"FORMULATION AND QUALITY EVALUATION OF SESAME-PUMPKIN BURFI"

Project work submitted to

DEPARTMENT OF PG STUDIES IN FOOD SCIENCE AND NUTRITION

BESANT WOMEN'S COLLEGE, MANGALORE

in partial fulfilment of requirement for the award of DEGREE OF MASTER OF SCIENCE IN FOOD SCIENCE AND NUTRITION

Submitted By,

K. MAYA KUDVA

Reg. No:189044801

Department of PG studies in Food Science and Nutrition

Besant Women's College, M.G. road, Kodialbail

Mangalore-575003

September 2020

CERTIFICATE

This is to certify that the dissertation titled "Formulation and Quality Evaluation of Sesame-Pumpkin Burfi" is a bona fide record of independent research work done by ***Ms. K. MAYA. KUDVA (REGISTER NO. 189044801)*** under my supervision during **January 2020-April 2020**, submitted to the Mangalore university, in partial fulfilment for the award of the ***Degree of Master of Science in Food Science and Nutrition***, and that the dissertation has not previously formed the basis for the award of any other degree, diploma, associateship, fellowship or other tile.

Place: MANGALORE

DATE: 10 SEPTEMBER 2020

RESEARCH GUIDE

DR. ASHA RAI M.G.

M.Sc.,M.Phil.,PhD

Head

Department of PG Studies in Food Science and Nutrition

Besant Women's College

M.G. Road, Kodialbail

Mangalore 575003

Contact no: 8147985337

BESANT WOMEN'S COLLEGE

M.G. ROAD, MANGALORE-575003

(accredited by the NAAC with 'A' Grade)

CERTIFICATE

This is to certify that the project work entitled "***FORMULATION AND QUALITY EVALUATION OF SESAME-PUMPKIN BURFI"***, submitted to the ***Department of PG Studies in Food Science and Nutrition***, by ***Ms. K. MAYA KUDVA (Reg.no 189044801),*** towards the partial fulfilment of the degree of ***Master of Science in Food Science and Nutrition***, is a faithful record of original work carried out by her in the academic year 2019-2020.

HEAD OF THE DEPARTMENT

Place: MANGALORE

DATE: 10 SEPTEMBER 2020

DECLARATION

I, ***K. Maya. Kudva***, do hereby declare that the study recorded in this dissertation titled "***FORMULATION AND QUALITY EVALUATION OF SESAME-PUMPKIN BURFI***", was conducted by me under the guidance of ***DR. ASHA RAI M. G.,*** **Head of the Department of PG studies in Food Science and Nutrition, Besant Women's College, Mangalore**, in partial fulfilment of the requirement for the Degree of Master of Science in Food Science and Nutrition.

The information and the data given in the report is correct and true to the best of my knowledge. I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text.

I also declare that the work has not been submitted to any other university or institution for the award of any degree, diploma, or title of recognition.

Mangalore

September 2020 K. Maya. Kudva

ACKNOWLEDGEMENT

I would like to thank the following people without whom I would not have been able to complete this dissertation.

First, I would like to express my sincere gratitude to ***Dr. Asha Rai M.G.***, my guide and Head of the Department of PG Studies Food Science and Nutrition, Besant Women's College, for her constant guidance, support, encouragement, and patience.

I owe my sincere thanks to respected principal, ***Dr. Satish Shetty P.***, Besant Women's College, Mangalore, for his constant support and encouragement in my endeavours.

I would like to thank my lecturers, ***Mrs. Indira Badya, Mrs. Shwetha Singh, Mrs. Roopa Rao K,*** and ***Ms. Firhath*** for their recommendations and valuable inputs from time to time.

I wish to place on record my heartfelt thanks to ***Dr. Archana Prabhat*** (Head of the Department of Food Science and Nutrition, Alva's Centre for PG Studies and Research, Moodbidri) and ***Dr. Prabhavathi S.N.*** (Assistant professor, Department of M.Sc. Food Science and Nutrition, University of Mysore) for their willingness to impart knowledge and give practical suggestions whenever solicited.

I am grateful to ***Mrs. Sucharitha Suresh*** for her help with statistics.

I am thankful to my friends ***Sona Amin*** and ***Nireeksha. V*** for their considerate suggestions.

Finally, my biggest thanks to my mother ***Vaidehi R. Kudva*** and my father ***Radesh Kudva*** who set me off on the road to this M.Sc. degree. They have supported me positively and boosted my morale at every step of the way. I am indebted to them for their continuous encouragement and assistance in any way they could throughout the project.

Place : Mangalore K. Maya. Kudva

Date: 10 September 2020

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LIST OF ABBREVIATIONS

%	Percentage
g	Grams
mg	Milligrams
µg	Micrograms
ml	Milliliter
kJ	Kilo joule
kcal	Kilo calorie
°C	Degree Celsius
cfu	Colony forming unit
F1	Formulation 1
F2	Formulation 2
F3	Formulation 3
F4	Formulation 4

ABSTRACT

Sweets are indeed relished by the young and the old alike but are a source of empty calories. This project was an attempt to develop a sweet, which will not only be delicious, but will also provide nutrients to the body. The objective of this project was to formulate and develop a sesame-pumpkin burfi and determine its proximate composition and shelf life. The sesame seeds were subjected to roasting and germination, after which their calcium and phosphorus content was determined along with that of raw sesame seeds. The calcium content was highest in roasted sesame seeds and the phosphorus content was highest in raw sesame seeds. Four different formulations of burfi were made with varying ratios of sesame seed and pumpkin. Sensory evaluation of all the four formulations was done, in which Formulation 4 (F4) burfi was the most acceptable. F4 burfi and Control burfi (burfi made only with pumpkin) were subjected to proximate analysis and shelf life study. Proximate analysis of both the samples revealed that the F4 burfi had higher amounts of ash, protein, fat, carbohydrate, crude fiber, calcium and phosphorus. Consequently, the calorific value of F4 burfi was higher than that of Control burfi. The shelf life study was done with sensory evaluation and microbial analysis. In F4 burfi, highly significant difference ($p<0.01$) was found with respect to all the characteristics (appearance, color, texture, aroma, taste, and overall acceptability). Whereas, in Control burfi, highly significant difference ($p<0.01$) was found with respect to texture, aroma, taste, and overall acceptability. Though the Control burfi had higher rating than the F4 burfi for all the sensory characteristics, both had sensory ratings within the acceptable range. Both the burfis had a shelf life of 7 days. The microbial count of both the burfis, on the first day of storage and on the day of visible mould growth, was within the acceptable range.

Key words: sesame, calcium, pumpkin.

Chapter 1

INTRODUCTION

Product development is nothing but creation of an entirely new product or improving an existing product. Product development begins with deciding the potential of a new food product which will be not only economical but also accepted by the community. The product must be put through a series of checks such as, taste sampling, consumer sampling and shelf life study. Only after this, the product is produced on a large scale, packaged and marketed.

1.0. INTRODUCTION.

Sweets are an important and inseparable part of any joyous occasion. However, sweets are often high in empty calories and have less nutritive value. There is a need to develop sweets that are not only relished but also provide our body with the much-needed nutrients. Everyone likes sweets and developing such healthy sweets can be a great way of meeting the daily requirements of nutrients. The aim of this project is to develop a sweet which will not only taste good but will also provide nutrients to the body.

This study is an effort to create a product comprising essentially sesame seeds and pumpkin. Sesame seeds are a rich source of protein and calcium whereas pumpkin forms the base of the product as it is a locally grown vegetable and is easily affordable. The product is essentially in the nature of 'burfi'- an Indian dessert. In the traditional cuisine burfi is prepared using *khoa* (concentrated milk product). However, there are many burfis which are not milk-based such as besan burfi, coconut burfi etc.

Sesame is one of the most ancient oilseed crops known to mankind. It is a member of Pedaliaceae family **(Hegde, D. M. 2012)**. It is commonly known as *til* (hindi), *tal* (gujarati), *tili* (punjabi), *nuvvulu* (telugu), *ellu* (tamil) and *rasi* (oriya) and so on in different parts of the country. About 36 species of sesame are said to be existent. The major sesame producers are India, Myanmar, China and Sudan **(Nagendra Prasad, M. N. *et al* 2012)**. Sesame seeds are tiny, flat, oval with a nutty taste. They vary in size from small to large and come in a host of different colors depending upon the variety including white, yellow, lack, grey, brown and red **(Bisht, I. S. *et al* 1998)**.

Sesame has many domestic and industrial applications. The oil extracted from sesame seeds is used as cooking oil and in the production of margarine; in formulations of body massage creams soaps and lubricants; as a solvent for intramuscular injections and as an insecticide. Sesame oil meal, obtained after extraction, is a nutritious live-stock feed. Roasted sesame seeds are used for garnishing bread loaves, biscuits, bagels, chocolates. In the Middle East sesame seeds are mainly utilized in the production of sesame butter(tahina) **(Elleuch, M. *et al* 2011).** In India, sesame seeds are used in the manufacture of traditional confections such as laddu and chikki. They are eaten whole after roasting.

The seeds are rich in fat, protein, carbohydrate, fiber and some minerals. The seeds are an important source of oil (44-58%), protein (18-25%), carbohydrate (~13.5%) and ash (~5%). Sesame seed contains approximately 50 percent oil (out of which 35% is monounsaturated fatty acids and 44% polyunsaturated fatty acids) and 45 percent meal (out of which 20% is protein). The seed also contains significant amount of important minerals with the potassium concentration being the highest, followed by phosphorus, magnesium, calcium, and sodium **(Tenyang, N., *et al* 2017).**

Approximately 14% protein is present in a healthy young adult, most of that is present in muscle tissue. Formation of every tissue in the body including teeth and bones requires protein and amino acids. Proteins perform many structural, protective, and regulatory functions in the body. The human body needs protein to function normally and without protein, the most basic functions of life such as respiration, muscle contraction cannot be carried out. Proteins are involved in growth, maintenance, and repair of the body tissues. They maintain the fluid balance and acid-base balance. Proteins are required for synthesis of many enzymes, hormones, neurotransmitters, antibodies, lipoproteins etc. Inadequate intake of protein over a long period of time can lead to protein energy malnutrition.

Calcium is the most abundant mineral in the body. 99% of the body calcium is present in bones, the remaining 1% is found in the teeth and soft tissues and out of this, only 0.1% is distributed in extracellular fluid. It is important for the normal growth and development of the skeleton. Calcium present in extracellular fluid has an important role in blood clotting, muscle contraction and relaxation, nerve transmission and glandular secretion. Calcium deficiency caused by poor dietary intake affects bone growth and bone mineralization in childhood and adolescence and leads to loss of bone minerals in adults. Calcium deficiency causes rickets in children and osteoporosis and osteomalacia in adults **(Textbook of Human Nutrition, 2014).**

Pumpkin is from genus *Cucurbita* of the family *Cucurbitaceae*. It includes squash and cucumbers which are grown throughout the tropical and sub-tropical countries. There are three common types of pumpkin worldwide, namely *Curcurbita pepo*, *Curcurbita maxima* and *Cucurbita moschata*. Pumpkin can be found in many shapes, sizes, and colours. In India, these are mostly consumed in fresh vegetable preparations and are also used as a

thickening agent in soups **(Bhat, M. A. *et al* 2013)**. Pumpkin is a good source of carotene, pectin, mineral salts, vitamins, and other substances that are beneficial to health **(Saeleaw, M. *et al* 2011).** Pumpkin is a valuable source of functional components mainly carotenoids, lutein, zeaxanthin, vitamin E, ascorbic acid, phytosterols, selenium, and linoleic acid, which act as antioxidants in human nutrition **(Dhiman, A.K. *et al*., 2009).**

OBJECTIVES OF THE STUDY:

- ✓ To formulate and develop sesame-pumpkin burfi.
- ✓ To determine the proximate composition and shelf life of the sesame-pumpkin burfi.

Chapter 2

REVIEW OF LITERATURE

Developing a new product is a complex process. This requires knowledge of ingredients, processing methods, consumer demands and preferences etc. Having knowledge of these aspects helps one to make the right decision. This study focuses on developing a Sesame-Pumpkin burfi. In this study, pumpkin has been used as the base because it is a staple vegetable which is easily available and inexpensive. Review of available literature related to the two main components provides the foundation for the product development process.

2.0 REVIEW OF LITERATURE

2.1 SESAME SEEDS

Sesame seeds contain 6-7% moisture, 17-32% protein, 48-55% oil, 14-16% sugar, 6-8% fibre and 5-7% ash. The oil content varies from 37-63% depending upon the variety and growing season. Sesame seed is a good source of certain minerals, particularly calcium, phosphorus, and iron. The seeds contain approximately 4-7% minerals. Sesame seeds are also a good source of certain vitamins, particularly niacin, folic acid, and tocopherols **(Hegde, D.M. 2012).**

TABLE 1

Nutritive value of sesame seeds (white) per 100 grams

Energy (kJ)	2174±9
Protein (g)	21.70±0.44
Fat (g)	43.05±0.04
Carbohydrate(g)	10.83±0.50
Moisture (g)	3.30±0.28
Ash(g)	4.13±0.16
Dietary fibre(g)	16.99±0.30
Calcium (mg)	1283±149
Phosphorus (mg)	754±42.6
Iron (mg)	15.04±2.09

Source: Indian Food Composition Tables, 2017

2.1.1. EFFECT OF PROCESSING METHODS

Makinde, F.M., *et al*., (2013) studied the nutritional composition and effect of processing treatments on antinutritional factors of Nigerian sesame cultivars. The sesame seeds were used as whole, dehulled seeds and hulls. Proximate composition, minerals, vitamins, and anti-nutritional factors of whole seeds, dehulled seeds and hulls were determined. The whole and dehulled seeds were subjected to soaking, germination, roasting, auto claving and

cooking. The effect of these methods on the anti-nutritional factors were determined. The whole seeds contained moisture (4.18-5.41%), protein (21.9-23.6%), fat (45.6-46.1%), crude fiber (4.0-7.15%), ash (6.16-.34%) and carbohydrates (10.8-1.0%). Dehulled sesame seeds had 25.3-26.8% protein, 47.7-49.9% fat and 9.7-12.4% carbohydrate. The hulls had the lowest amount of protein, fat, and carbohydrate. The amount of calcium and phosphorus was higher in whole seeds compared to dehulled seeds and hulls. All processing treatments reduced the anti-nutritional factors in whole and dehulled seeds. But germination lead to maximum reduction in the anti-nutritional factors.

