reports claims that antioxidant activity is affected by phenolics and flavonoid in the sample(Calderón Bravo *et al.*, 2021; Juliani *et al.*, 2002; Khursheed *et al.*, 2023; S. Y. Kim *et al.*, 2020; Naimi *et al.*, 2015). Similar trait is evident in this paper as increase in TPC and TFC was observed and the increment was found in antioxidant activity too.

Part V

Conclusion and recommendations

5.1 Conclusion

As per the objectives of this work, methodologies were followed as mentioned, calculations and analyses were performed and based on the results and discussions, following conclusions were made:

1. Basil seed was found to be highly nutritious with fat, protein, ash, and crude fiber content of 28.05%, 21.86%, 4.58% and 37.95%.
2. Basil seed gum demonstrated good WHC of 68.09 g/g BSG.
3. As per sensory analysis using ANOVA at 5% level of significance, BSB sample B with 0.1% BSG was found to be superior than other samples
4. TPC, TFC, DPPH radical scavenging activity of seeds were commendable and that of BSB sample B were found to be greater than the control sample A.

5.2 Recommendations

The recommendations from this dissertation work that can be used for further experiments are as follows:

1. The best sample, sample B can be commercialized as a functional beverage.
2. Shelf-life estimation of the BSB supplemented with BSB can be done.
3. Effect of different process variables on the quality of BSB can be studied.
4. Incorporation of other species of basil seed in BSB can be done and studied.
5. Incorporation of basil seeds in dairy beverages such as flavored milk or whey products can be studied.
6. Analysis of phytochemicals and antioxidant activity using several other organic solvents such as petroleum ether, n-hexane, ethanol and others can be performed.
7. Other methods of analysis for the determination of antioxidant capacity such as FRAP, CUPRAC, ABTS can be used.

Part VI

Summary

Basil seeds are one of the commendable sources of nutrition and biochemical compounds. It has attracted scientific interests from all around the globe because of its chemical properties and its mucilaginous polysaccharide released on soaking. Number of research works have been carried out on both utilization of the seeds and its gum (BSG).

In this study, basil seed was incorporated in mango flavored beverage along with BSG as a hydrocolloid for stability of seeds in the suspension. Among four different formulations prepared, namely A (0% BSG), B (0.1% BSG), C (0.2% BSG) and D (0.3% BSG), sample B was found to be best as per sensory evaluation. The sensory evaluation was carried out based on color, taste, mouthfeel, appearance and overall acceptability. The obtained data were statistically analyzed using ANOVA at 5% level of significance.

The proximate composition of the seeds was 5.50%, 28.05%, 21.86%, 4.58%, 37.95% and 7.56% for moisture, fat, protein, ash, crude protein and carbohydrate content which confers the seeds to be highly nutritious. The TPC and TFC content of the seeds were 25.010 mg GAE/g seeds (DM) and 11.635 mg QE/g seeds (DM), implying the seeds to be rich in biochemical compounds. The proximate composition of the best BSB sample for TSS, Ph, ascorbic acid, reducing sugar, total sugar, nonreducing sugar, acidity and sedimentation height were 11 °Bx, 3.28, 6.537mg/100g, 5.26%, 16.85%, 11.59%, 0.273% and 96.49%, respectively. The total plate count and yeast and mold count, both were zero for the BSB as it was properly pasteurized. Sedimentation height of sample B was drastically higher than control (sample A), which provide strong evidence for the hydrocolloid property of BSG. The WHC of BSG was found to be 14.26 g/g BSG.

The TPC, TFC and DPPH RSA of the BSB samples were 476.858 µg GAE/ml BSB, 73.409 µg QE/ml BSB and 45.64%, respectively for sample A and 568.892 µg GAE/ml BSB, 86.790 µg QE/ml BSB and 49.16%, respectively for sample B. The values indicate that with addition of BSG, increase in TPC, TFC and DPPH RSA is evident implying BSG to contains certain amounts of phytochemicals too. Overall, the study suggested basil seed to be good source of nutrient and sample B (with 0.1% BSG) to be best sample of BSB with excellent sensory characteristics, nutritional value and biochemical profile.

References

Adamu, H. M., Abayeh, O., Agho, M., Abdullahi, A., Uba, A., Dukku, H. and Wufem, B. J. J. o. e. (2005). An ethnobotanical survey of Bauchi State herbal plants and their antimicrobial activity. **99** (1), 1-4.

