

**DEVELOPMENT OF MILKSHAKE BLENDED WITH PUMPKIN
SEED (*Cucurbita moschata*)**

by

Miss. Kalyani Subhash Auti

(Reg. No. 2021/134)

A Thesis submitted to the
**MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA**

in partial fulfilment of the requirements for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

DAIRY SCIENCE



DEPARTMENT OF ANIMAL HUSBANDRY AND DAIRY SCIENCE

**POST GRADUATE INSTITUTE,
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
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2024

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APPROVED BY

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DEPARTMENT OF ANIMAL HUSBANDRY AND DAIRY SCIENCE

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MAHARASHTRA, (INDIA)**

2024

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part
there of has not been submitted
by me or other person to any
other University or Institute
for a Degree or
Diploma

Place : MPKV, Rahuri

(Kalyani S. Auti)

Dated : / /2024

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Head,

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Rahuri – 413 722, Dist. Ahmednagar,

Maharashtra, India

CERTIFICATE

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The assistance and help received during the course of this investigation have been duly acknowledged.

Place : MPKV, Rahuri

Date : / /2024

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Dr. D.K. Kamble

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Department of Animal Husbandry and Dairy Science,
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Place : MPKV, Rahuri

Date : / /2024

(D.K. Kamble)

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Associate Dean,
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Place : MPKV, Rahuri

Date : / /2024

(S.A. Ranpise)

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“If I have seen further, it is by standing on the shoulders of Giants”

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(Kalyani S. Auti)

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LIST OF ABBREVIATIONS

%	:	Per cent
^o C	:	Degree Celcius
@	:	At the rate
μ	:	Micron
μg	:	Microgram
β	:	Beta
AAS	:	Atomic Absorption Spectrometry
AOAC	:	Association of official analytical collaboration
AR	:	Analytical Reagent
AD	:	Anno Domini
BOD	:	Biological Oxygen Demand
CC	:	Coliform Count
CD	:	Critical Difference
cm	:	Centimetre (s)
CMC	:	Carboxymethylcellulose
<i>et al.</i>	:	And Other (et alli)
FAO	:	Food and Agriculture Organization
Fig.	:	Figure
g	:	Gram
GOI	:	Government of India
GR	:	Guaranteed Reagent
h	:	Hour (s)
HDL	:	High density lipoprotein
i.e.	:	That is
IS	:	Indian standards
ISO	:	International Organization for Standardization
Kcal	:	kilo calorie
LDL	:	Low density lipoprotein
lit.	:	Litre
LA	:	Lactic acid
LR	:	Laboratory Reagent
MBR	:	Mean Burette Reading
mg	:	Milligram

min	:	Minutes
ml	:	Millilitre
MPKV	:	Mahatma Phule Krishi Vidyapeeth
MG	:	Microbial Growth
N	:	Normality
NA	:	Nutrient Agar
ND	:	Not Detected
NIR	:	Near Infrared Radiation
No.	:	Number (s)
N.D.R.I.	:	National Dairy Research Institute
NHB	:	National Horticulture Board
NPU	:	Net Protein Utilization
PUFA	:	Polyunsaturated fatty acid
PDA	:	Potato Dextrose Agar
pH	:	Per centage of H ⁺ ions
SPSF	:	Sprouted Pumpkin Seed Flour
RH	:	Relative Humidity
RSM	:	Response Surface Methodology
S.E.	:	Standard Error
SMP	:	Skim Milk Powder
SNF	:	Solid not fat
SPC	:	Standard Plate Count
TCC	:	Total Carotenoid Content
TPC	:	Total Phenolic Content
TS	:	Total Solid
UHT	:	Ultra high temperature
UV	:	Ultra violet
viz.	:	Namely
VRBA	:	Violet Red Bile Agar
Wt.	:	Weight
YMC	:	Yeast and Mold Count

ABSTRACT

DEVELOPMENT OF MILKSHAKE BLENDED WITH PUMPKIN SEED (*Cucurbita moschata*)

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Miss. Kalyani Subhash Auti

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Research Guide : Dr. D.K. Kamble

Department : Animal Husbandry and Dairy Science

Milkshake is a product of western origin which is obtained by preparing mix containing milk, skim milk powder, stabilizer and sugar and speed mixing the product in mixer to make it pourable and generate foam in it. The milkshakes that are commonly sold in the Indian sub-continent consist of sweetened cold milk added with colorings and flavoring agents without freezing but vigorously shake.

The pumpkin seeds are unique in flavor and nutty in taste and rich in iron, protein, manganese, magnesium, zinc, potassium, copper, phosphorous, polyunsaturated fatty acids and carotenoids thus consumed in various regions. Pumpkin seeds play important role in relaxing vessels and lowering blood pressure, anti-diabetic effect, cancer management, gynaecological effect and also for natural treatment of parasites. Blending of pumpkin seeds in milkshake is beneficial for malnourished children, pregnant women and aged people due to its nutraceutical importance.