Makinde, F.M., *et al*., (2014) compared the nutritional quality of flour obtained from raw, roasted, and fermented sesame seeds grown in Nigeria. Nutritional value of all the three sesame flours (raw, roasted and fermented) were determined. The proximate content of sesame was protein 15.4-26.5g/100g, fat 52.4-62.8g/100g, crude fiber 3.34-3.89g/100g, ash 3.93-6.78g/100g, carbohydrate 11.7-13.4g/100g and energy 550-593. Kcal/100g. The sesame had high amounts of calcium (464-56mg/100g), phosphorus (442-508mg/100g), magnesium (399-455mg/100g) and potassium (336-489mg/100g). The phytate and oxalate content of roasted sesame and fermented sesame was considerably lower than raw sesame. The study reveals that roasting and fermentation process significantly increases the nutritional quality of sesame. It was found that calcium content of sesame increased when subjected to fermentation and roasting.

Makinde, F.M., *et al*., (2016) studied the effect of different roasting techniques (open pan roasting and microwave roasting) on the chemical composition of sesame seed flour and physico-chemical properties of its oil. The open pan roasted sesame flour (ORSF) and microwave roasted sesame flour (MRSF) were analyzed for proximate composition, mineral, vitamin, anti-nutritional factors. The seed oil was analyzed for peroxide and iodine value. It was seen that the crude fat, ash and fiber and energy vale was higher in MRSF and ORSF compared to raw sesame flour. The protein and carbohydrate values of roasted flour were lower than that of raw sesame flour. The concentration of calcium, potassium, phosphorus, and magnesium was lowest in raw sesame flour. The thiamine, riboflavin and niacin value were low in roasted flour. The phytate and oxalate content was significantly low in the roasted sesame flour when compared to raw sesame flour. The peroxide value increased in

roasted flour and iodine value decreased in roasted flour when compared to raw flour. This study reveals that roasting significantly increases the nutritional value of sesame. And, the anti-nutritional content also significantly reduces on roasting.

Ghavidel, R.A. *et al*., (2007) studied the impact of germination and dehulling on nutrient, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds (green gram, cowpea, lentil and chickpea). The variations were- control, germinated, and germinated + dehulled. It revealed that germination increases the protein, thiamine content and in-vitro iron and calcium bioavailability as well as in-vitro starch and protein digestibility in all the legume samples. There was a further increase observed in all the samples that were dehulled after germination. This study infers that germination increases nutrient content and nutrient bioavailability.

Poneros-Schneier, A.G., *et al*., (1989) studied the bioavailability of calcium from sesame seeds, almond powder, whole wheat bread, spinach, and non-fat dairy milk in rats. The study revealed that calcium bioavailability was highest in non-fat dairy milk and lowest in spinach. The relative bioavailability of the products was $CaCO_3$(100%), non-fat dairy milk (100%), whole wheat bread (95%), almond powder (65%), sesame seeds (65%), non-fat dairy milk and spinach mixture (52%) and spinach (47%). This study suggests that sesame seeds contain an intermediate level of bioavailable calcium.

OM, A. *et al*., (2020) studied the antinutrients, bio accessibility and mineral balance of cookies produced from processed sesame seed flour blends. The sesame seeds were subjected to defatting, cooking, roasting, germination and fermentation and processed into flour. The effect of the above used processing methods on anti-nutritional content of the flour was analyzed. The total mineral content, in-vitro bioaccessibilty and mineral balance of the developed cookies was determined. The calcium, magnesium and iron content of the cookies ranged from 2220.95-2916.9mg/100g, 25.21-278.58mg/100g and 30.57-45.55mg/100g respectively. Percent mineral bioaccessibility ranged from 40.60-97.85%, 15.07-92.14%, 25.05-97.67%, 2.54-89.81% and 25.13-98.18 % for zinc, copper, calcium, magnesium and iron respectively. Mineral balance of the cookie samples for zinc, copper, calcium, magnesium and iron was 2.14-59.3%, .85-86.33%, 2.32-74.94%, 39.95-97.54% and 3.75-74.87% respectively. Increased percentage of substitution of cooked, roasted and fermented

sesame flour increased the bioaccessibility of minerals in the cookies. It infers that processing methods like roasting, germination increase the bioaccessibility of calcium.

Alobo, A.P. (2001) studied the effect of sesame seed flour on millet biscuit characteristics. Millet flour was replaced with defatted sesame seed flour at 30, 40 and 50% and biscuits were prepared. Proximate composition of the flours was determined. The physicochemical characteristics and sensory characteristics of the biscuits were analyzed. Sesame flour had more protein, fat, ash and crude fiber than millet flour. The sesame-millet blend flour has higher protein contents. The biscuit made with blend flour were lighter than those prepared only with millet flour. Diameter and weight of biscuits reduced with increase in the level of sesame replacement. In sensory evaluation, the biscuit had high scores for flavor and crispness but low scores for color. The low scores were because of darkening occurring due to sugar caramelization.

2.2 CALCIUM

Calcium is essential for normal growth and development of the skeleton. During skeletal growth, the calcium accumulates in the skeleton at the average rate of 150mg/day. Adequate amount of calcium is crucial for achieving optimal peak bone mass and alters the rate of bone loss associated with aging **(Introduction to Human Nutrition, 2nd edition,2009).**

A high proportion of body calcium is present in bones and hence its development and maintenance are the major determinants of calcium needs. Calcium requirements differ in various stages of life. The requirements are greatly increased in childhood and adolescence and during pregnancy, lactation and after the onset of menopause **(Flynn,A. 2003).**

2.2.1. CALCIUM INTAKE

Harinarayan, C.V. *et al*., (2015) studied the dietary calcium intake pattern in South Indian rural, urban and metropolitan city subjects. In this study, the dietary intake of calcium and phytate intake of 524 healthy subjects from Bengaluru was recorded and was compared with the dietary calcium intake of 325 healthy subjects from rural areas around Tirupati, and 508 healthy subjects from urban Tirupati. The intake was recorded by recalling the diet consumed in the previous 5 to 7 days. It was found that the dietary calcium intake was significantly lower in the rural subjects compared to the urban and metropolitan city subjects. Also, the

dietary [phytate was found to significantly differ in both the rural, urban as the metropolitan city groups. The consumption of milk, milk products and vegetables were higher in the metropolitan and urban groups compared to the rural groups. Hence, there is a need to improve the dietary calcium intake which can be done by enrichment or supplementation.

Calcium intake influences the risk of osteoporosis by affecting the genetically determined peak bone mass. Peak bone mass is accumulated during the first two decades of life. It has a huge impact of the bone density during old age (**Nicklas, T.A., *et al.*, 2003**).

2.2.2. CALCIUM DEFICIENCY

A study by **Sandler, R.B. *et al.*, (1985)** correlates postmenopausal bone density and milk consumption in childhood and adolescence. The study involved 255 women. The primary criteria for admission to the study required the women to be at least one year after the menopause and to refrain from estrogen replacement therapy. The bone density was assessed, current calcium intake was estimated by the method of the three-day diet record and calcium intake in childhood, adolescence and adulthood was assessed retrospectively. The data obtained indicated that women who reported drinking milk with every meal during childhood and adolescence had significantly higher bone density than women who reported drinking milk less frequently. This suggests that prevention begins in childhood and adolescence during growth and skeletal development.

Aggarwal, V. *et al.*, (2012) studied the role of calcium and vitamin D deficiency in causation of nutritional rickets in young Indian children. In this study, 67 children with nutritional rickets were compared with 68 age-and-sex matched healthy controls for demographic factors, nutritional status, dietary calcium and phytate intake, sun exposure and biochemical parameters. The data disclosed that the calcium intake of cases was lower than that of the controls. The serum levels of 25- hydroxyvitamin D was low in both the case and controls. The phytate intake was also higher in the cases. This study infers that coexistence of low dietary calcium with a low or borderline vitamin D nutrition status leads to rickets.

Marwaha, R.K. *et al.*, (2011) conducted a study to evaluate bone health status in elderly Indians. The study involved 1600 subjects (>50 years of age) staying in Delhi, India. They were evaluated for anthropometric, biochemical and hormonal parameters. It revealed that

out of 1600 healthy subjects, 35.1% (24.6% males and 42.5 % females) were osteoporotic and 49.5% (54.3% males and 44.9% females) had osteopenia. This conveys that a large population of elderly Indian subjects are osteoporotic.

2.3. PUMPKIN:

Pumpkin belongs to the family *Cucurbitaceae*. Commonly grown species are *Cucurbita pepo, Cucurbita moschata* and *Cucurbita maxima. C.moschata* grows throughout all the regions of India, whereas *C.maxima* grows in hills and subtropical regions. The summer and rainy season are the main growing season, but winter pumpkins are also cultivated in southern and western India. India is one of the leading producers of pumpkin in the world. The pumpkin contains approximately 85-90% water, 70-86% edible portion, 2.0-2.1% protein, 0.3-0.6% fat, 1.4-3.5% starch, 1.1-2.7% dietary fiber and 179-190kJ of energy(40-45kcal). Pumpkin pulp is low in protein but rich in sodium, potassium, iron, manganese and phosphorus and pectin **(Dhiman, A.K. *et al*., 2009)**

TABLE 2

Nutritive value of pumpkin per 100 grams.

Energy (kJ)	97±8
Protein (g)	0.84±0.21
Fat (g)	0.16±0.02
Carbohydrate(g)	4±0.64
Moisture (g)	91.85±0.45
Ash(g)	0.58±0.09
Dietary fibre(g)	2.56±0.11
Calcium (mg)	23.06±4.30
Phosphorus (mg)	22.18±7.47
Iron	0.36±0.11

Source: Indian Food Composition Tables, 2017

Azizah, A.H., *et al*., (2009) studied the effect of various cooking methods on antioxidant activity of pumpkin. The pumpkin was subjected to two methods of cooking: stir-

frying(180ºC) and boiling (100ºC); each for 2, 4 and 6 minutes. The beta-carotene and lycopene content were determined using HPLC method and total phenolics was measured using Folin-Ciocalteau method. The radical scavenging activity of the sample was determined by 1, 1-diphenyl-2-picrylhydrazyl assay. Both the cooking methods led to an increase in beta-carotene (2-4 times) and lycopene (17 to 40 times). But there was a decrease in the total phenolics (18 to 54%) in the pumpkin after cooking by boiling and stir-frying. However, the cooked pumpkin exhibited high radical scavenging activity (81.1%-94.6%).

Dhiman Anju,K. (2017) formulated an Instant Halwa Mix from dehydrated pumpkin and studied its stability during storage. Instant halwa mix was prepared using dehydrated pumpkin in the form of granulated powder and shreds. The dehydrated powder was used individually and in combination with pumpkin seed powder. The Instant Halwa Mix prepared with dehydrated pumpkin shreds and seed powder had the highest amount of crude protein and fiber. Halwa prepared from dehydrated pumpkin shreds had the highest amount of beta-carotene (6.90mg/100g). the crude protein was found to increase significantly when prepared by addition of pumpkin seed powder. Halwa prepared from granulated pumpkin powder had the highest score in sensory evaluation. With the passage of time the score of sensory evaluation decreased. The instant halwa mix was found to have a shelf life of 6 months at ambient conditions. Also, the instant halwa mix stored in aluminium laminated pouch had lower score in sensory evaluation when to compared to that stored in polyethylene pouches.

2.4. PRODUCT DEVELOPMENT

Agrahar-Murugkar, D. *et al*., (2018) developed cookies naturally fortified with calcium and protein rich ingredients – sesame seeds, soy butter, dried *moringa* leaves and coconut powder. The effect of these ingredients on the texture, nutritional quality and sensory acceptance of the cookies was analyzed. Two types of cookie were prepared- salted and sweet. Both these varieties were fortified with sesame seeds and soy butter. The *moringa* leaves powder and coconut powder was added to fortified salted cookies and fortified sweet cookies respectively. It was revealed that the fortified cookies had improved nutritional and functional properties. There were no unfavorable changes in the sensory characteristics. 100 grams of salted sesame cookies provide 64% of the Recommended Dietary Allowances (RDA) of protein in Indian children. Similarly, salted soy butter cookies provide 55% of the

RDA for protein, whereas sweet sesame cookies provide 41% RDA for protein and sweet soy butter cookies provide 37% of the RDA for protein. 100 grams of sesame salted cookies provide 52%, soy salted cookies provide 46%, sweet sesame cookies provided 45% and sweet soy cookies provide 40% of the RDA for calcium in Indian children. These cookies are a healthy evening snack, which will partially meet the RDA for calcium and protein.

Olagunju, A.I., et al., (2013) formulated wheat cookies incorporated with germinated sesame seeds and evaluated its nutritional and sensory attributes. There was a noticeable increase in the protein content of sesame seeds due to germination. The process of germination also reduced the antinutrients significantly. There was an increase in the protein fat and ash content of wheat-sesame cookies with an increase in sesame supplementation. Cookies supplemented with 5% germinated sesame flour was highly acceptable by the consumers. By this we can conclude that germination increases the protein content and decreases the antinutritional content. Also, sesame supplementation improved the nutritive value of wheat cookies.

Gayathri, G. *et al*., (2017) formulated a low-cost food supplement by using leaves of agathi, sesame seeds, ragi, soybean and black gram dal. The ingredients were subjected to various processing techniques like soaking, germination, sun-drying, roasting and powdering. Laddu was prepared by combining the powdered ingredients in three different proportions – A (15:25:30: 25:5), B (15:20:30:25:10) and C (10:15:30:25:10). The laddu was analyzed for calcium. Sensory evaluation and shelf life study of the product was done. The combination A (15:25:30: 25:5) was found to have the highest calcium content (701mg/100mg) and was highly acceptable on sensory evaluation. The shelf life of the product was 75 days. Developing such type of low-cost calcium-rich product can be beneficial in delaying the onset and the progression of different bone diseases.