Akshatha, D. S., Naik, R. S., Chethana, B. and Brundha, A. (2019). A study on nutritional, functional and anti-nutritional properties of basil seed incorporated products.

Alqahtani, N. K., Ashton, J., Katopo, L., Haque, E., Jones, O. A., Kasapis, S. J. B. c. and fibre, d. (2014). Consistency of UHT beverages enriched with insoluble fibre during storage. **4** (1), 84-92.

Altıok, E. (2010). Recovery of phytochemicals (having antimicrobial and antioxidant characteristics) from local plants. Izmir Institute of Technology (Turkey),

Anjaneyalu, Y. V. and Gowda, D. C. J. C. R. (1979). Structural studies of an acidic polysaccharide from Ocimum basilicum seeds. **75**, 251-256.

Arora, P., Ansari, S. and Arora, S. J. A. J. P. R. (2019). Nutritional beverages. **9**, 1-28.

Avlani, D., Agarwal, V., Khattry, V., Biswas, G. R. and Majee, S. B. J. I. J. A. P. (2019). Exploring properties of sweet basil seed mucilage in development of pharmaceutical suspensions and surfactant-free stable emulsions. **11** (1), 124-129.

Azuma, J.-i., Sakamoto, M. J. T. i. G. and Glycotechnology. (2003). Cellulosic hydrocolloid system present in seed of plants. **15** (81), 1-14.

Bever, B. O. J. J. o. E. (1980). Oral hypoglycaemic plants in West Africa. **2** (2), 119-127.

Biglarian, N., Rafe, A., Shahidi, S. A. J. J. o. t. S. o. F. and Agriculture. (2021). Effect of basil seed gum and κ-carrageenan on the rheological, textural, and structural properties of whipped cream. **101** (14), 5851-5860.

Bilal, A., Jahan, N., Ahmed, A., Bilal, S. N., Habib, S., Hajra, S. J. I. J. o. C. R. and Review. (2012). Phytochemical and pharmacological studies on Ocimum basilicum Linn-A review. **4** (23), 73-83.

Blois, M. S. J. N. (1958). Antioxidant determinations by the use of a stable free radical. **181** (4617), 1199-1200.

Brand-Williams, W., Cuvelier, M.-E., Berset, C. J. L.-F. s. and Technology. (1995). Use of a free radical method to evaluate antioxidant activity. **28** (1), 25-30.

Calderón Bravo, H., Vera Céspedes, N., Zura-Bravo, L. and Muñoz, L. A. J. F. (2021). Basil seeds as a novel food, source of nutrients and functional ingredients with beneficial properties: A review. **10** (7), 1467.

Capra, S. (2006). Nutrient reference values for Australia and New Zealand: Including recommended dietary intakes.

Cherian, R. J. I. J. S. R. S. E. T. (2019). Health benefits of basil seeds. 511-515.

Choi, J.-Y., Heo, S., Bae, S., Kim, J. and Moon, K.-D. J. L. (2020). Discriminating the origin of basil seeds (Ocimum basilicum L.) using hyperspectral imaging analysis. **118**, 108715.

Chopra, R. N. and Nayar, S. L. (1956). "Glossary of Indian medicinal plants". Council of Scientific and Industrial Research.

Christ, B. and Müller, K. J. A. d. P. (1960). Zur serienmäßigen Bestimmung des Gehaltes an Flavonol-Derivaten in Drogen. **293** (12), 1033-1042.

Darrah, H. H. J. E. B. (1974). Investigation of the cultivars of the basils (Ocimum). 63-67.

Domínguez-Ávila, J. A., Olivas-Aguirre, F. J., de la Rosa, L. A., Wall-Medrano, A., González-Aguilar*, G. A. J. F., Chemistry, V. P. and Human Health, n. E. (2017). Flavonoids and Their Relation to Human Health. 139-154.

Gajendiran, A., Thangaraman, V., Thangamani, S., Ravi, D. and Abraham, J. J. B. P. R. (2016). Antimicrobial, antioxidant and anticancer screening of Ocimum basilicum seeds. **6** (3), 114-119.

Genovese, D. B. and Lozano, J. E. J. F. H. (2001). The effect of hydrocolloids on the stability and viscosity of cloudy apple juices. **15** (1), 1-7.

Ghasemzadeh, A., Jaafar, H. Z. and Rahmat, A. J. M. (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (Zingiber officinale Roscoe). **15** (6), 4324-4333.