It is observed that as other number of milk products, milkshake is also lacking in mineral content. Due to growing health awareness, there has been huge demand for nutrient enriched food. Considering the nutritional importance of pumpkin seed and consumer demand towards western products, the effort has been made to prepare milkshake blended with pumpkin seed.

Initially four different forms of pumpkin seed viz., roasted pumpkin seed powder, sundried pumpkin seed paste, sun dried pumpkin seed paste + roasted pumpkin seed powder (1:1) and sprouted seed paste taken at rate of 5 per cent were studied where sugar 10 per cent and cow milk standardized for 4.5 per cent fat was kept constant. On the basis of sensory evaluation most accepted pumpkin seed form i.e. roasted pumpkin seed powder was selected for further study.

During second phase of investigation, three types of sweetener viz., 10 per cent Sugar, 0.03 per cent Natural Sweetener (Stevia) and 10 per cent Honey were taken where selected pumpkin seed form and cow milk standardized for 4.5 per cent fat was kept constant.

At final stage, of experiment pumpkin seed milkshake samples prepared by adopting optimized processing steps using three levels of roasted pumpkin seed powder viz., 3 (K₁), 5 (K₂), 7 (K₃) per cent of milk and two levels of sugar viz., 8 (S₁) and 10 (S₂) per cent of milk. The treatment combinations were T₀, T₁ (K₁S₁), T₂ (K₁S₂), T₃ (K₂S₁), T₄ (K₂S₂), T₅ (K₃S₁) and T₆ (K₃S₂). The milkshake prepared under various stages of experiment were evaluated for sensory analysis for color and appearance, body and texture, flavour and overall acceptability on 9 points hedonic scale and analysed for physico-chemical and microbial qualities. Further the data was analysed statistically by using completely randomized design (CRD). The result showed that out of six treatment combinations, the colour and appearance, body and texture, flavour and overall acceptability score was recorded maximum for treatment T₅ (K₃S₁).

The standardized formulated milkshake (7 per cent roasted pumpkin seed powder and 8 per cent sugar) had 4.12 per cent fat, 3.22 per cent protein, 11.48 per cent carbohydrate, 1.45 per cent crude fiber, 1.15 per cent ash, 21.42 per cent total solid, 0.12 per cent acidity, 6.49 pH, 10.37 mg/g calcium, 5.47 mg/g magnesium, 5.44 mg/g zinc, 1.13 mg/g iron, 6.27 mg/g potassium and 11.95 mg/g phosphorous. The SPC count was 1.19×10^5 cfu/ml, while yeast and mould and coliform growth was not detected.

The shelf life of milkshake samples was evaluated at interval of 3 days at $5\pm 1^{\circ}\text{C}$ and it was observed that physico-chemical and microbial quality deteriorate at 9th day of storage and product was unacceptable at 12th day. During storage the fat, protein, carbohydrate, crude fiber, ash and total solid and mineral content viz., calcium, magnesium, zinc, iron, potassium and phosphorous decreased with increase in storage period. While, acidity increased with decreased pH. The SPC and YMC increased as storage period progressed while, no colliform growth was observed during 9th days of storage. The data was analysed statistically by using factorial completely randomized design (FCRD).

Developed milkshake blended with roasted pumpkin seed powder was subjected to consumer (N=100) acceptance test and they rated the product between “very good” to “excellent”. The cost of production of experimental milkshake blended with roasted pumpkin seed powder was found Rs. 119.32/lit.

1. INTRODUCTION

Dairy sector in India has grown substantially over the years and it is still world's largest producer of milk. The amount of milk produced in 2022-23 estimated at 230.58 million tonnes, representing yearly increase of 5.29 per cent. About 46 per cent of the milk produced in India is either consumed by farmers directly or sold to non-farmers in rural areas, leaving 54 per cent of the milk accessible for sale to both organized and unorganized players (GOI, 2022-23).

Milk and milk products are important parts of the daily diet, especially for vulnerable populations including infants, school-age children and the elderly. Milk is regarded as a practically complete food since it is an excellent supply of protein, fat and essential minerals (Davies *et al.*, 1986). Various animal milks provide the required constituents in varying amounts (Kholif *et al.*, 1994). Milk components provide nutritional security as well as capable of providing potential health benefits in their various forms (Burrington, 1999).

Being a perishable commodity, milk spoils quickly if not handled appropriately. Along with to being used and consumed directly as market milk, surplus milk is turned into a variety of milk products to suit the preferences of individuals from various regions of the nation. Processing of milk into dried, coagulated, fermented, or frozen milk products, delectable foods can be created from it. The process of turning milk into milk products has been around since ancient times and is being used today. The Indian milk production system has recently advanced in response to market needs, which ultimately represent customer preferences.

The enrichment of food products is a consequential idea to treat explicit nutritional insufficiencies. Food enrichment improves human health and helps prevent chronic illnesses. Consumption of dairy products has recently been linked to health benefits that are the direct antitheses of diseases and complexity that related to overweight and obesity. About 50 per cent of India's milk production is utilized for making Indigenous milk products such as Milkshake, ghee, makhan, khoa, paneer, channa and curd. These products provides profitable outlet to the organized sector (Aneja *et al.*, 2002).