Chapter 3

MATERIALS AND METHODS

The study aims to develop a nutritious yet tasty Sesame-Pumpkin burfi. The processed sesame seeds were analysed for changes in calcium and phosphorus content. The burfi was made in different formulations. The burfi which was the most accepted in sensory evaluation, was further subjected to proximate analysis and shelf life study.

3.0. MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Raw Ingredients

Pumpkin, white sesame seeds, sugar, ghee, and cardamom were purchased from Central market, Mangalore.

3.1.2. Equipment

Weighing balance (Essae-Teraoka Pvt. Ltd), Hot air oven (B.D. instrumentation, Ambala Cantt) , muffle furnace (Rotek instruments, Kerala), 2375 Double beam spectrophotometer, water bath (Labotech instruments, B.D. instrumentation, Ambala Cantt) , centrifuge machine (Remi elektrotechnik, Vasai)

3.1.3. Reagents and Chemicals

All the reagents and chemicals used were of AR grade procured from Fisher Scientific, Mumbai; Medilise, Kerala; Emplura, Mumbai.

Copper sulphate penta hydrate, concentrated Sulphuric acid, Sodium hydroxide pellets, concentrated Hydrochloric acid, Glucose, Anthrone reagent, solid Sodium carbonate, Ammonium oxalate, Glacial acetic acid, Ammonia, Potassium permanganate, Silver nitrate, Ammonium molybdate, 1-amino-2-naphthol-4-sulphonic acid, Anhydrous mono potassium phosphate, Sodium sulphite, Sodium bisulphite, Distilled water, Double distilled water, Folin-Ciocalteau reagent, sodium potassium tartarate, di-potassium hydrogen phosphate, potassium dihydrogen phosphate, hexane

3.1.4. Glassware used

Test tubes, beakers, conical flasks, glass rod, burette, standard flask, measuring cylinder, watch glass, Petri dish, pipettes, micropipettes, conical flask

3.2. METHODS

3.2.1. Processing of sesame seeds and preparation of sesame seed powder.

3.2.1.1 Roasted sesame powder

The sesame seeds were cleaned washed and sun-dried. The seeds were roasted in an open pan on a medium flame for about 10-15 minutes till the seeds started popping. After cooling to room temperature, the seeds were blended in a home mixer to give a coarse powder. The coarse powder was stored in an airtight container and refrigerated until further analysis.

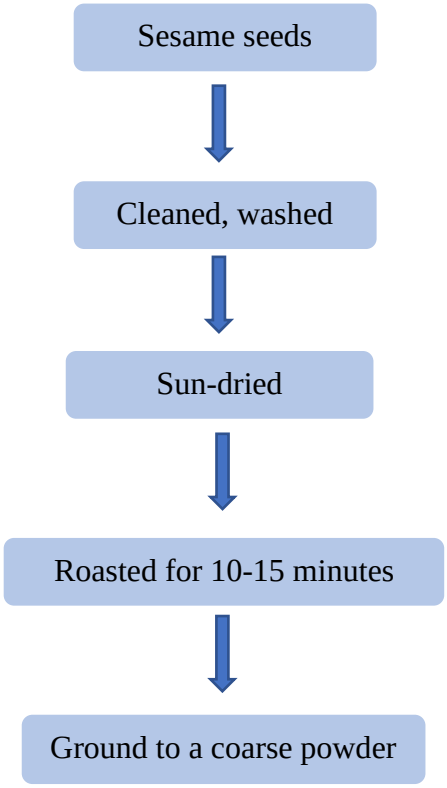


Figure 1: Schematic representation of preparation roasted sesame powder

3.2.1.2. Germinated sesame powder

The sesame was cleaned, washed, and soaked in water for 4 hours. The water was drained, and the seeds were tied in a wet muslin cloth and left overnight (7-8 hours) for germination. The germinated seeds were sundried and powdered in a home mixer to form a coarse powder. The powder was stored in an airtight container and was refrigerated until further analysis.

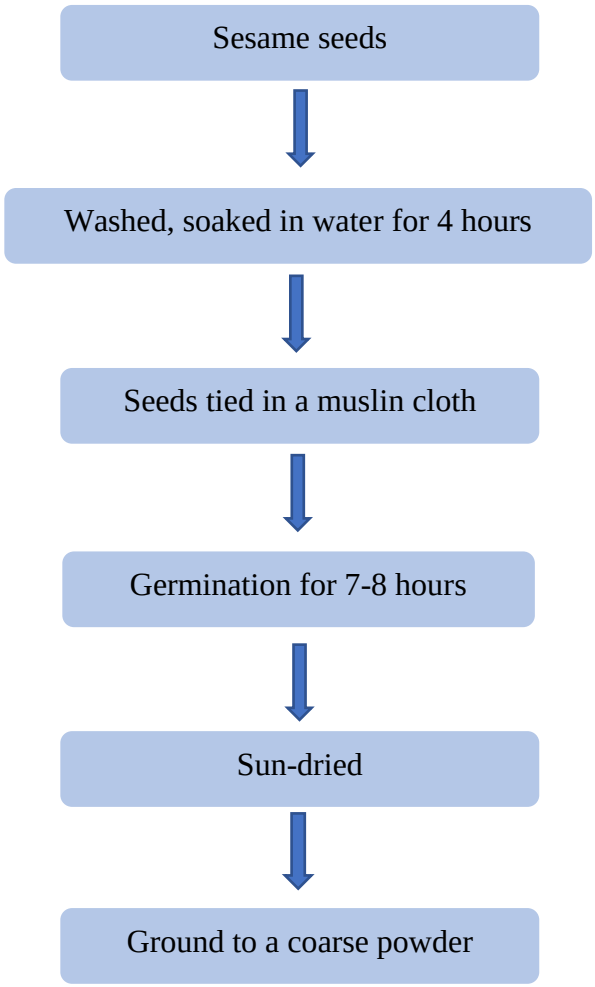
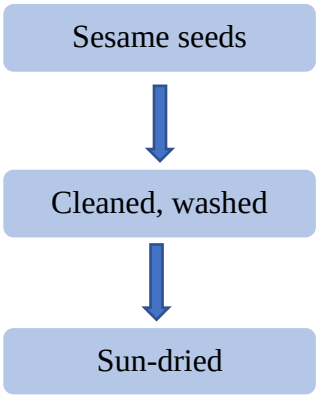


FIGURE 2: Schematic representation of the preparation of germinated sesame powder

3.2.1.3. Raw sesame powder

The raw sesame seeds were cleaned, washed, sun-dried and ground to a coarse powder. The powder was stored in an airtight container and was refrigerated until further analysis



Ground to a coarse powder

FIGURE 3: Schematic representation of the preparation of raw sesame powder

Plate 1: Raw Sesame seeds

Plate 2: Roasted Sesame seeds

Plate 3: Germinated Sesame seeds

3.2.2. Estimation of calcium and phosphorus content in processed sesame seeds

The calcium and phosphorus content of the raw, roasted, and germinated sesame seeds were determined. The calcium content of the raw, roasted, and germinated sesame seeds was estimated by titration against potassium permanganate (AOAC,1980). The phosphorus content of the samples was estimated by the Fiske Subbarow method (Raghuramulu et.al., 2003).

3.2.3. Development of sesame-pumpkin burfi:

3.2.3.1. Formulation of sesame-pumpkin burfi

The burfi was prepared according to different formulations given in Table 3.

In formulation F1, the percentage of roasted sesame powder incorporated is 20%.

In formulation F2, the percentage of roasted sesame powder incorporated is 25%.

In formulation F3, the percentage of germinated sesame powder incorporated is 20%.

In formulation F4, the percentage of germinated sesame powder incorporated is 25%.

TABLE 3

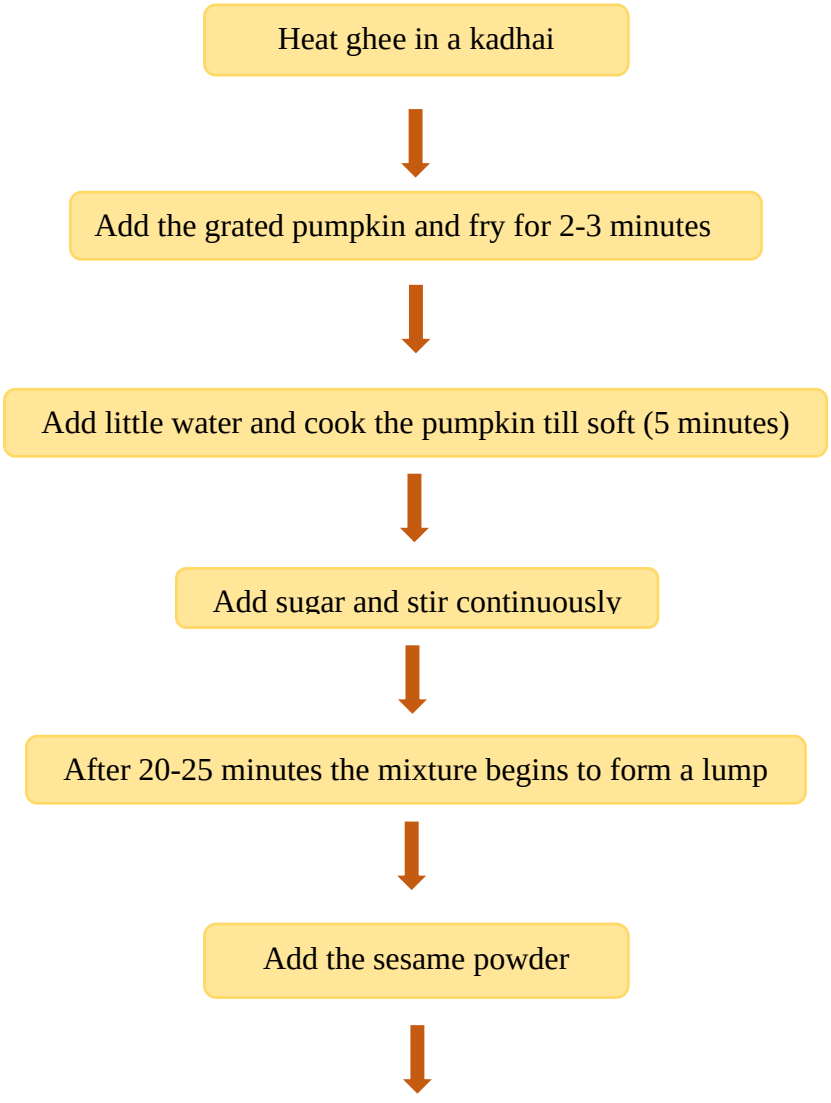
Formulation of sesame pumpkin burfi

Ingredients	Formulation (g)				
	F1	F2	F3	F4	Control
Grated pumpkin	30	25	30	25	50
Roasted sesame	20	25	-	-	-
Germinated sesame	-	-	20	25	-
Sugar	45	45	45	45	45
Ghee	5	5	5	5	5

F = formulation

3.2.3.2. Preparation of sesame-pumpkin burfi

The pumpkin was washed, peeled. The inside contents of the pumpkin (fibrous strands, seeds) were removed. The pumpkin was grated. Ghee was heated in a kadhai. The grated pumpkin and fried in the ghee for 2 minutes. A little amount of water was added, and the pumpkin was cooked until it was soft (approximately 5 minutes). Sugar was added and the mixture was mixed continuously. After 20-25 minutes, when the mixture began to form a lump leaving the sides of the kadhai, the sesame seed powder and cardamom powder were added. The mixture was mixed thoroughly. The mixture was transferred to a plate greased with ghee. The mixture was flattened with a spatula. The mixture was cut with a knife into pieces in the shape of a diamond.



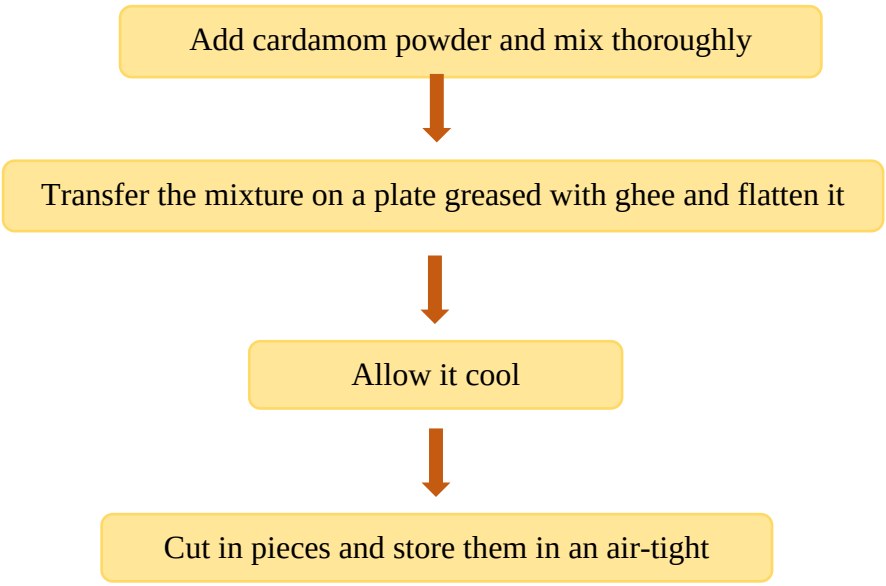
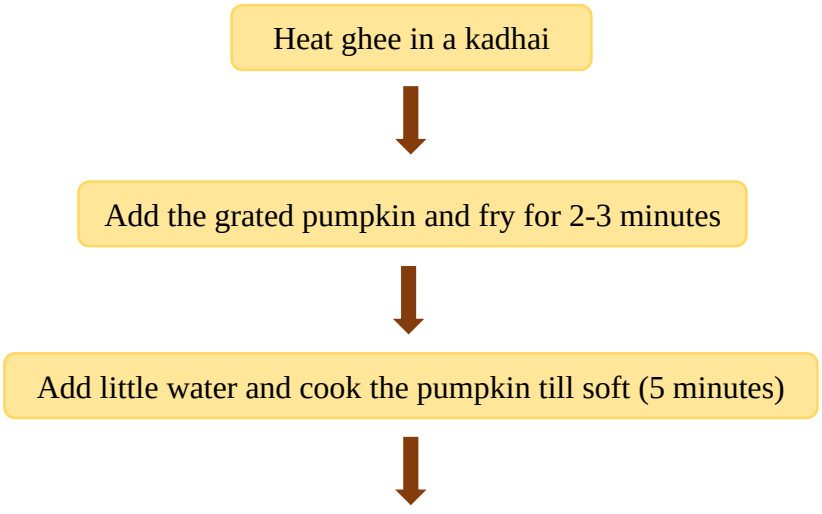


FIGURE 4: Schematic representation of the preparation of sesame-pumpkin burfi

3.2.3.3. Preparation of Control burfi

The pumpkin was washed, peeled. The inside contents of the pumpkin (fibrous strands, seeds) were removed. The pumpkin was grated. ghee was heated in a kadhai. The grated pumpkin and fried in the ghee for 2 minutes. A little amount of water was added, and the pumpkin was allowed to cook until it was soft (approximately 5 minutes). Sugar was added and the mixture mixed continuously. After 20-25 minutes, the mixture began to form a lump leaving the sides of the kadhai, cardamom powder was added. The mixture was transferred to a plate greased with ghee. The mixture was flattened with a spatula. The mixture was cut with a knife into pieces in the shape of a diamond.