Gudej, J. and Tomczyk, M. J. A. o. p. r. (2004). Determination of flavonoids, tannins and ellagic acid in leaves from Rubus L. species. **27**, 1114-1119.

Hajmohammadi, A., Pirouzifard, M., Shahedi, M. and Alizadeh, M. J. L. (2016). Enrichment of a fruit-based beverage in dietary fiber using basil seed: Effect of Carboxymethyl cellulose and Gum Tragacanth on stability. **74**, 84-91.

Halliwell, B. J. A. i. p. (1996). Antioxidants: the basics-what they are and how to evaluate them. **38**, 3-20.

Hasler, C. M. J. T. J. o. n. (2002). Functional foods: benefits, concerns and challenges—a position paper from the American Council on Science and Health. **132** (12), 3772-3781.

Heim, K. E., Tagliaferro, A. R. and Bobilya, D. J. J. T. J. o. n. b. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. **13** (10), 572-584.

Hendrawan, Y., Sabrinauly, S., Hawa, L. C., Argo, B. D. and Rachmawati, M. J. A. E. I. C. J. (2019). Analysis of the phenol and flavonoid content from basil leaves (Ocimum Americanum L) extract using pulsed electric field (PEF) pre-treatment. **21** (2), 149-158.

Hesarinejad, M. A., Arefkhani, A., Rafe, A., Javidi, F. and Sadeghian, A. (2021). Investigating the qualitative, textural and sensory characteristics of low fat Iranian white cheese containing a mixture of basil seed gum with xanthan or guar.

Horwitz, W. and Latimer, G. J. A. O. m. o. a. o. A. I. A. I., Gaithersburg, MD, USA. (2005). AOAC Official methods of analysis of AOAC International.

Hosseini-Parvar, S., Matia-Merino, L., Goh, K., Razavi, S. M. A. and Mortazavi, S. A. J. J. o. f. e. (2010). Steady shear flow behavior of gum extracted from Ocimum basilicum L. seed: Effect of concentration and temperature. **101** (3), 236-243.

HUSSAIN, N., ISHAK, I., ABDULLAH, M. F., ABD RAUH, A. and AZHAR, N. J. M. A. B. (2019). Water soluble hydrocolloid from basil seed (Ocimum basilicum L.) mucilage. **48** (2), 97-101.

Idris, A. A., Nour, A. H., Ali, M. M., Erwa, I. Y., Omer Ishag, O. A., Nour, A. H. J. A. J. o. P. and Sciences, C. (2020). Physicochemical properties and fatty acid composition of Ocimum basilicum L. seed oil. **8** (1), 1-12.

James, J. (2020). Characterization of basil (Ocimum basilicum L.) seed flour and its functionality in ice cream: a thesis presented in partial fulfilment of the requirements for the degree of Master of Technology in Food Technology at Massey University, Palmerston North, New Zealand. Massey University,

Jaros, D., Rohm, H., Strobl, M. J. L.-F. S. and Technology. (2000). Appearance properties—A significant contribution to sensory food quality? **33** (4), 320-326.

Javanmardi, J., Stushnoff, C., Locke, E. and Vivanco, J. J. F. c. (2003). Antioxidant activity and total phenolic content of Iranian Ocimum accessions. **83** (4), 547-550.

Javed, M., Ahmed, W., Shahbaz, H. M., Rashid, S., Javed, H. J. B. A. o. B. and Technology. (2022). Techno-Functional Assay and Quality Assessment of Yogurt Supplemented with Basil Seed Gum Powder. **65**, e22200814.

Juliani, H., Simon, J. J. T. i. n. c. and uses, n. (2002). Antioxidant activity of basil. **575** (9).

Kc, J. and Rai, B. J. P. P. P. L. K., Nepal. (2007). Basic food analysis handbook.

Khaliq, R., Tita, O. and Sava, C. S. (2017). A comparative study between seeds of sweet basil and psyllium on the basis of proximate analysis.

Khursheed, T., Fatima, T., Qadri, T., Rafiq, A., Malik, A., Naseer, B. and Hussain, S. Z. J. I. J. o. F. P. (2023). Biochemical, nutraceutical and phytochemical characterization of chia and basil seeds: A comparative study. **26** (1), 1-13.

Kim, H.-J., Chen, F., Wang, X., Rajapakse, N. C. J. J. o. A. and Chemistry, F. (2006). Effect of methyl jasmonate on secondary metabolites of sweet basil (Ocimum basilicum L.). **54** (6), 2327-2332.