Traditional dairy products and sweets are an integral part of Indian heritage. Milkshake is a product of western origin which is obtained by preparing mix containing milk, skim milk powder, stabilizer and sugar and speed mixing the product in mixer to make it pourable and generate foam in it. The milkshakes that are commonly sold in the Indian sub-continent consist of sweetened cold milk added with colorings and flavoring agents without freezing but vigorously shake (Ubale *et al.*, 2014).

A milkshake is a western dairy product with lower fat and sugar contents and higher milk solids not fat (MSNF) than ice cream. It is made by freezing a mix and quickly mixing the frozen product in a mixer to make it pourable and generate foam in it (Sharma and Gupta, 1978).

A milkshake is a sweet, cold beverage which is usually made from milk, ice cream or iced milk and flavorings or sweeteners such as fruit syrup or chocolate sauce. Milkshakes, like soft-frozen products, are usually prepared for consumption at the retail outlet directly from milkshake mixes that have been processed at a dairy processing facility and packaged and shipped in bulk containers (Karki *et al.*, 2015).

Pumpkin belongs to family Cucurbitaceae grown worldwide as a vegetable. Several species of pumpkin present viz., *Cucurbita pepo*, *Cucubita maxima*, *Cucurbita moschata* (Lee *et al.*, 2003). The pumpkin seeds are utilized for cure of different diseases. The pumpkin is one of the famous edible plant that is utilized as cure of many disorders due to occurrence of many edible components and phytochemicals (Yadav *et al.*, 2010).

India is the second largest country producing pumpkin in the world after China. The area and production of pumpkin in India during 2021-22 are 1,09,000 hectares and 2299 million tonnes respectively (NHB, 2022).

As long as, Indian diet has included the flesh of many plants, but despite the high nutritional worth of the seeds, they have nearly always been thrown away as trash. Pepitas or pumpkin seeds, are one kind of such seed. Pumpkin seeds are regarded as a by-product in Asian nations. The seeds are frequently thrown away, broken up for fertilizer, or fed to animals after harvest. In India, only little portions of salted and roasted seeds are consumed; the rest are wasted. Typically, high-quality pumpkin seeds are obtained by allowing the fruit to reach full maturity. Pumpkin fruit has a seed content that

ranges from 3.52 to 4.27 per cent. The semi-flat, ovoid-shaped pumpkin seeds have a conical tip and are filled with olive-green kernels that have a nutty, buttery and sweet flavor. They can be used to savory meals or eaten as a snack. In the past, pumpkin seeds have been used to make oil, strengthen breads, eat them as a snack and even treat medical conditions. A distinct flavor that is well-known worldwide, pumpkin seeds and pumpkin seed oil help to generate an aromatic flavor when they are roasted. The nutritional and health-protective qualities of pumpkin seeds have drawn a lot of attention.

The pumpkin seeds are unique in flavor and nutty in taste and rich in iron, protein, manganese, magnesium, zinc, potassium, copper, phosphorous, polyunsaturated fatty acids and carotenoids thus consumed in various regions. Pumpkin seeds play important role in relaxing vessels and lowering blood pressure, anti-diabetic effect, cancer management, Gynaecological effect and also for natural treatment of parasites. Blending of pumpkin seeds in milkshake is beneficial for malnourished children, pregnant women and aged people due to its nutraceutical importance. Very scanty work had been carried out by using pumpkin seeds in milk products. Hence, for considering the nutritional importance of milk and pumpkin seeds the attempt has been made to “Develop milkshake blending with pumpkin seed” with following objectives

1. To optimize the level of pumpkin seed in milkshake.
2. To study sensory, physico-chemical and microbial qualities of milkshake.
3. To assess the shelf life of milkshake blended with pumpkin seed.

2. REVIEW OF LITERATURE

Milkshake is one of the important fluid milk product consumed mainly in Western countries. There had been a limited research work carried out on different aspects of this product. The Milkshake shows wide variation in method of manufacture, chemical and microbial quality, packaging and shelf life. The paucity of adequate published information of milkshake is the main constraint in determining the legal standard. The work done so far on production of milkshake and similar products is reviewed as under

2.1 Nutritional Importance of Milk and Milk Products

Milk has been known as nature's most complete food (Park, 2009) for millennia, playing currently an important role in the diet of over 6 billion people in the world (Haug *et al.*, 2007). Milk and dairy products are nutrient-dense foods, supplying energy and high-quality protein with a range of essential micronutrients (especially calcium, magnesium, potassium, zinc and phosphorus) in an easily absorbed form (Pfeuffer *et al.*, 2017). Milk minerals are crucial for human health and development as well as in dairy processes as cheese making and for all traits involving salt-protein interactions (Franzoi *et al.*, 2017). They play a key role in healthy human nutrition and development throughout life, but especially in childhood (Muehlhoff *et al.*, 2013).