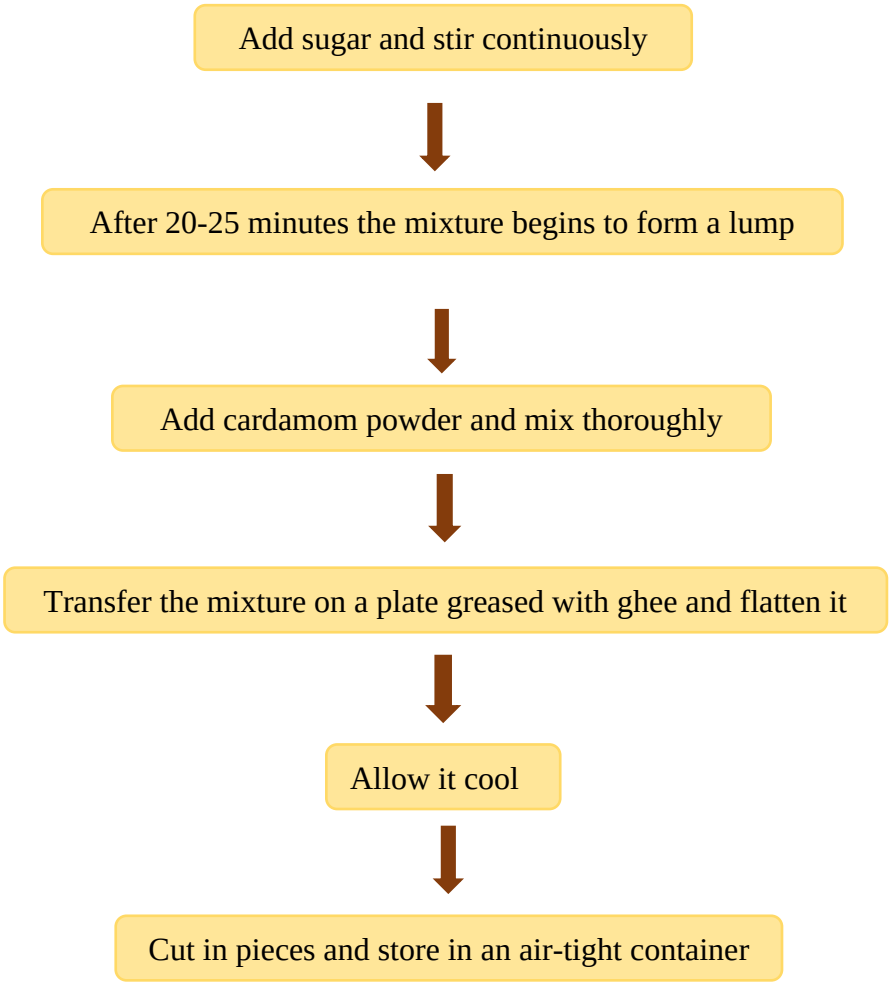


Figure 5: Schematic representation of the preparation of Control burfi

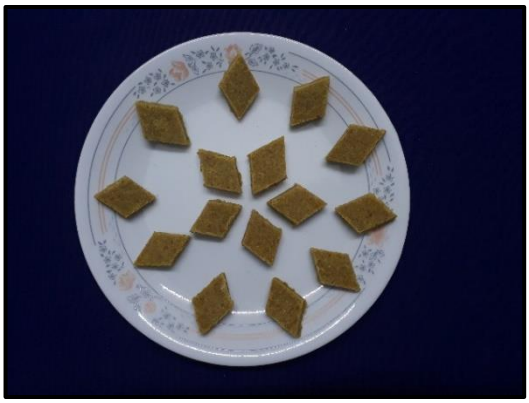


Plate 4: F1 burfi

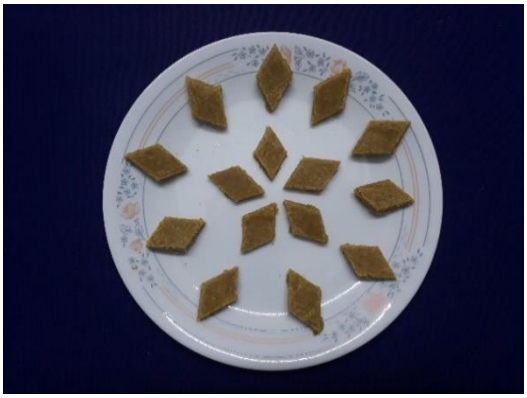


Plate 5: F2 burfi

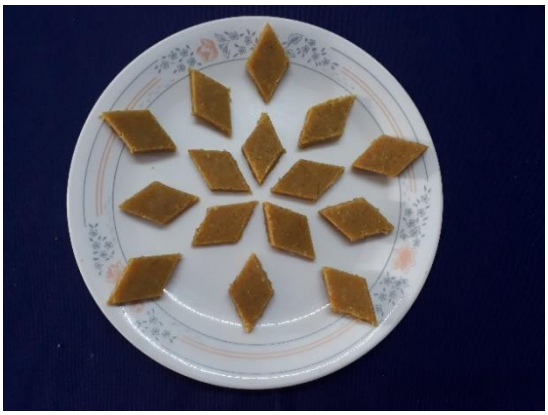


Plate 6: F3 burfi

Plate 7: F4 burfi

Plate 8: Control burfi

3.2.4. Sensory evaluation of sesame-pumpkin burfi

A sensory evaluation of all the four variations of burfi was carried out. The burfis were rated on a 5-point hedonic scale by 50-panel members. The panel members were selected by random sampling. Out of the four burfis, the most acceptable burfi was subjected to proximate analysis and shelf-life study. The samples were coded by a random three-digit number and presented in clean odor-free containers. The burfis were rated from 5 to 1, keeping 5 for like a lot and 1 for dislike a lot as per the method given in (PDST, *Sensory Analysis Teacher's Manual,* Dublin, 2017).

The scores were assigned as follows:

5-like a lot

4-like a little

3-neither like nor dislike

2-dislike a little

1-dislike a lot

3.2.5. Proximate analysis

3.2.5.1.Estimation of moisture content (AOAC,2000)

Empty petri dish and lid were dried in the oven at 105ºC for 3 hours and transferred to desiccator to cool. Weight of empty dish and lid was noted. About 3 grams of the sample was weighed in the dish. The dish was placed in the oven at 105ºC for 3 hours. After drying, the petri dish was cooled in the desiccator. The dish containing the dried sample was reweighed.

Calculation:

$$\text{Moisture (\%)} = \frac{W1-W2}{W2} \times 100$$

Where,

W1 = weight (g) of the sample before drying

W2 = weight (g) of the sample after drying

3.2.5.2.Determination of ash content (AOAC, 2000)

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a foodstuff. The crucible was heated, cooled and weighed to a constant weight. About 5 grams of the food sample was weighed into the crucible. The crucible was heated over low Bunsen flame. When the fumes were no longer produced, the crucible was heated in the muffle furnace for about 5-6 hours at 600ºC. The crucible was cooled in a desiccator and weighed. To ensure complete ashing, the crucible was again heated in the muffle furnace for 1 hour, cooled and weighed.

Calculation:

$$\text{Ash content (g/100g sample)} = \frac{Weight\ of\ the\ ash}{Weight\ of\ the\ sample\ taken} \times 100$$

3.2.5.3.Estimation of protein (Waterborg, J. H., 2009)

1 g of powdered sample was mixed with 4ml of potassium phosphate buffer and was centrifuged at 5000rpm for 15 minutes. 1 ml of the supernatant was used for the protein estimation. 0.2, 0.4, 0.6, 0.8, and 1ml of the working standard solution was pipetted out in a series of test tube. The volume was made up to 1ml in all the test tubes with distilled water. A test tube with 1 ml of distilled water served as the blank. 5ml of alkaline copper sulphate solution was added to each test tube mixed well and allowed to stand for 10 minutes. 0.5ml of Folin-Ciocalteau Reagent was added to all the test tubes, mixed well, and incubated at room temperature in the dark for 30 minutes. The absorbance was read at 660nm and the concentration was determined using spectrophotometer.

3.3.5.4. Estimation of fat (AOCS, 2000)

10 g of the food sample was weighed and transferred to a 250ml conical flask. The flask was filled with hexane till the food sample was completely submerged. The mouth of the conical flask was covered with aluminum foil. The conical flask was shaken for a minute, every half an hour. The next day, the hexane in the conical flask was slowly decanted in a pre-weighed beaker. The beaker was kept in open, for the hexane to get evaporated. The conical flask was

refilled with hexane and the same procedure was repeated for 3 days. After 3 days, when the hexane had completely evaporated from the beaker, the weight of the beaker (along with the fat) was noted.

Calculation:

$$\text{Fat (g/100g of sample)} = \frac{weight\ of\ beaker\ with\ fat - weight\ of\ empty\ beaker}{amount\ of\ sample\ taken} \times 100$$

3.2.5.5. Estimation of carbohydrates (Sadasivam, S., 1996)

100mg of powdered sample was taken in test tube and was hydrolyzed by keeping in a boiling water bath for 3 hours with 5ml of 2.5N HCl and cooled to room temperature. It was neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 100ml and centrifuged. 1 ml of the supernatant was taken for analysis. 0.2-1.0ml of the standard solution was pipetted out in a series of tube. All the tubes were made up to 1ml with distilled water. 1ml of distilled water in test tube served as blank. 4ml of Anthrone reagent was added to all the test tubes. The test tubes were heated in boiling water bath for 8 minutes. The absorbance was read at 630nm and the concentration was determined using spectrophotometer.

3.2.5.6. Estimation of crude fiber (Raghuramulu et al., 2003)

2g of the sample was weighed in a 500ml conical flask. 200ml of 0.255N sulphuric acid was added and heated gently on a hot plate and boiled for exactly 30 minutes. The mixture was filtered in another conical flask through a muslin cloth over a funnel. The residue on the cloth was washed with 200-300ml of hot water until it was free from acid. The material was transferred from the cloth to the same beaker. 200ml of 0.313N sodium hydroxide was added and was boiled exactly for 30 minutes. The mixture was filtered through the same cloth through a funnel. The residue was washed with 200-300ml of hot water until it was free from alkali. The residue was transferred to a crucible and was heated at 150-200ºC in a hot air oven for a minimum of 4 hours. It was cooled and weighed. The crucible was heated in a muffle furnace at 600ºC for 30 minutes. It was cooled and weighed.

Calculation:

$$\text{Crude fiber (g/100g sample)} = \frac{weight\ of\ the\ crucible\ with\ contents\ before\ ashing - weight\ of\ the\ crucible\ with\ contents\ after\ ashing}{sample\ weight\ (grams)}$$

3.2.5.7. Estimation of total energy content

The energy content was estimated by the factorial method.

Energy (kcal) = (4 × protein) + (9 × fat) + (4 × carbohydrate)

3.2.5.8. Preparation of ash solution (Raghuramulu et al., 2003).

The sample was defatted using hexane. 5g of the defatted sample was weighed in the crucible. The crucible is heated on a low flame until the sample is completely charred. The crucible was heated in the muffle furnace at 500-600ºC for 5-6 hours. It is then cooled in a desiccator and weighed. The ash was moistened with a small amount of distilled water (0.5-1 ml) and 5 ml of concentrated hydrochloric acid was added to it. The mixture is evaporated to dryness on low flame. Another 5ml of the concentrated hydrochloric acid is added again and the solution is evaporated to dryness. 4ml of concentrated hydrochloric acid and a few ml of distilled water was then added and the solution was warmed and filtered in a 100ml volumetric flask using Whatman No. 40 filter paper. After cooling, the volume is made up to 100ml with distilled water.