Kim, S. Y., Hyeonbin, O., Lee, P. and Kim, Y.-S. J. J. o. d. s. (2020). The quality characteristics, antioxidant activity, and sensory evaluation of reduced-fat yogurt and nonfat yogurt supplemented with basil seed gum as a fat substitute. **103** (2), 1324-1336.

Kišgeci, J., Jelačic, S., Beatovic, D., Levic, J., Moravčevic, D., Zaric, V. and Gojkovic, L. J. A. f. e. z. (2011). Evaluation of basil seed (Ocimum basilicum L.). **14** (2), 41-44.

Kumar, S. and Pandey, A. K. J. T. s. w. j. (2013). Chemistry and biological activities of flavonoids: an overview. **2013**.

Lim, J. J. F. q. and preference. (2011). Hedonic scaling: A review of methods and theory. **22** (8), 733-747.

Lv, R., Kong, Q., Mou, H. and Fu, X. J. I. J. o. B. M. (2017). Effect of guar gum on stability and physical properties of orange juice. **98**, 565-574.

Mabood, F., Gilani, S. A., Hussain, J., Alshidani, S., Alghawi, S., Albroumi, M., Alameri, S., Jabeen, F., Hussain, Z., Al-Harrasi, A. J. S. A. P. A. M. and Spectroscopy, B. (2017). New design of experiment combined with UV–Vis spectroscopy for extraction and estimation of polyphenols from Basil seeds, Red seeds, Sesame seeds and Ajwan seeds. **178**, 14-18.

Majdinasab, M., Niakousari, M., Shaghaghian, S. and Dehghani, H. J. F. H. (2020). Antimicrobial and antioxidant coating based on basil seed gum incorporated with Shirazi thyme and summer savory essential oils emulsions for shelf-life extension of refrigerated chicken fillets. **108**, 106011.

Manach, C., Regerat, F., Texier, O., Agullo, G., Demigne, C. and Remesy, C. J. N. R. (1996). Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. **16** (3), 517-544.

Martirosyan, D. M., Singh, J. J. F. f. i. h. and disease. (2015). A new definition of functional food by FFC: what makes a new definition unique? **5** (6), 209-223.

Mathews, S., Singhal, R. and Kulkarni, P. J. F. c. (1993). Ocimum basilicum: A new non-conventional source of fibre. **47** (4), 399-401.

Munir, M., Qayyum, A., Raza, S., Siddiqui, N. R., Mumtaz, A., Safdar, N., Shible, S., Afzal, S. and Bashir, S. J. P. J. o. A. R. (2017). Nutritional assessment of basil seed and its utilization in development of value added beverage. **30** (3), 266-271.

Nadeem, F., Hanif, M. A., Bhatti, I. A., Jilani, M. I. and Al-Yahyai, R. (2020). Basil. *In:* "Medicinal Plants of South Asia".). pp. 47-62. Elsevier.

Naimi, F., Bousta, D., Balouiri, M. and Meskaoui, A. E. J. J. o. A. P. S. (2015). Antioxidant and free radical-scavenging properties of seeds flavonoids extract of Cedrus

atlantica Manetti, Linum usitatissimum L. and Ocimum basilicum L. species. **5** (8), 095-099.

Naji-Tabasi, S. and Razavi, S. M. A. J. F. H. (2017a). Functional properties and applications of basil seed gum: An overview. **73**, 313-325.

Naji-Tabasi, S. and Razavi, S. M. A. J. F. H. (2017b). New studies on basil (Ocimum bacilicum L.) seed gum: Part III–Steady and dynamic shear rheology. **67**, 243-250.

Nazir, S., Wani, I. A. J. J. o. A. R. o. M. and Plants, A. (2021). Physicochemical characterization of basil (Ocimum basilicum L.) seeds. **22**, 100295.

Nicolas, L., Marquilly, C., O'Mahony, M. J. F. q. and preference. (2010). The 9-point hedonic scale: Are words and numbers compatible? **21** (8), 1008-1015.

Paton, A., Harley, R. and Harley, M. J. B. (1999). Ocimum: an overview of classification and relationships. 11-46.

Pedro, A. C., Moreira, F., Granato, D. and Rosso, N. D. J. A. d. A. B. d. C. (2016). Extraction of bioactive compounds and free radical scavenging activity of purple basil (Ocimum basilicum L.) leaf extracts as affected by temperature and time. **88**, 1055-1068.