Dairy products are rich in nutrients that are essential for good bone health, including calcium, protein, vitamin D, potassium and phosphorus (Rizzoli, 2014). Adequate calcium intake influences skeletal calcium retention during growth and thus affects peak bone mass achieved in early adulthood (Bonjour, 2005). The high levels of calcium play an important role in the development, strength and density of bones for children and in the prevention of bone loss and osteoporotic fractures in elderly people (Park, 2009). Studies show that frequent consumption of dairy foods and milk should be recommended in order to prevent periodontal disease (Adegboye *et al.*, 2012). Calcium also has been shown to be beneficial in reducing cholesterol absorption and in controlling body weight and blood pressure (Park, 2009).

Dairy industry has developed a large scale of new, nutritionally improved products which have achieved a very good success in the market. Therefore, traditional

dairy products as we have known them for ages are evolving into the new generation of dairy products with different characteristics and better health and nutritional properties. Milk based beverages belong certainly to this group of new products, milk based beverages includes flavoured milk, milkshakes etc.

Murphy *et al.* (2008) had conducted a research on health effects associated with consumption of flavoured milk in US. They reported that milk is a key source of macronutrients such as calcium, magnesium, phosphorus, vitamin D, vitamin A, riboflavin, vitamin B-12, zinc and potassium for US children and teenagers and milk drinkers are more likely to meet dietary recommendations for many nutrients. Other beverages contribute to energy intakes but provide fewer nutrients. Several of the nutrients provided by milk are important for optimal health and growth. The dietary guidelines for Americans recommend that children aged 2 to 8 years consume 2 c/day fat-free or low-fat milk or equivalent dairy servings and that all people aged 9 years and older consume three dairy servings per day.

Milk consumption is frequently included as important elements in a healthy and balanced diet. It is the first food for mammals and provides all the necessary energy and nutrients to ensure proper growth and development, being crucial in respect to bone mass formation. However, several controversies arise from consumption of milk products during adulthood, especially because it refers to milk from other species. Despite these controversies, epidemiologic studies confirm the nutritional importance of milk in the human diet and reinforce the possible role of its consumption in preventing several chronic conditions like cardiovascular diseases, some forms of cancer, obesity and diabetes. Lactose malabsorption symptoms and cow milk protein allergy are generally considered to be the adverse reactions to milk consumption (Pereira *et al.*, 2014).

Milk is a considerable resource of products whose composition varies. Four components are dominant in quantitative terms: water, fat, protein and lactose; while the minor components are minerals, enzymes, vitamins and dissolved gases. It satisfies the demand of the consumer who seeks more and more innovative products with consistent quality. The dairy industry needs to utilize all the riches of this raw material, which is both simple in appearance and complex in composition. In general, cow milk is

less rich in lactose, fat content and protein. On the other hand, the mineral content is similar (Guétouache *et al.*, 2014).

2.2 Technology of Manufacture of Milkshake

Pakalwad *et al.* (2010) standardized procedure for preparation of milkshake from buffalo milk blended with papaya pulp and studied for its acceptability. Milkshake was prepared from different proportions of buffalo milk and papaya pulp i.e. 100:0 (T₀), 90:10 (T₁), 85:15 (T₂) and 80:20 (T₃). The sensory score for overall acceptability of papaya milkshake of treatments T₀, T₁, T₂ and T₃ were 8.18, 8.56, 8.23 and 8.03, respectively. It was observed that the papaya milkshake prepared from 90 parts of buffalo milk and 10 parts of papaya pulp was most acceptable and ranked between like very much to like extremely.

Ubale *et al.* (2014) prepared sapota milkshake wherein they added sapota pulp at three levels i.e. 7, 8 and 9 per cent and sweetened with jaggery at 7, 8 and 9 per cent of milkshake reported that the sapota milkshake prepared with 7 per cent jaggery and 8 per cent pulp was accepted by the all judges as this combination scored 6.10 to 7.17 and ranked between like very much to like extremely.

Karki *et al.* (2015) prepared milkshake by using wheat bran and banana pulp extract. Initially they washed wheat bran and subsequently dried at 50°C for 2 hrs followed by moist heat stabilization at 120°C for 15 min. At last they grind the bran and pass through 0.3 mm sieve. Simultaneously ripened banana pre treated and pulp was extracted. Both these ingredients were mixed in hot milk at 60°C along with sugar. Eight different formulations were made using factorial equation and sample with 27 per cent banana, 7 per cent sugar and 6 per cent wheat bran had highest mean acceptance by all the judged. Then mixture was stir and cooled at room temperature. To control the acidity of product sodium by carbonate was added @ 0.02 per cent. At last milkshake was filled in glass bottles and kept at 5°C.