3.2.5.9. Estimation of calcium (AOAC 1980)

An aliquot of 25ml ash solution was pipetted out in a clean conical flask. The solution was diluted to 150ml with double distilled water. A few drops of methyl red indicator were added, and the mixture was neutralized with ammonia till the pale pink colour changed to yellow. The solution was heated to a boiling point and 10ml of ammonium oxalate was added. The mixture was boiled for a few more minutes. Glacial acetic acid was added until the colour of the solution was distinctly pink. The mixture was allowed to stand for 4 hours or preferably overnight. It was filtered through Whatman No. 42 filter paper and washed with warm water, till the filtrate was oxalate free. (since HCl had been used for preparing ash solution, it was

convenient to test for absence of chloride using $AgNO_3$). 5-10ml of dilute H_2SO_4 was added on the filter paper and the point of the filter paper was broken with a pointed glass rod. The filter paper was transferred into the conical flask. The solution was heated to 70ºC and titrated against 0.01N $KMnO_4$ to a permanent pale pink colour**.**

Calculation:

1 ml of 0.01N $KMnO_4$ = 0.2004 mg of Calcium

Calcium (mg)/ 5g food sample = $\frac{titre\ value \times 0.2004 \times 100}{25}$

3.2.5.10. Estimation of phosphorus (Raghuramulu *et al.*, 2003).

The method prescribed by Raghuramulu *et.al.,* 2003 was modified and used. Different aliquots of the standard phosphorus solution (0.5ml, 1ml, 2ml, and 4ml) were taken and the volume was made up to 4ml with double distilled water. 1 ml of the test sample was taken, and the volume was made up to 4 ml with double-distilled water. To all test tubes, 1ml of 2.5% ammonium molybdate and 0.4ml of aminonaphtholsulphonic acid reagent was added. The blank was prepared by adding 4ml of double-distilled water, 1ml of 2.5% ammonium molybdate and 0.4ml of aminonaphtholsulphonic acid reagent. The test tubes were incubated at room temperature for 10 minutes and the absorbance was read at 660nm and the concentration was obtained in a spectrophotometer.

3.2.6. Shelf life study

3.2.6.1. Sensory evaluation

The Control burfi and the F4 burfi was stored at room temperature in an air-tight container. A sensory evaluation of both the samples was conducted with 30 panel members. Attributes like appearance, colour, texture, aroma, taste, and overall acceptability were evaluated using a 9-point hedonic scale. The Control burfi and the F4 burfi were rated from 9 to 1, keeping 9 for like extremely and 1 for dislike extremely as per the method given in (**Singh-Ackbarali *et.al.*, 2014**). Collected data was assessed by mean and standard deviation. Repeated measures ANOVA with Post hoc analysis was performed to ascertain the significance across

the days. Analysis was performed using SPSS 16 software. Level of significance in the present study was 0.05.

3.2.6.2. Microbial analysis

The Control and the F4 burfi were tested for standard plate count, E. coli, yeast and mould count. The samples were given to a NABL accredited laboratory (Mangalore Biotech Laboratory, Pumpwell Circle, Mangalore) for microbial testing. The samples were tested for the microbial growth on the first day of the product development and on the day of visible mould growth. The Standard Plate Count was determined by IS: 5402 (PART 1): 2018. The E. coli. count was determined by IS:5887 (PART 1): 2005. The yeast and mould count was determined by IS: 5403: 2018.

Chapter 4

RESULTS AND DISCUSSION

The section depicts the outcome and results of the methods that were used for development and quality evaluation of the sesame-pumpkin burfi. Based on the sensory evaluation the formulation F4 was selected, which was subjected to proximate analysis and shelf life study. The results reveal that the sesame-pumpkin burfi was rich in calcium and had an approximate shelf life of 7 days.

4.0. RESULTS AND DISCUSSION

4.1. Estimation of calcium and phosphorus content in processed sesame seeds

On cooking, the components of food undergo various changes. In our traditional cooking, foods are subjected to various types of processing such as boiling, roasting, germination, fermentation, etc. All these processes result in changes the nutritional components of food. In this study, the sesame seeds were added to the burfi in two processed forms, roasted and germinated. Calcium and phosphorus are two minerals which occur in sesame in high proportions. These two minerals are also particularly important for growth and development. Hence, the change that may occur in the calcium and phosphorus content, on subjecting the sesame to roasting and germination, was analysed. The calcium and the phosphorus contents are presented in table 4 and table 5 respectively.

Table 4

Calcium content of raw, roasted, and germinated sesame seeds (mean± SD, n=3)

Food sample	Calcium content (mg/100grams)
Raw sesame	2351.3 ± 2.97
Roasted sesame	2634.53 ± 2.51
Germinated sesame	2388.73 ± 3.05

Table 4 shows the calcium content of the raw, roasted and germinated seeds. The calcium content was in a range of 2351.3-2634.53 mg/100g. The highest concentration of calcium was seen in roasted seeds (2634.53±2.51mg/100g) which was significantly different from the raw and germinated sesame seeds. Makinde *et al.*, 2016 also reported an increase in calcium content on roasting. Germination also lead to a slight increase in the calcium content. Hence, both the processing techniques increased the calcium content of sesame.

Table 5

Phosphorus content of raw, roasted, and germinated seeds (mean ± SD, n=3)

Food sample	Phosphorus content (mg/100g)
Raw sesame	530.13 ± 5.5
Roasted sesame	460.93 ± 1.85
Germinated sesame	515.26 ± 2.04

Table 5 shows that the phosphorus content was in the range of 460.93-530.13mg/100g. There was a significant decrease in phosphorus content in the roasted and germinated sesame seeds. The decrease in phosphorus content was slightly more in roasted sesame than in germinated sesame. Both the processing techniques reduced the phosphorus content of the sesame.

4.2. Sensory evaluation of sesame-pumpkin burfi

Sensory evaluation is a process whereby, the quality of food is judged by a panel of judges. When a product is developed for the consumers from the viewpoint of health, one must make sure that the taste and other characteristics of the food are also well accepted by the consumers. Only then, will people choose a healthy product over any other unhealthy food. The color, taste, texture, flavor etc., are some of the factors that determine the overall acceptability of the product. The panel members were asked to taste each burfi and rate the overall acceptability. The overall acceptability was rated on a 5-point hedonic scale. The scores for the 4 different formulated burfis is given below in the table 6.

Table 6

Sensory evaluation of sesame-pumpkin burfis using 5-point Hedonic scale rating

BURFI	5-Point Hedonic Scale rating
F1	3.26 ± 1.26
F2	3.6 ± 1.14
F3	3.84 ± 0.96
F4	**4.42 ± 0.70**

(mean± S.D, n=50), F = formulation

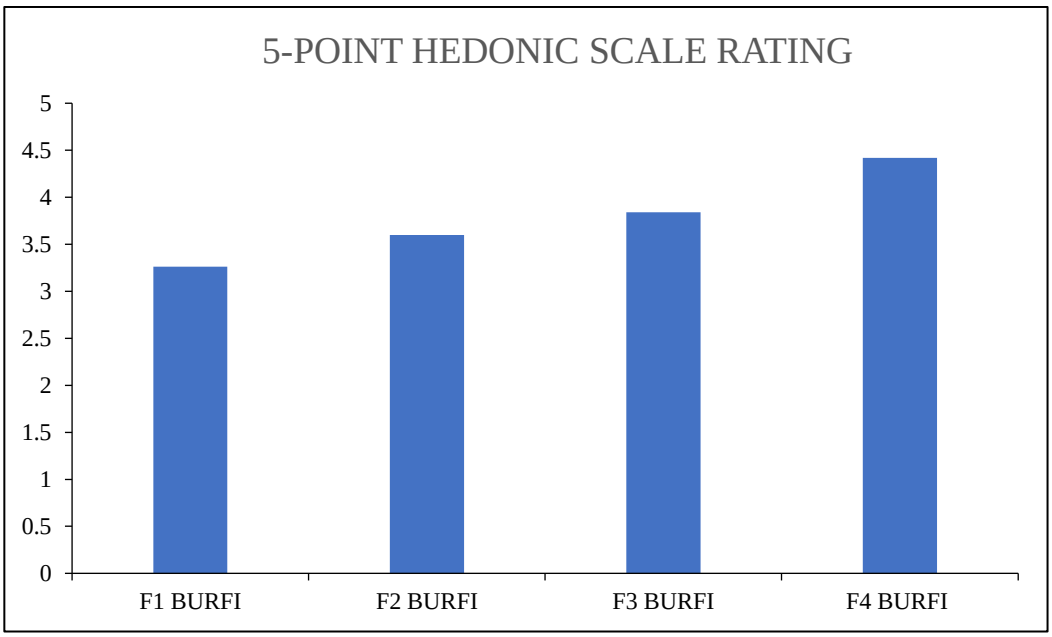


Figure 6: 5-point Hedonic scale rating of the four burfis

From the Table 6 and Figure 6 above it is seen that F4 burfi containing germinated sesame at 25% level scored highest in sensory evaluation. The burfi F4 had the highest score of 4.42 ± 0.70 among the four burfis.

4.3. Proximate analysis

Table 7

Proximate composition of Control burfi and F4 burfi per 100 grams

Parameter	Control burfi	F4 burfi
Moisture(g)	25.853 ± 0.58	23.367 ± 0.31
Ash(g)	0.282 ± 0.01	1.936 ± 0.10
Protein(g)	1.365 ± 0.42	6.52 ± 0.35
Fat(g)	6.241 ± 0.22	21.58 ± 0.05
Carbohydrate(g)	42.133 ± 0.75	44.9 ± 0.56
Crude fibre(g)	2.183 ± 0.39	4.433 ± 0.23
Calcium(mg)	26.720 ± 3.34	520.505 ± 5.63
Phosphorus(mg)	194.733 ± 0.38	487.467 ± 0.50
Energy(kcal)	230.16	399.9

Mean ± SD (n=3)

The proximate analysis of both, the control and the F4 burfi, was conducted. Table 7 gives a comparative picture of the proximate composition of both the Control and F4 burfi. It was seen that the F4 burfi contained higher amounts of ash, protein, fat, carbohydrates, fiber, calcium, and phosphorus. The protein content of F4 burfi (6.52g/100g) was nearly five times more than that of the Control burfi (1.365g/100g). Similarly, the fat content was also considerably higher in the F4 burfi (21.58g/100g). The crude fiber content of the F4 burfi (4.433g/100g) was twice as much as that of Control burfi (2.183g/100g). The calcium content of the F4 burfi (520.505mg/100g) is nearly twenty times more than that of Control burfi (26.720mg/100g). Similarly, the phosphorus content of the F4 burfi (487.467mg/100g) was approximately twice that of Control burfi (194.733mg/100g). Further, the F4 burfi had significantly higher calorific value (399.9 kcal/100g) than the control burfi (230.16 kcal/100g). The moisture content, on the other hand, was seen to be lesser in the F4 burfi (23.367g/100g) when compared to Control burfi (25.853g/100g).

4.4. Shelf life study

Each product has a shelf life. Shelf life study is an important aspect of product development. Shelf life was determined based on sensory evaluation and microbial testing.

4.4.1. Sensory evaluation

The sensory attributes have a profound effect on consumer's preference. Different food products undergo deterioration in sensory profile because of various chemical and biochemical changes that progress during storage. Sensory evaluation was conducted on a 9-point hedonic scale rating. The burfi was evaluated for its appearance, colour, texture, aroma, taste, and overall acceptability every alternate day (day 1, day 3, day 5, day 7). Both the burfis had a shelf life of 7 days. Fungal growth was observed on both the burfis by the 8^{th} day.

The data obtained by the sensory evaluation was subjected to repeated measures ANOVA test and Bonferroni post hoc analysis. The repeated measures ANOVA is a member of the ANOVA family. All the ANOVAs compare one or more mean scores with each other: they are tests for difference in mean scores. The repeated measures ANOVA compare the means across one or more variables that are based on repeated observations. The ANOVA test tells whether there is any significant difference between the means. If there is any significant difference, post hoc test are run to confirm where exactly the differences occur. Repeated measure ANOVA was conducted because the same sample was tested at different time intervals (the burfi was tasted every alternate day).

Effect of storage on the appearance:

Table 8

Effect of storage on the appearance (repeated measures ANOVA)

Parameter	Burfi	Storage period	N	Mean	Standard Deviation	Repeated Measures ANOVA	
						F value	p
Appearance	Control burfi	Day 1	30	8.17	0.91	1.094	0.356
		Day 3	30	8.07	0.94		
		Day 5	30	8.10	0.88		
		Day 7	30	8.07	0.87		
	F4 burfi	Day 1	30	7.60	0.89	9.667	0.000
		Day 3	30	7.57	0.97		
		Day 5	30	7.50	0.94		
		Day 7	30	7.20	0.85		

From table 8, it is seen that in the Control burfi, there was no significant difference ($p>0.05$) in the appearance from day 1 to day 7. The burfi had an acceptable score of 8 throughout the 7 days, i.e., the appearance of the burfi was liked very much till the 7^{th} day. In F4 burfi, the difference was highly significant ($p<0.01$) in the appearance from day 1 to day 7.

Table 9

Effect of storage on the appearance (Post hoc Bonferroni Test)

Parameter	Burfi		Paired differences			
			Mean	Standard deviation	Change (%)	Bonferroni test
						p value
Appearance	Control burfi	Day 1 - Day 3	0.100	0.403	1.22	1.000
		Day 1 - Day 5	0.067	0.365	0.82	1.000
		Day 1 - Day 7	0.100	0.403	1.22	1.000
		Day 3 - Day 5	0.033	0.320	0.41	1.000
		Day 3 - Day 7	0.000	0.371	0.00	1.000
		Day 5 - Day 7	0.033	0.183	0.41	1.000
	F4 burfi	Day 1 - Day 3	0.033	0.320	0.44	1.000
		Day 1 - Day 5	0.100	0.403	1.32	1.000
		Day 1 - Day 7	0.400	0.563	5.26	0.003
		Day 3 - Day 5	0.067	0.254	0.88	0.965
		Day 3 - Day 7	0.367	0.556	4.85	0.007
		Day 5 - Day 7	0.300	0.535	4.00	0.028

From Table 9, it is observed that significant difference occurred in appearance ($p<0.05$) from day 5 to day 7.