Pękal, A. and Pyrzynska, K. J. F. A. M. (2014). Evaluation of aluminium complexation reaction for flavonoid content assay. **7**, 1776-1782.

Phuyal, N., Jha, P. K., Raturi, P. P. and Rajbhandary, S. J. J. o. E. (2019). Zanthoxylum armatum DC.: Current knowledge, gaps and opportunities in Nepal. **229**, 326-341.

Prakasa Rao, E., Puttanna, K., Ganesha Rao, R., Ramesh, S. J. J. o. S. and Crops, A. (2011). Nitrogen and potassium nutrition of French basil (Ocimum basilicum Linn.). **16** (2).

Purushothaman, B., PrasannaSrinivasan, R., Suganthi, P., Ranganathan, B., Gimbun, J. and Shanmugam, K. J. J. o. N. R. (2018). A comprehensive review on Ocimum basilicum. 71-85.

Pushpangadan, P. and George, V. (2012). Basil. *In:* "Handbook of herbs and spices".). pp. 55-72. Elsevier.

Ramarathnam, N., Osawa, T., Ochi, H., Kawakishi, S. J. T. i. F. S. and Technology. (1995). The contribution of plant food antioxidants to human health. **6** (3), 75-82.

Ranganna, S. (1986). "Handbook of analysis and quality control for fruit and vegetable products". Tata McGraw-Hill Education. [0074518518].

Razavi, S. M., Mortazavi, S. A., Matia-Merino, L., Hosseini-Parvar, S. H., Motamedzadegan, A., Khanipour, E. J. I. j. o. f. S. and Technology. (2009). Optimisation study of gum extraction from Basil seeds (Ocimum basilicum L.). **44** (9), 1755-1762.

Razavi, S. M. and Naji-Tabasi, S. (2023). Rheology and texture of basil seed gum: A new hydrocolloid source. *In:* "Advances in food rheology and its applications".). pp. 413-458. Elsevier.

Rezapour, R., Ghiassi Tarzi, B., Movahed, S. J. J. o. F. B. and Technology. (2016). The effect of adding sweet basil seed powder (Ocimum basilicum L.) on rheological properties and staling of baguette bread. **6** (2), 41-46.

Roberfroid, M. B. J. T. A. j. o. c. n. (2000). Concepts and strategy of functional food science: the European perspective. **71** (6), 1660S-1664S.

Salehi, F., Kashaninejad, M., Tadayyon, A., Arabameri, F. J. J. o. F. S. and Technology. (2015). Modeling of extraction process of crude polysaccharides from Basil seeds (Ocimum basilicum l.) as affected by process variables. **52**, 5220-5227.

Sarfraz, Z., Anjum, F., Khan, M., Arshad, M., Nadeem, M. J. A. J. o. F. S. and Technology. (2011). Characterization of basil (Ocimum basilicum L.) parts for antioxidant potential. **2** (9), 204-213.

Schwartz, S. J., Cooperstone, J. L., Cichon, M. J., von Elbe, J. H. and Giusti, M. M. (2017). Colorants. *In:* "Fennema's food chemistry".). pp. 681-752. CRC press.

Shahidi, F. and Naczk, M. (2003). "Phenolics in food and nutraceuticals". CRC press. [0203508734].

Shahmoradi, Z., Khaledabad, M. A. and Amiri, S. J. F. B. (2023). Effect of co-encapsulation of Lactobacillus acidophilus LA5 and selenium in hydrogelated matrix of basil seed mucilage/sodium caseinate on properties of set yogurt. **55**, 103039.

Shraim, A. M., Ahmed, T. A., Rahman, M. M. and Hijji, Y. M. J. L. (2021). Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. **150**, 111932.

Simon, J. E., Morales, M. R., Phippen, W. B., Vieira, R. F., Hao, Z. J. P. o. n. c. and uses, n. (1999). Basil: A source of aroma compounds and a popular culinary and ornamental herb. **16**, 499-505.

Singh, S. and Majumdar, D. J. I. j. o. p. (1995). Anti-inflammatory and antipyretic activities of Ocimum sanctum fixed oil. **33** (4), 288-292.

Singleton, V. L., Orthofer, R. and Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *In:* "Methods in enzymology" (Vol. 299).). pp. 152-178. Elsevier. [0076-6879].