Bakane *et al.* (2016) prepared milkshake with the pulp of custard apple fruits. To have uniformity in the trial, they extracted custard apple pulp and stored at -20°C. Custard apple milkshake was prepared from different proportion of milk and custard apple pulp i.e. 100:0, 90:10, 80:20, 75:25, 70:30, 65:35, 60:40. The sensory evaluation of custard apple milk was carried out by 9 point Hedonic scale and milkshake

with 35 per cent custard apple pulp and 65 per cent milk was most acceptable and ranked between like very much to like extremely i.e. from 8 to 9.

Kadav (2001) prepared fruit flavoured milkshake with the fortification of pineapple syrup and jamun juice @ 5, 10, 15 and 20 per cent level each. Milkshake was made by adding 5 per cent skim milk powder, 10 per cent sugar and 0.5 per cent gelatin in milk. He concluded that milkshake prepared using 15 per cent pineapple syrup and 10 per cent jamun juice was superior over rest of the treatments.

Taware (2002) prepared custard apple milkshake with different concentrations (10, 20 per cent of custard pulp and two sugar levels i.e. 7 and 9 per cent) the research work was conducted in two phases viz. the 15 preliminary trials (phase-I) and experimental trials (phase-II). Milkshake with 15 per cent custard apple pulp and 9 per cent sugar was acceptable and ranked between like moderately to like very much for all sensory attributes.

Jadhav *et al.* (2003) manufactured a sapota milkshake with different concentrations (10, 20 and 30 per cent sapota pulp). Cow milk was filtered, pasteurized (63°C for 30 minutes), then cooled to 5°C. Sapota pulp and sugar were added to the milk. The pulp-milk blend was mixed in a blender then cooled to 5°C. Milkshake with 20 per cent sapota pulp and 80 per cent cow milk was most acceptable than rest of the treatments.

Kashid (2005) manufactured golden milkshake from cow milk blended with safflower milk from different proportions 100:0 (T₀), 70:30 (T₁), 60:40 (T₂), 50:50 (T₃) was prepared and studied for its acceptability and chemical composition. Blending of safflower milk lowers the cost of production. The maximum score for colour and appearance, flavour, consistency, mouthfeel and overall acceptability were 8.45, 8.27, 8.03, 8.53 and 8.32, respectively. The golden milkshake contained fat, protein, sugar, ash, TS, acidity, pH as 4.5, 3.22, 14.08, 0.68, 23.44, 0.13 and 0.49, respectively. Golden milkshake prepared from 70 parts cow milk and 30 parts safflower milk was closer to control in acceptability; Golden milkshake prepared from 50 parts of cow milk and 50 parts of safflower milk was also acceptable, scoring between like moderately to like very much, for all sensory attributes. The cost of production of Golden milkshake prepared from safflower milk : cow milk (50:50) blend was Rs. 11.30 and was more economical

than control. Good quality Golden milkshake could be prepared by blending cow milk with safflower milk (50:50) with addition of 8 per cent cane sugar.

Sankhla and Bhatnager (2006) prepared recipe of anjeer shake using different combination of ingredients and studied its organoleptic acceptability and suitability for drying. The shake having 12.5 per cent anjeer and 17.0 per cent total solids gave best quality product, in the form of thin uniform dried sheets when dried in roller shake prepared by reconstituting instant mix was well accepted by the selected panel of judges.

Poul *et al.* (2009) manufactured milkshake from buffalo milk blended with custard apple pulp from different proportion 100:00 (T₀), 90:10 (T₁) 85:15 (T₂) and 80:20 (T₃) and studied for its acceptability and chemical composition. The maximum score for colour, appearance, flavour, consistency, test and overall acceptability were 8.25, 8.52, 8.65, 8.70 and 8.51, respectively. He concluded that milkshake prepared from 90:10 blends of buffalo milk and custard apple pulp was most acceptable and economical.

Kuchekar (2011) manufactured milkshake from buffalo milk blended with almond at different proportion of 95:5 (T₁), 90:15 (T₂) and 85:20 (T₃) and studied for acceptability and chemical composition. It was observed that the maximum score for colour appearance, flavor consistency, taste and overall acceptability were 8.25, 8.52, 8.65, 8.70 and 8.51, respectively. Milkshake blended with 5 per cent crushed almond and 95 per cent buffalo milk rated maximum score for overall acceptability and economical to consumers.

Mule (2012) prepared milkshake from buffalo milk with constant level of sugar and different levels of crushed fig (5, 7.5, 10 %). It was observed that score for overall acceptability ranged in between 8.11-8.30. Fig Milkshake for different treatments are T₁, T₂, T₃ and T₄ resulted for overall acceptability as 8.11, 8.25, 8.32 and 8.13, respectively. He found that blending of fig 5 per cent with buffalo milk result in superior quality and highest overall acceptability.

2.3 Chemical composition of milkshake

Sharma and Gupta (1978) reported the chemical composition of milkshake as fat 4.0, SNF 13.0, sugar 10.0 and sodium alginate 0.4 per cent. Further they reported

that the blending the powder with crashed ice gave milkshake with 28 per cent total solids.