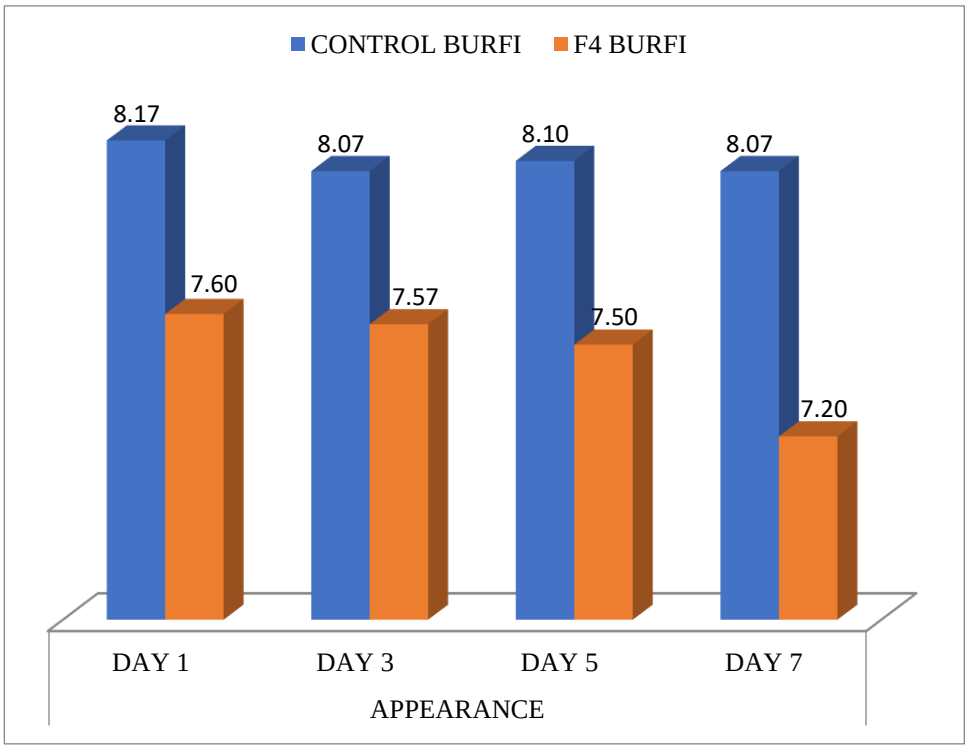


Figure 7: Effect of storage on appearance of Control burfi and F4 burfi

The above Figure 7 illustrates changes in the scores for appearance of Control burfi and F4 burfi over the days of storage. There was minimal change in the scores of Control burfi for appearance till day 7; whereas in the case of the F4 burfi there is a slight decrease in the scores for appearance on the Day 5 and further noticeable change on Day 7. There was a slight change in the appearance of the F4 burfi which explains the drop in the scores. The Control burfi however, registered no such noticeable change.

Effect of storage on the color:

Table 10

Effect of storage on the color (repeated measures ANOVA)

Parameter	Burfi	storage period	N	Mean	Standard Deviation	Repeated Measures ANOVA	
						F value	p
Color	Control burfi	Day 1	30	8.43	0.73	0.225	0.879
		Day 3	30	8.43	0.73		
		Day 5	30	8.47	0.73		
		Day 7	30	8.43	0.77		
	F4 burfi	Day 1	30	7.60	0.93	18.882	0.000
		Day 3	30	7.60	0.93		
		Day 5	30	7.53	0.86		
		Day 7	30	7.10	0.76		

In Table 10, it is seen that in the Control burfi, there was no significant difference ($p>0.05$) in the color from day 1 to day 7. The burfi had an acceptable score of 8.4 throughout the 7 days which means the color of the burfi was liked very much till the day 7. In F4 burfi, the difference was highly significant ($p<0.01$) in the color from day 1 to day 7. The scores for the color of the F4 burfi noticeably changed from 7.60± 0.93 on day 1 to 7.10± 0.76 on day 7. The score for color of the F4 burfi was 7, which means it was liked moderately.

Table 11

Effect of storage on the color (Post hoc Bonferroni Test)

Parameter	Burfi		Paired differences			
			Mean	Standard deviation	Change (%)	Bonferroni test
						p value
Color	Control burfi	Day 1 - Day 3	0.000	0.263	0.00	1.000
		Day 1 - Day 5	0.033	0.183	0.40	1.000
		Day 1 - Day 7	0.000	0.371	0.00	1.000
		Day 3 - Day 5	0.033	0.183	0.40	1.000
		Day 3 - Day 7	0.000	0.263	0.00	1.000
		Day 5 - Day 7	0.033	0.320	0.39	1.000
	F4 burfi	Day 1 - Day 3	0.000	0.263	0.00	1.000
		Day 1 - Day 5	0.067	0.365	0.88	1.000
		Day 1 - Day 7	0.500	0.572	6.58	0.000
		Day 3 - Day 5	0.067	0.254	0.88	0.965
		Day 3 - Day 7	0.500	0.509	6.58	0.000
		Day 5 - Day 7	0.433	0.504	5.75	0.000

The Table 11, shows that, there was a highly significant difference ($p<0.01$) in color after day 3.

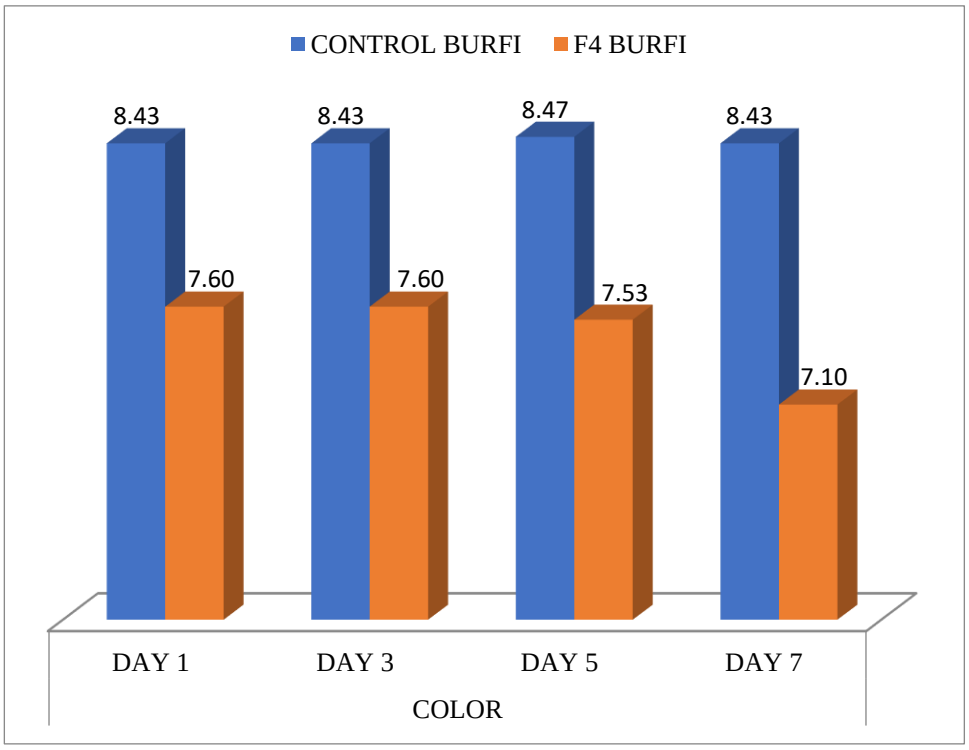


Figure 8: Effect of storage on color of Control burfi and F4 burfi

From the above Figure 7, it is evident that there was very minute change in the scores for color of the Control burfi. The scores for the color of the F4 burfi remained almost the same till Day 3. There was a slight decrease in the scores on Day 5 and Day 7. The color of the Control burfi remained nearly the same whereas that of F4 burfi changed to a duller shade towards day 7.

Effect of storage on the texture:

Table 12

Effect of storage on the texture (repeated measures ANOVA)

Parameter	Burfi	storage period	N	Mean	Standard Deviation	Repeated Measures ANOVA	
						F value	p
Texture	Control burfi	Day 1	30	7.97	0.85	5.960	0.001
		Day 3	30	7.97	0.81		
		Day 5	30	7.93	0.83		
		Day 7	30	7.70	0.75		
	F4 burfi	Day 1	30	7.83	0.91	14.901	0.000
		Day 3	30	7.67	0.88		
		Day 5	30	7.53	0.75		
		Day 7	30	7.07	0.69		

From the Table 12, it is seen that in Control burfi, there was a high significant difference observed in the texture ($p<0.01$) from day 1 to day 7. The score for texture of the Control burfi was 7, which means it was liked moderately. But the score dropped drastically from 7.97±0.81 on day 1 to 7.0±0.75 on day 7. In F4 burfi, there was high significant difference observed in the texture ($p<0.01$) from day 1 to day 7. The score for texture of the F4 burfi was 7, which means it was liked moderately. The scores dropped from 7.83 ± 0.91 on day 1 to 7.07 ± 0.69 on day 7.

Table 13

Effect of storage on the texture (Post hoc Bonferroni Test)

Parameter	Burfi		Paired differences			
			Mean	Standard deviation	Change (%)	Bonferroni test
						p value
Texture	Control burfi	Day 1 - Day 3	0.000	0.263	0.00	1.000
		Day 1 - Day 5	0.033	0.414	0.42	1.000
		Day 1 - Day 7	0.267	0.521	3.35	0.045
		Day 3 - Day 5	0.033	0.320	0.42	1.000
		Day 3 - Day 7	0.267	0.450	3.35	0.018
		Day 5 - Day 7	0.233	0.430	2.94	0.035
	F4 burfi	Day 1 - Day 3	0.167	0.648	2.13	1.000
		Day 1 - Day 5	0.300	0.651	3.38	0.104
		Day 1 - Day 7	0.767	0.858	9.79	0.000
		Day 3 - Day 5	0.133	0.346	1.74	0.260
		Day 3 - Day 7	0.600	0.724	7.83	0.001
		Day 5 - Day 7	0.467	0.629	6.19	0.002

From Table 13, it is seen that in Control burfi a significant difference occurred after day 3, whereas in the F4 burfi the significant difference occurred from day 5 to day 7.

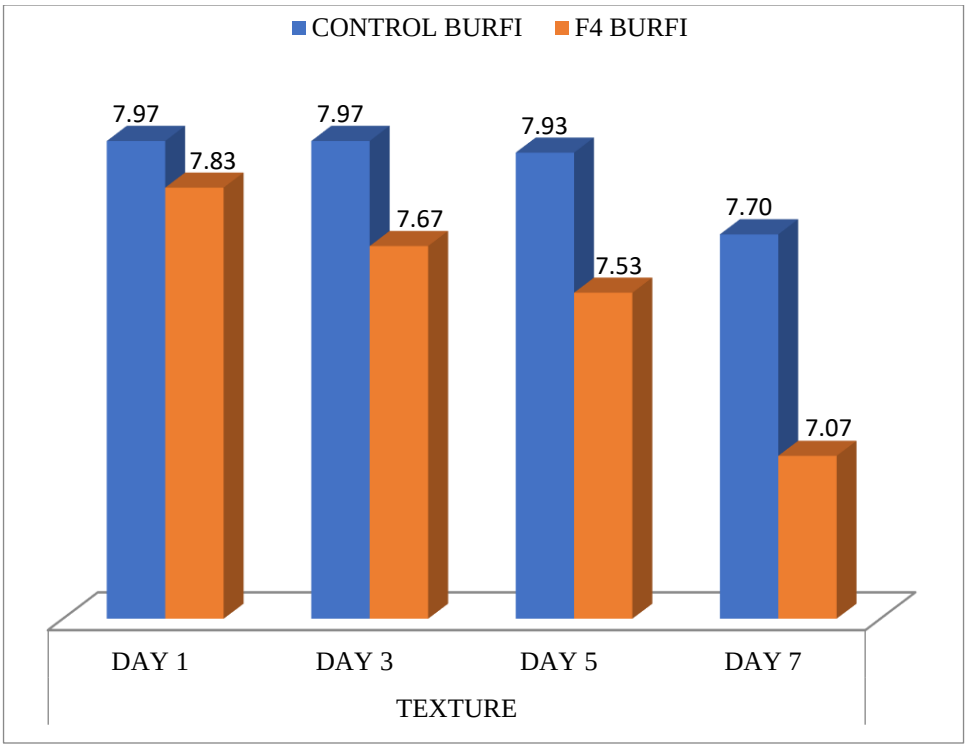


Figure 9: Effect of storage on texture of Control burfi and F4 burfi

The above Figure 9 depicts the change in the scores of the two burfis related to texture. It was seen that there was very slight change in the scores of the Control burfi till Day 5. However, there was a drop in the scores on the Day 7. In the case of the F4 burfi there was a steady and gradual decrease over 5 days and a significant drop in the score on Day 7. During the 7 days, both the burfis gradually became grainy and liable to be crushed easily.

Effect of storage on the Aroma:

Table 14

Effect of storage on the aroma (repeated measures ANOVA)

Parameter	Burfi	storage period	N	Mean	Standard Deviation	Repeated Measures ANOVA	
						F value	p
Aroma	Control burfi	Day 1	30	7.87	0.73	17.543	0.000
		Day 3	30	7.43	0.73		
		Day 5	30	7.37	0.61		
		Day 7	30	7.07	0.69		
	F4 burfi	Day 1	30	7.03	0.96	15.808	0.000
		Day 3	30	7.33	0.99		
		Day 5	30	7.13	0.82		
		Day 7	30	6.80	0.66		

According to Table 14, there was highly significant difference ($p<0.01$) in the aroma of the Control burfi from day 1 to day 7. The score for aroma was 7, which means it was liked moderately. The score for aroma dropped from 7.87±0.73 on day 1 to 7.07±0.69 on day 7. In the F4 burfi, there was highly significant difference($p<0.01$) in the aroma from day 1 to day 7. The score for the aroma of the F4 burfi was 7 (it was liked moderately) till the day 5, but the score reduced to 6(it was liked slightly) on day 7.