Snoussi, M., Dehmani, A., Noumi, E., Flamini, G. and Papetti, A. (2016). Chemical composition and antibiofilm activity of Petroselinum crispum and Ocimum basilicum essential oils against Vibrio spp. strains. **90**, 13-21. 10.1016/j.micpath.2015.11.004.

Thanushree Prabhuswamy, T. P., Kathiravan Tamilselvan, K. T., Michael, M., Moses, J. and Anandharamakrishnan Chinnasamy, A. C. (2019). Influence of different hydrocolloids on dispersion of sweet basil seeds (Ocimum basilicum) in fruit-flavoured beverages.

Uematsu, Y., Ogata, F., Saenjum, C., Nakamura, T. and Kawasaki, N. J. S. (2020). Removing Sr (II) and Cs (I) from the aqueous phase using basil seed and elucidating the adsorption mechanism. **12** (7), 2895.

Veberič, R. (2010). "Bioactive compounds in fruit plants". Biotehniška fakulteta Ljubljana. [9616275356].

Wang, H., Cao, G., Prior, R. L. J. J. o. a. and chemistry, f. (1996). Total antioxidant capacity of fruits. **44** (3), 701-705.

Zamani Ghaleshahi, A., Ezzatpanah, H., Rajabzadeh, G., Ghavami, M. J. J. o. f. s. and technology. (2020). Comparison and analysis characteristics of flax, perilla and basil seed oils cultivated in Iran. **57**, 1258-1268.

Appendices

Appendix A

A.I List of chemicals used

S.N.	Chemicals
1	2,6-dichlorophenol indophenol
2	Aluminum trichloride
3	Boric acid
4	Buffers
5	Citric acid
6	Dextrose
7	DPPH
8	Ethanol
9	Folin-Ciocalteu
10	Gallic acid
11	Hydrochloric acid
12	L-ascorbic acid
13	Metaphosphoric acid
14	Methanol
15	Methyl red
16	Methyl orange
17	n-hexane
18	Quercetin
19	Sodium carbonate
20	Sodium hydroxide
21	Sulfuric acid

B.II List of equipment used

S.N.	Materials used
1	Autoclave
2	Buchner filtration set
3	Burette/pipette/beakers and other glassware
4	Centrifuge
5	Cuvette
6	Digital balance
7	Electric cabinet dryer
8	Grinder
9	Heating mantle
10	Hot air oven
11	Incubator
1	Magnetic stirrer
13	Micro-Kjeldahl
14	Micropipette
15	Mortar-pestle
16	Muffle furnace
17	pH meter
18	Refractometer
19	Rotary shaker
20	Sintered glass crucible
21	Spectrophotometer
22	Thermometer
23	Thermal gun
24	Utensils, knives, etc.
25	Vortex mixture

Appendix B

Sensory Analysis Score Card

Name of the panelist: Date:

Name of the product: Basil seed drink

Dear panelist, you are provided with 4 samples of basil seed drink on each proportion with variation on basil seed gum. Please, test the following samples and check how much you prefer for each of the samples. Give the point for your degree of preference for each sample as shown below.

Judge the characteristics on the 1-9 scale as below:

Like extremely – 9	Like slightly – 6	Dislike moderately – 3
Like very much – 8	Neither like nor dislike – 5	Dislike very much – 2
Like moderately – 7	Dislike slightly – 4	Dislike extremely – 1

Parameters	Product code			
	A	B	C	D
Color				
Taste				
Mouthfeel				
Appearance				
Overall acceptability				

Any comments:

……...…….

……

Signature:

Appendix C

C.I. One way ANOVA for color

Source of variation	DF	Sum of squares	Mean square	F ratio	Prob>F
Sample	3	1.0938	0.3646	0.75	0.532
Residual	28	13.6250	0.4866		
Total	31	0.4866			

Tukey's 95% confidence intervals

Sample		Mean
A	a	6.625
D	a	6.875
C	a	7.000
B	a	7.125

C.II. One way ANOVA for taste

Source of variation	DF	Sum of squares	Mean square	F ratio	Prob>F
Sample	3	7.2500	2.4167	8.20	<.001
Residual	28	8.2500	0.2946		
Total	31	15.5000			

Tukey's 95% confidence intervals

Sample		Mean
D	a	6.250
A	ab	6.625
C	ab	6.875
B	b	7.375

C.III. One way ANOVA for mouthfeel

Source of variation	DF	Sum of squares	Mean square	F ratio	Prob>F
Sample	3	7.0938	2.3646	4.61	0.010
Residual	2	14.3750	0.5134		
Total	31	21.4688			