Holsinger *et al.* (1987) conducted study to evaluate the acceptability of low sugar chocolate milkshake. On an average the low sugar milkshake and a commercial milkshake, contained milk fat 3.25 and 3.25, SNF 11 and 11, sweetener 6 and 11, TS 21.55 and 27.05, cocoa 1.0 and 1.5, stabilizer 0.3 and 0.3 per cent, vanilla flavour 3 and 3 drops, respectively.

Planovskii and Golovach (1988) studied the development of acidity and manufacturing technique of the dried mixture of skim milk and apple juice. The composition of product was given as fat 1.1, protein 25.30, carbohydrates 55.00, ash 13.50 and moisture 5.00 per cent.

Varpe (1992) studied flavoured milkshake with fortification of mango, sapola, apple and orange pulp at the 5, 10 and 15 per cent level, in which 15 to 10 per cent level of mango pulp and 10 per cent level of orange squash was found to be more acceptable. He reported the composition of plain milkshake as fat 3.63, TS 26.55 and acidity 0.189 per cent.

Kshirsagar (1996) studied the fruit flavoured milkshake with fortification of mango, sapota, coco and mango essence in which milkshake with 12 per cent level of mango fruit pulp had superior sensory quality and acceptability than the other milkshakes. He stated the chemical composition of plain milkshake as fat 2.12, TS 24.32 and acidity 0.18 per cent.

Kadav (2001) prepared fruit flavoured milkshake with pineapple syrup and jamun juice in which milkshake with 15 per cent pineapple syrup level and reported chemical composition of plain milkshake as fat 2.67, total solids 25.27, acidity 0.18 per cent.

Jadhav *et al.* (2003) studied on blending of milkshake with different combination of sapota pulp. He reported average composition of milkshake as fat 3.72 to 3.41, TS 25.40 to 21.13, protein 3.23 to 2.59 per cent.

Kashid (2005) prepared golden milkshake from cow milk and studied chemical properties. He reported chemical composition of golden milkshake as fat 4.5, protein 2.80 to 3.22, TS 21.54 to 23.44 per cent.

Poul *et al.* (2009) prepared milkshake from buffalo milk blended with custard apple pulp and studied chemical properties. He reported composition of milkshake as fat 3.95, protein, 3.84, sugar 13.38, ash 0.81 and TS 24-26 per cent.

Pakalwad *et al.* (2010) prepared papaya milkshake from buffalo milk and studied chemical composition as, fat 3.95, protein 3.87, ash 0.79, carbohydrate 17.17 and TS 18.00 per cent.

Kuchekar (2011) prepared almond milkshake from buffalo milk and studied chemical properties. He reported composition of milkshake as fat 7.40, protein 4.44, sugar 12.27, ash 1.59 and TS 25.70, Acidity 0.15 per cent.

Mule (2012) prepared milkshake from buffalo milk blended with fig and studied mean chemical composition as 6.04, 4.52, 1.25, 12.78, 24.59, 0.14 per cent for fat, protein, ash, sugar, total solids and acidity, respectively.

2.4 Sensory Quality of Milkshake

Wheeler and Gillies (1973) formulated an organoleptically acceptable banana milkshake by mixing ripe Cavendish bananas and whole or partially skimmed milk in the ratio of 1:3 by weight. During preliminary trials, fat content of the milkshake, homogenization pressure, banana type, ripeness and concentration as well as fresh and preserved purees, were evaluated by a trained panel. Acceptability of banana milkshake containing additives was also assessed.

Bhandari *et al.* (1976) reported that rose and pineapple flavoured milk prepared from admixture of 50 per cent filled milk and 50 per cent standardized milk was highly acceptable.

Sharma and Gupta (1978) used different combinations as fat 3, 4 and 5 per cent, MSNF 12, 13 and 14 per cent with spray dried and roller dried skim milk, of these combinations, 4 per cent of milk fat and 13 per cent SNF with 10 per cent sugar and 0.4 per cent sodium alginate gave milkshake with good whipping ability, foam stability and good sensory evaluation.

Patil (1983) studied the organoleptic quality of banana milk beverages. He observed the average score of 7.0 for flavour character, consistency score ranged between 6.0 to 8.5 and score for overall acceptability ranged between 5.5 to 8.0.

Varpe (1992) studied flavoured milkshake with fortification of mango, sapota, apple and orange pulp @ 5, 10 and 15 per cent level, amongst all the treatments 10 to 15 per cent level of mango pulp and 10 per cent level of orange squash was found to be most acceptable.

Kshirsagar (1996) prepared the fruit flavoured milkshake with fortification of mango, sapota, coco and mango essence in which milkshake with 12 per cent level of mango fruit pulp had superior overall acceptability than the other milkshakes.

Gonzalez *et al.* (2000) conducted study on acceptability of 13 vanilla milkshakes, containing 31.72 - 88.28 g sugar and 0.17-5.83 g colouring, per liter of low fat (1.55 %) and subjected to sensory evaluation using RSM before being tested by a panel of 40 consumers, aged 20-25 years. All milkshakes contained 40 g of vanilla flavourings per liter of milk. The most acceptable milkshakes contained 60 g sugar and 2.2 g of colouring per liter of milk.