Table 15

Effect of storage on the aroma (Post hoc Bonferroni Test)

Parameter	Burfi		Paired differences			
			Mean	Standard deviation	Change (%)	Bonferroni test
						p value
Aroma	Control burfi	Day 1 - Day 3	0.433	0.626	5.51	0.004
		Day 1 - Day 5	0.500	0.630	6.36	0.001
		Day 1 - Day 7	0.800	0.847	10.17	0.000
		Day 3 - Day 5	0.067	0.254	0.90	0.965
		Day 3 - Day 7	0.367	0.615	4.93	0.017
		Day 5 - Day 7	0.300	0.535	4.07	0.028
	F4 burfi	Day 1 - Day 3	0.300	0.466	3.93	0.009
		Day 1 - Day 5	0.500	0.572	6.55	0.000
		Day 1 - Day 7	0.833	0.874	10.92	0.000
		Day 3 - Day 5	0.200	0.484	2.73	0.188
		Day 3 - Day 7	0.533	0.860	7.27	0.012
		Day 5 - Day 7	0.333	0.711	4.67	0.094

From Table 15, it is evident that in the Control burfi a significant difference occurred on all the days except from day 3 to day 5. Whereas, in the F4 burfi the significant difference was observed on all days.

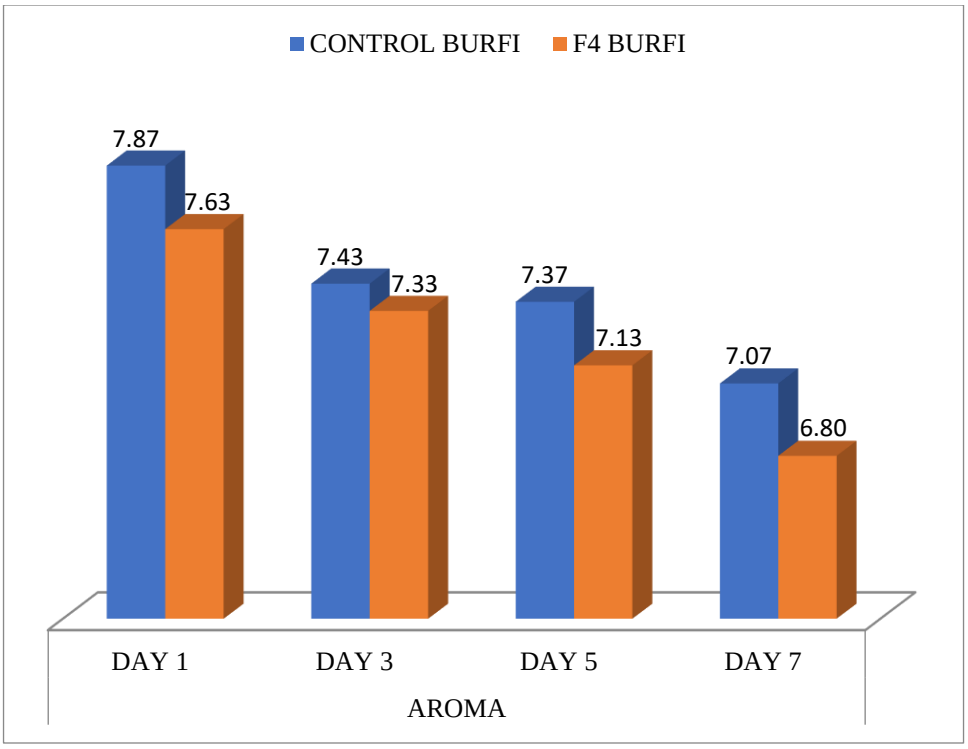


Figure 10: Effect of storage on aroma of Control burfi and F4 burfi

The Figure 10 is indicative of the fact that scores for the aroma of the burfi steadily decreased over all the 7 days. For both the burfis, there was a significant difference in the scores from Day 1 to Day 3, as well from Day 5 to Day 7. The change in scores from 3 to day 5 however was not much. On Day 1, the Control burfi had the sweet fragrance of pumpkin which gradually became weaker day by day. In the case of F4 burfi, the aroma was a balance of pumpkin and sesame. However, with each day the fragrance of pumpkin decreased and the strong odour of sesame tended to predominate.

Effect of storage on the taste:

Table 16

Effect of storage on the taste (repeated measures ANOVA)

Parameter	Burfi	storage period	N	Mean	Standard Deviation	Repeated Measures ANOVA	
						F value	p
Taste	Control burfi	Day 1	30	8.4	0.73	7.367	0.000
		Day 3	30	8.40	0.86		
		Day 5	30	8.33	0.84		
		Day 7	30	8.03	0.85		
	F4 burfi	Day 1	30	8.37	0.96	26.153	0.000
		Day 3	30	8.07	0.78		
		Day 5	30	7.97	0.76		
		Day 7	30	7.40	0.86		

In Table 16, it is observed that in the Control burfi, there was a highly significant difference ($p<0.01$) in the taste from Day 1 to Day 7. The score for taste was 8 which means it was liked very much. A drop was observed in the scores from 8.47±0.73 on day 1 to 8.03±0.85 on Day 7. In the F4 burfi, there was a highly significant difference ($p<0.01$) in the taste from Day 1 to Day 7. The score for taste was 8 (it was liked very much) till Day 3 after which the score further reduced to 7 (it was liked moderately). The score for the taste of the F4 burfi reduced from 8.37±0.96 on Day 1 to 7.40±0.86 on Day 7.

Table 17

Effect of storage on the taste (Post hoc Bonferroni Test)

Parameter	Burfi		Paired differences			
			Mean	Standard deviation	Change (%)	Bonferroni test
						p value
Taste	Control burfi	Day 1 - Day 3	0.067	0.450	0.79	1.000
		Day 1 - Day 5	0.133	0.507	1.57	0.965
		Day 1 - Day 7	0.433	0.728	5.12	0.017
		Day 3 - Day 5	0.067	0.365	0.79	1.000
		Day 3 - Day 7	0.367	0.556	4.37	0.007
		Day 5 - Day 7	0.300	0.596	3.60	0.060
	F4 burfi	Day 1 - Day 3	0.300	0.596	3.59	0.060
		Day 1 - Day 5	0.400	0.675	4.78	0.018
		Day 1 - Day 7	0.967	0.765	11.55	0.000
		Day 3 - Day 5	0.100	0.305	1.24	0.498
		Day 3 - Day 7	0.667	0.606	8.26	0.000
		Day 5 - Day 7	0.567	0.626	7.11	0.000

According to Table 17, in the Control burfi, a significant difference occurred on Day 5. In F4 burfi, highly significant difference occurred around Day 5 till Day 7.

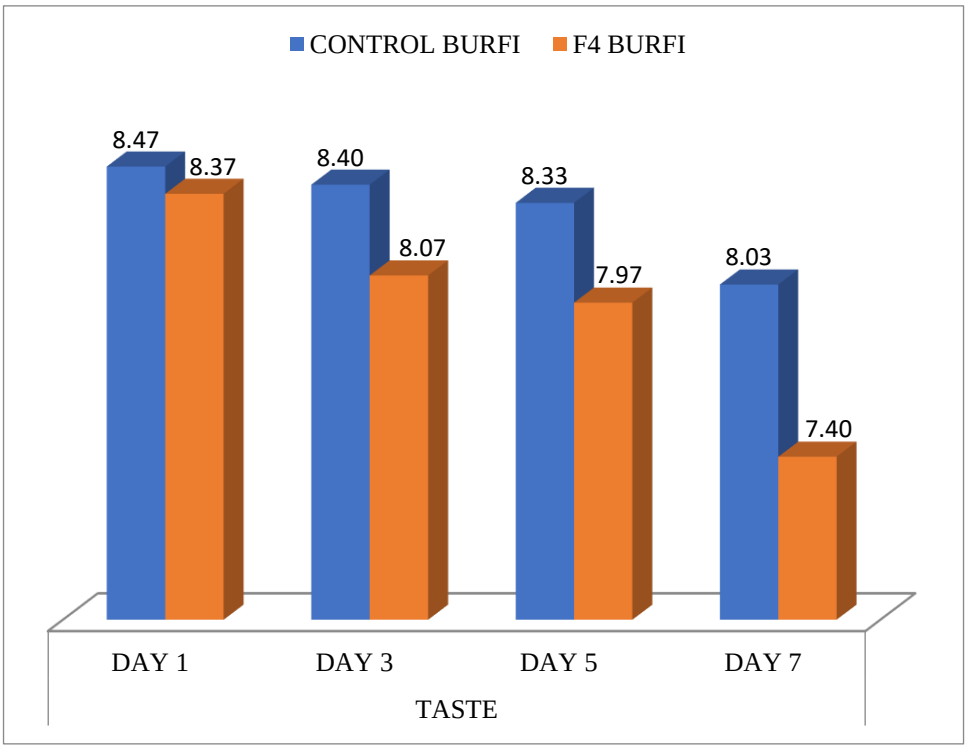


Figure 11: Effect of storage on taste of Control burfi and F4 burfi

Figure 11 shows the change in scores for taste of the two burfis from Day 1 to Day 7. It is seen that there was a small change in the scores till the Day 5 in the Control burfi. However, after Day 5 there was a significant decrease in the score. In the F4 burfi, the scores for the taste gradually declined till day 5. The decline was particularly drastic after day 5.

In the case of the Control burfi, in the later days of storage, the taste of pumpkin was not as strong or distinguished as it was on the first day. The F4 burfi on the other hand, had a more predominant taste of sesame towards the end.

Effect of storage on the Overall acceptability:

Table 18

Effect of storage on the overall acceptability (repeated measures ANOVA)

Parameter	Burfi	storage period	N	Mean	Standard Deviation	Repeated Measures ANOVA	
						F value	p
Overall Acceptability	Control burfi	Day 1	30	8.18	0.52	14.367	0.000
		Day 3	30	8.07	0.55		
		Day 5	30	8.04	0.54		
		Day 7	30	.87	0.57		
	F4 burfi	Day 1	30	7.81	0.67	38.883	0.000
		Day 3	30	7.65	0.69		
		Day 5	30	7.53	0.60		
		Day 7	30	7.11	0.57		

According to Table 18, in the Control burfi, there was a highly significant difference ($p<0.01$) in the overall acceptability from day 1 to day 7. The Control burfi had an overall acceptability of 8.18±0.52 (it was liked very much) on day 1 which dropped to 7.87±0.67 (it was liked moderately) on day 7. In the F4 burfi, there was a highly significant difference ($p<0.01$) in the overall acceptability from day 1 to day 7. The F4 burfi had an overall acceptability of 7.81 ±0.67 (it was liked moderately) on day 1 which reduced to 7.11±0.57 on day 7.

Table 19

Effect of storage on the overall acceptability (Post hoc Bonferroni Test)

Parameter	Burfi		Paired differences			
			Mean	Standard deviation	Change (%)	Bonferroni test
						p value
Overall acceptability	Control burfi	Day 1 - Day 3	0.107	0.221	1.30	0.079
		Day 1 - Day 5	0.104	0.236	1.71	0.017
		Day 1 - Day 7	0.307	0.367	3.75	0.000
		Day 3 - Day 5	0.033	0.092	0.41	0.344
		Day 3 - Day 7	0.200	0.268	2.48	0.002
		Day 5 - Day 7	0.167	0.293	2.07	0.025
	F4 burfi	Day 1 - Day 3	0.160	0.299	2.05	0.039
		Day 1 - Day 5	0.273	0.358	3.50	0.001
		Day 1 - Day 7	0.693	0.483	8.88	0.000
		Day 3 - Day 5	0.113	0.187	1.48	0.015
		Day 3 - Day 7	0.533	0.418	6.97	0.000
		Day 5 - Day 7	0.420	0.391	5.58	0.000

In Table 19, it is seen that, in the Control burfi, a significant difference occurred from day 5. In F4 burfi, a significant difference was observed on all days.

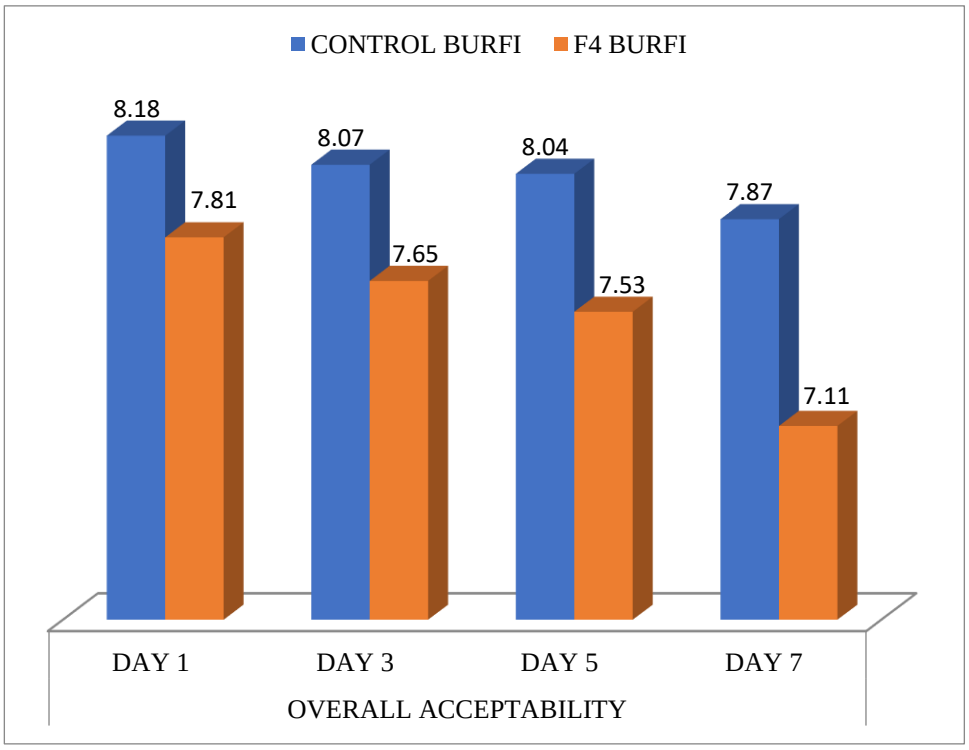


Figure 12: Effect of storage on overall acceptability of Control burfi and F4 burfi

In the Figure 12, the scores for overall acceptability of the Control burfi is seen to be slight and gradual over the period of observation. In case of the F4 burfi also, the pattern of decline of score was gradual and slight till Day 5. After Day 5, the scores for F4 dropped significantly by Day 7.