Tukey's 95% confidence intervals

Sample		Mean
D	a	6.250
A	a	6.500
C	ab	6.875
B	a	7.500

C.IV. One way ANOVA for appearance

Source of variation	DF	Sum of squares	Mean square	F ratio	Prob>F
Sample	3	16.0938	5.3646	9.32	<.001
Residual	28	16.1250	0.5759		
Total	31	32.2188			

Tukey's 95% confidence intervals

Sample		Mean
A	a	5.625
C	b	7.125
B	b	7.250
D	b	7.375

C.V. One way ANOVA for overall acceptability

Source of variation	DF	Sum of squares	Mean square	F ratio	Prob>F
Sample	3	6.0938	2.0312	4.33	0.0013
Residual	28	13.1250	0,4688		
Total	31	19.2188			

Tukey's 95% confidence intervals

Sample		Mean
A	a	6.250
D	a	6.375
C	ab	6.625
B	b	7.375

Appendix D

D.I calibration curve for TPC

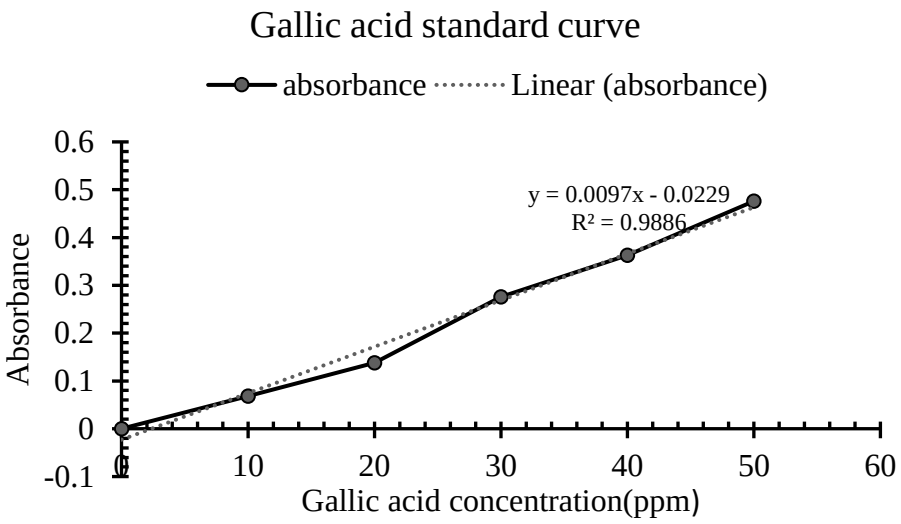

Figure D.I Standard Gallic acid curve

D. II. Calibration curve for flavonoid

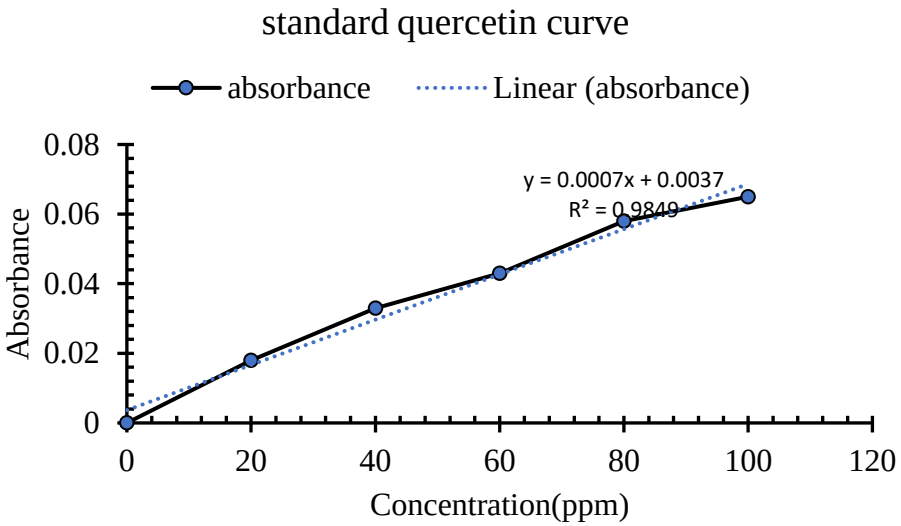

Figure D.II Standard quercetin curve