Kadav (2001) prepared fruit flavoured milkshake with pineapple syrup and jamun juice. The milkshake with 15 per cent pineapple syrup had score for overall acceptability of 7.82 closely followed by 10 per cent level of jamun flavoured milkshake scoring 7.19.

Jadhav *et al.* (2003) conducted study on the acceptability of milkshake (colour, appearance, flavour, consistency, overall acceptability) with different concentrations (10, 20 and 30 %) of sapota pulp with cow milk. The milkshakes were subjected to sensory analysis. Results showed that the scores for colour and appearance of sapota milkshake decreased with increasing level of sapota pulp. The milkshake with 10 per cent sapota pulp had a very light brown colour which was acceptable to the judges. Increasing the level of sapota pulp resulted in increased viscosity which was not preferred by the judges. Increasing the level of sapota pulp from 10 to 20 per cent did not affect the overall acceptability to a great extent, however incorporation of 30 per cent sapota pulp significantly decreased the overall acceptability of the milkshakes. The maximum score for colour, flavour, consistency, overall acceptability were 8.22, 8.22, 8.05 and 8.16, respectively.

Kashid (2005) prepared golden milkshake and studied for its organoleptic quality. He reported that blending for golden milkshake can be done to maximum extent

of 50 per cent safflower milk + 50 per cent cow milk, this combination was acceptable to the judges. Golden milkshake manufactured from cow milk (control) had maximum score for overall acceptability of 8.32 followed by T₁, T₂ and T₃ (8.21, 7.94 and 7.46).

Rebate *et al.* (2010) prepared flavoured milk by blending safflower milk with cow milk. The flavoured milk prepared from cow milk served as control (T₀), 80 per cent cow milk + 20 per cent safflower milk (T₁), 70 per cent cow milk + 30 per cent safflower milk (T₂), 60 per cent cow milk + 40 per cent safflower milk (T₃), sugar was added @ 8 per cent and orange colour and flavour was used in flavoured milk. He reported that treatment (T₃) was most acceptable.

2.5 Keeping Quality of Milkshake

Wheeler and Gillies (1973) formulated organoleptically acceptable banana milk by mixing ripe bananas and whole or partially 23 skimmed milk in the ratio of 1:5 by weight. The product, when stored at 3°C and 10°C, has a keeping quality of 2 weeks. When banana milk was made into yoghurt, manufacture pattern (and fermentation rate) was normal and the product was acceptable to tasters.

Nolan (1982) studied development of banana delight dry mix, i.e. mixed with ice or ice + water to give a milkshake product. The dry mix has a shelf life of approximately one year at storage temperature < 85°F, has various other applications, e.g. imitation, ice-cream mix, whipped toppings and its content in final product is approximately 15-40 per cent.

Khan and Lynch (1985) studied freezer stable whipped ice cream and milkshake food products. The product is substantially non crystalline and is spoonable at about 0°C and volume of the product remains substantially constant during storage at about 0°C for at least 6 months. Optionally, a polyhydric alcohol may replace part of sugar. The products can be used like a soft serve ice-cream at 0.15°C, or as a milkshake at higher temperature.

2.6 Microbial Quality of Milkshake

Comi *et al.* (1993) studied bacteriological quality of milkshakes. Over 2 years (1990-92), 1402 milkshake samples were analysed for total counts of aerobic flora, coliforms *staphylococcus aureus*, *salmonella species*, yeasts and moulds. The microbiological counts varied according to the milk-base used: chocolate, banana and

strawberry flavourings. Chocolate flavoured samples were contaminated than others and this correlated with high yeast count in chocolate intermediates and bases. Mean counts of total yeasts and coliforms in pasteurized and non-pasteurized chocolate milkshakes showed no significant differences on the basis of microbiological analysis. Despite this poor hygienic quality, it is concluded that pathogen count was too low to cause alimentary toxicity.

Barnard *et al.* (1995) investigated the bacteriological quality of flavoured milkshakes. Eight hundred and eight milkshakes were collected and tested for bacteria within 24 hours. Milkshake samples tested, 39 per cent contained < 1 coliform /g and 42 per cent contained >10 coliform /g, 34 per cent of the milkshakes had standard plate count (SPC) of < 1000 organisms per g, however 26 per cent of the samples exceeded the regulatory maximum of 50,000 organisms/g.

Al-Haq *et al.* (1996) formulated a apple milkshake with 13 per cent cane sugar, 3 per cent apple pulp, 2 per cent SMP, 0.3 per cent Givaudan's stabilizer, 0.2 per cent citric acid, 0.02 per cent sodium citrate, 0.01 per cent potassium sorbate, 0.07 per cent pineapple flavour, colour as desired and water to 100 per cent. This was manufactured at a.UHT installation, using direct injection of steam at 144 °C and packed in 250 ml brick packs. The results of the triangular test showed that the product was admired by the panelists and it remained good up to 75 days' ambient storage or 90 days refrigerated storage.