4.4.2. Microbiological analysis

According to BIS (IS: 5550:2005) Standards laid down for burfi the standard plate count should not be more than 30,000/g and the yeast and mould count not more than 10/g burfi. The microbial count influences the acceptability and hence, shelf life of any product affecting its colour and appearance, taste, and texture of the product. The sesame-pumpkin burfi stored at room temperature was subjected to analysis for standard plate count, coliform count, and yeast and mould count. The analysis was conducted on the 1st day of the storage period and on the day when there was visible mould growth.

Table 20

Microbial analysis of the burfi sample on day 1 and day 8 of the storage period

Microbial analysis	Control burfi		F4 burfi	
	Day 1	Day 8	Day 1	Day 8
Standard plate count (cfu/g)	210	340	<10	<10
E. coli (cfu/25gm)	Absent	absent	Absent	absent
Yeast and Mould (cfu/g)	< 10	< 10	<10	<10

Table 20 shows the microbial count of the Day 1 and Day 8 of the storage period of both the burfis. The standard plate count of the Control burfi on day 1 was 210 cfu/g and it increased to 340 cfu/g by Day 8. In the Control burfi, the E. coli was absent on Day 1 as well Day 8 of the storage period. Also, the yeast and mould count on Day 1 and Day 8 was less than 10 cfu/g. The standard plate count of the F4 burfi on Day 1 and Day 8 was less 10cfu/ g. In the F4 burfi, the E. coli was absent on Day 1 as well Day 8 of the storage period. The yeast and mold count on both, Day 1 and Day 8, was less than 10 cfu/g. Thus, it was concluded that both the Control and the F4 burfi had a microbial count within the limits set by the BIS standards.

Chapter 5

SUMMARY AND CONCLUSION

This section gives an insight into the outcome of this study. The sesame-pumpkin burfi developed was rich in calcium. The burfi had a shelf life of 7 days. Hence, this burfi is a healthier alternative to other sweets which provide only empty calories.

5.1. SUMMARY

- The project endeavored to formulate a unique combination of sesame and pumpkin with the aim of providing a nutritive snack.
- The sesame seeds were subjected to roasting and germination. The calcium and phosphorus content of the raw, roasted, and germinated sesame seeds was analysed. It was found that the roasted sesame had the higher calcium level than the raw and germinated sesame. However, the phosphorus content was more in raw sesame as compared to roasted and germinated sesame seeds.
- 4 different formulation of burfi were developed, by incorporating roasted and germinated sesame in varying levels namely, F1 with 20% roasted sesame, F2 with 25% roasted sesame, F3 with 20% germinated sesame and F4 with 25% germinated sesame.
- Sensory evaluation was done for all the 4 formulations of burfi, in which F4 burfi was liked the most by the panel members. The most liked burfi, i.e., F4 burfi was further subjected to proximate analysis and shelf life study.
- Along with F4 burfi, the proximate analysis and shelf life study of Control burfi was done. The burfi which served as Control comprised of pumpkin only.
- Proximate analysis of both the burfis revealed that the F4 burfi had higher amounts of ash, protein, fat, carbohydrate, calcium and phosphorus than the Control burfi.
- The shelf life study was a two-pronged approach utilizing sensory evaluation on the one hand and microbial analysis on the other.
- The sensory evaluation showed that both the burfis had a shelf life of 7 days. Highly significant difference was noted in the texture aroma taste, and overall acceptability of the Control burfi. On the other hand, significant changes were seen with respect to all characteristics, namely, appearance, color, texture, aroma taste and overall acceptability of the F4 burfi. Though the Control burfi had higher rating in all characteristics when compared to F4 burfi, both had sensory ratings within the acceptable range.
- The microbial analysis was conducted on the basis of standard plate count(cfu/g), E. coli count (cfu/25g) and yeast and mold count (cfu/g). The microbial count of both the burfis,

on the first day of storage and on the day of visible mould growth, was within the acceptable range.

5.2. CONCLUSION

The study intended to develop a healthy sesame-pumpkin burfi, which would not only have a novel taste, but also will be more nutritious. The sesame-pumpkin burfi can serve as a healthy alternative to other sweets which are a pool of empty calories. The burfi was prepared using germinated sesame seeds, which further increased the calcium content of the burfi. 100g of the sesame-pumpkin burfi approximately fulfills the recommended dietary allowances for calcium in adults and children. 100 grams of the sesame-pumpkin burfi fulfills two thirds of the recommended dietary allowances for calcium in adolescents. This study can have significant implication for community nutrition. Both the ingredients of the burfi, i.e., sesame seeds and pumpkin, are not only inexpensive but also available all year round. Hence, the burfi can be used as a nutritional supplement to provide calcium on a widespread basis. Although the burfi had a high calcium content, it was noticeable that the fat content was also considerably high.

RECOMMENDATIONS:

- The fat content in sesame-pumpkin burfi was slightly high. Further studies can be done to find out ways and means to reduce the fat without changing the taste of the burfi. This will make the burfi healthier.
- The burfi is a good source of calcium, but it proved to have a shelf life of only 7 days. The shelf life of the burfi can be increased using any permissible preservatives.

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APPENDIX

APPENDIX -1

SENSORY EVALUATION OF BURFI USING 5-POINT HEDONIC SCALE

NAME: _____

AGE: _____

Taste each of the following samples and tick how much you like it or dislike it.

Instructions: Rinse your mouth with water and have a piece of cracker before tasting each sample.

	232	413	507	985
Like a lot	_____	_____	_____	_____
Like a little	_____	_____	_____	_____
Neither like nor dislike	_____	_____	_____	_____
Dislike a little	_____	_____	_____	_____
Dislike a lot	_____	_____	_____	_____

SIGNATURE: _____

APPENDIX-2

Reagents used and preparation:

1. PROTEIN ESTIMATION BY LOWRY'S METHOD:

- Standard protein solution:

  20mg of bovine serum albumin was weighed and transferred to a 100ml standard flask. The standard flask was filled up to mark with distilled water. The concentration of the protein solution was 200µg/ml.

- Alkaline copper sulphate solution:

  The alkaline copper sulphate solution was prepared by mixing 50ml of solution A and 1ml of freshly prepared solution B.

- 0.1N sodium hydroxide solution:

  0.4 g of sodium hydroxide pellets were transferred to a 100ml standard flask. It was mixed with distilled water and shaken well till it was completely dissolved. The standard flask was filled to the mark with distilled water

- Solution A:
  - ✓ 2% Sodium carbonate solution: 2 g of sodium carbonate was transferred to a 100ml standard flask. The standard flask was filled up to mark with 0.1N sodium hydroxide solution.
- Solution B:
  - ✓ 1% sodium potassium tartarate solution: 1 g of sodium potassium tartarate crystals were transferred to a 100ml standard flask. It was filled up to mark with distilled water (it was freshly prepared).
  - ✓ 0.5% Copper sulphate solution: 0.5g of anhydrous copper sulphate was transferred to a 100ml standard flask. It was made up to mark with 1% sodium potassium tartarate solution.
- Folin- ciocalteau reagent:

  25ml of Folin-ciocalteau reagent was added to a 50ml measuring cylinder and it was filled up to mark with 25ml distilled water.

- Potassium phosphate buffer (pH 7.4):

3.03g of di-potassium hydrogen phosphate crystals and 1.035g of potassium dihydrogen phosphate crystals are weighed and transferred to a 250ml standard flask. It was filled up to mark with distilled water. The pH was adjusted sing a pH meter. The pH was adjusted with 0.1N sodium hydroxide (if pH less than 7.4) or with 0.1M hydrochloric acid (if pH more than 7.4)

2. ESTIMATION OF FAT BY SOXHLET METHOD:

- Hexane

3. ESTIMATION OF CARBOHYDRATES:

- Glucose stock solution:
  100mg of glucose was dissolved in 100ml of water in standard flask.
- Glucose working standard solution:
  10ml of stock was diluted to 100ml.1ml of this solution contains 100µg of glucose.
- Anthrone reagent:
  200mg of Anthrone was dissolved in 100ml of ice-cold 95% sulphuric acid.
- 2.5N HCl
- Solid sodium carbonate
- Distilled water

4. ESTIMATION OF CRUDE FIBER:

- 0.255N Sulphuric acid:
  7ml of concentrated sulphuric acid was dissolved in one-liter distilled water in a standard flask.
- 0.313N sodium hydroxide:
  12.78g of sodium hydroxide was dissolved and made up to mark with distilled water in a one-liter standard flask.
- Distilled water

5. PREPARATION OF ASH SOLUTION

- Concentrated hydrochloric acid
- Double distilled water

6. ESTIMATION OF CALCIUM

- 6% Ammonium oxalate solution:

  6g of ammonium of ammonium oxalate was dissolved and made up to the mark with distilled water in a 100ml standard flask.
- Methyl red indicator solution
- Dilute sulphuric acid (2N):

  5.55ml of concentrated sulphuric acid was dissolved and made up to mark with double distilled water in a 100ml standard flask.
- Glacial acetic acid
- Ammonia solution
- 0.1N Potassium permanganate:

  1.65 g of potassium permanganate was transferred to a 500ml standard flask. The flask was filled up to mark with double distilled water.
- 0.01N Potassium permanganate:

  10ml of 0.1N $KMnO_4$ was diluted to 100ml with distilled water in a standard flask. The solution was freshly prepared before use.
- Dilute silver nitrate solution
- Double distilled water

7. ESTIMATION OF PHOSPHORUS

- 10N sulphuric acid:

  10ml of concentrated sulphuric acid was diluted to 100ml with double distilled water in a standard flask.
- 15% sodium bisulphite:

  15g of sodium bisulphite was dissolved and made up to mark with double distilled water in a 100ml standard flask.

- 20% sodium sulphite:

  20g of sodium sulphite was dissolved and made up to mark with double distilled water in a 100ml standard flask.

- Standard phosphorus solution:

  35.1mg of anhydrous mono potassium phosphate (KH_2PO_4) was dissolved in double distilled water. To this 1ml of 10N sulphuric acid was added and the solution was made up to 100ml with double distilled water and mixed.

- 2.5% ammonium molybdate:

  2.5g of reagent grade ammonium molybdate was dissolved and made up to 100ml with double distilled water.

- Aminonaphtholsulphonic acid reagent:

  195ml of 15% sodium bisulphite solution was taken in a standard flask and 0.5g of 1-amino-2-naphthol-4-sulphonic acid was added to it followed by 5ml of 20% sodium sulphite. It was mixed until the powder was dissolved.

- Double distilled water.

APPENDIX -3

1. Estimation of protein by Lowry's method

Observation table

PARTICULARS	Blank (B)	STANDARD PROTEIN SOLUTION					TEST SAMPLE (T)
		S1	S2	S3	S4	S5	
Volume of standard protein solution (ml)	-	0.2	0.4	0.6	0.8	1	-
Concentration of standard protein solution (µ/ml)	-	40	80	120	160	200	-
Volume of test sample (ml)	-	-	-	-	-	-	1
Volume of distilled water (ml)	1	0.8	0.6	0.4	0.2	0	-
Volume of alkaline copper solution (ml)	5	5	5	5	5	5	5
Volume of Folin-ciocalteau reagent (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Incubate at room temperature in dark for 30 minutes							
Optical density at 660nm.							

2. Estimation of carbohydrate
Observation table

PARTICULARS	Blank (B)	Standard Glucose solution					Test sample (T)
		S1	S2	S3	S4	S5	
Volume of standard glucose solution (ml)	-	0.2	0.4	0.6	0.8	1	-
Concentration of standard glucose solution (ml)	0	20	40	60	80	100	-
Volume of test sample (ml)	-	-	-	-	-	-	1
Volume of distilled water (ml)	1	0.8	0.6	0.4	0.2	0	-
Volume of Anthrone reagent (ml)	4	4	4	4	4	4	4
Incubate in boiling water in water bath for 8 minutes							
Optical density at 630nm							

3. Estimation of phosphorus

Observation table

PARTICULARS	Blank	Standard phosphorus Solution				Test Solution (T)
	B	S1	S2	S3	S4	
Volume of standard phosphorus solution (ml)	-	0.5	1	2	4	-
Concentration of standard phosphorus solution (µg/ml)	-	40	80	160	320	-
Volume of test sample (ml)	-	-	-	-	-	1
Volume of double distilled water (ml)	4	3.5	3	2	0	3
Volume of 2.5% ammonium molybdate (ml)	1	1	1	1	1	1
Volume of 1-amino-2-naphthol-4 sulphonic acid reagent (ml)	0.4	0.4	0.4	0.4	0.4	0.4
Incubate at room temperature for 10 minutes						
Optical density 660nm						

4. Estimation of calcium

Observation:

Burette : 0.01N Potassium permanganate solution

Conical flask: Precipitate on the filter paper + 5-10ml dilute sulphuric acid

Endpoint: appearance of permanent pale pink colour.

Trial no.	Initial burette reading	Final burette reading	Volume of $KMnO_4$ used (ml)
1.			
2.			
3.			

APPENDIX-4

SHELF LIFE STUDY OF BURFI USING 9-POINT HEDONIC SCALE

Name:

Age:

Taste each sample given and rate the characteristics given below on a scale of 1 to 9.

9-Like extremely

8-Like very much

7-Like moderately

6-Like slightly

5-Neither like nor dislike

4-dislike slightly

3- dislike moderately

2- dislike very much

1- dislike extremely

Control Burfi:

Sensory characteristics	Day 1	Day 3	Day 5	Day 7
Appearance				
Color				
Texture				
Aroma				
Taste				
Overall accepatabilty				

F4 Burfi:

Sensory characteristics	Day 1	Day 3	Day 5	Day 7
Appearance				
Color				
Texture				
Aroma				
Taste				
Overall accepatabilty				