More (2016) prepared aloe vera added custard apple milkshake with cow milk, 12 per cent custard apple pulp, 10 per cent sugar and three levels of aloe vera pulp viz. 3, 4 and 5 per cent, respectively. Among that milkshake with cow milk, 12 per cent custard apple pulp, 10 per cent sugar and 4 per cent aloe vera pulp showed highest overall acceptability. He observed that SPC, YMC and CC of milkshake were 2.25×10^6 , 3×10^5 and 0.75×10^4 (cfu/g), respectively.

Hemant *et al.* (2021) formulated milkshake using acid modified psyllium husk with 630 ml of milk, 270 gm apple pulp and 100 gm sugar. Acid modification of psyllium husk was done using HCL in ethanol as solvent. Acid modified psyllium husk of 0.75% HCL of ratio 1:6 in ethanol solvent had better functional properties, hence it was used in milkshake samples at different percentages of 0.4, 0.6 and 0.8. Ageing of mix was

done at 6-10°C for 2-3 hours followed by deep Freezing of Mix -2 to -6 °C and after the addition of modified psyllium husk, milkshake was stored at -18°C for 24 hours. He found that TPC growth was 1.6×10^5 , 3.5×10^5 and 4.2×10^5 (cfu/g) for the first, second and third week, respectively. There was no yeast and mold growth in the first week but in the second and third week growth of 2.1×10^5 and 2.9×10^5 (cfu/g) were seen. Coliform count was not found in the first two weeks under good hygienic condition during refrigeration of milkshake.

2.7 Chemical Composition and Nutritional Importance of Pumpkin Seed

Hamed *et al.* (2008) analysed the defatted pumpkin seed flour in unroasted and roasted form which showed the proximate composition of unroasted and roasted flour as moisture 5.47 and 6.10 per cent, crude protein 65.05 and 60.17 per cent, crude fiber 2.98 and 3.75 per cent, crude ash 9.04 and 8.78 per cent, carbohydrate 15.63 and 18.68 per cent, respectively.

Kaur (2017) developed and evaluated pumpkin seed supplemented products also analysed proximate composition of raw and roasted pumpkin seed flour as moisture 6.98 and 2.80 per cent, protein 22.05 and 23.45 per cent, fat 30.80 and 91.90 per cent, crude fiber 7.68 and 7.56 per cent, ash 8.92 and 8.04 per cent, carbohydrates 31.25 and 33.81 per cent, respectively.

Lim (2012) reported that dried pumpkin seed kernels called pepitas had nutrient composition/100 g edible portion as energy 559 kcal, protein 30.23 g, total lipids 49.05 g, ash 4.88 g, carbohydrates 10.71 g, total dietary fiber 6 g, iron 8.82 mg, magnesium 592 mg, zinc 7.81 mg, total polyunsaturated fatty acids 20.976 g, β -carotene 9 μ g, leutin + zeaxanthin 74 μ g. Pumpkin seeds were considered as most nutritious and excellent source of vitamin A, all the minerals, protein and fair sources of thiamine and niacin.

Elinge *et al.* (2012) analysed the pumpkin seeds for their nutritional and antinutritional composition and the results obtained were: moisture content 5.00 per cent, ash 5.50 per cent, crude lipid 38.00 per cent, crude fiber 1.00 per cent, crude protein 27.48 per cent, carbohydrate, 28.03 per cent, energy 564 Kcal/100g. Mineral analysis showed that the iron and zinc content were 3.75 mg and 14.14 mg per 100 g.

Abd El-Ghany *et al.* (2010) found that the percentage values of protein, fat, ash, fiber, moisture and carbohydrate in pumpkin seeds were 31.57, 29.01, 3.89, 6.36, 5.11 and 24.06 per cent, respectively. The values of zinc, iron, calcium, magnesium and sodium were also analysed which were 7.99, 9.76, 78.18, 90.69 and 20.56 mg/100 gm.

Rodriguez-Miranda *et al.* (2012) studied the chemical composition of whole pumpkin seed meal and defatted pumpkin seed meal and results were as protein 35.45 and 64.13 per cent, fat 49.14 and 7.01 per cent, crude fiber 2.30 and 1.59 per cent, ash 5.27 and 9.13 per cent, carbohydrates 7.85 and 18.14 per cent, energy 2611.07 and 1702.42 Kcal per 100gm.

Milovanovic *et al.* (2014) found the chemical composition of pumpkin seeds as moisture 5.26 per cent, ash 3.26 per cent, protein 24.46 per cent, fat 38.53 per cent and crude fiber 14.77 per cent.

Kim *et al.* (2012) revealed that oleic, palmitic, linoleic and stearic acids were the main fatty acids found in pumpkin seeds whereas seeds of *Cucurbita pepo* and *Cucurbita moschata* variety had considerably more gamma tocopherol than the seeds of *Cucurbita maxima* which had the highest beta-carotene content. *Cururbita pepo* seeds had notably more beta-sitosterol content than the other varieties of pumpkin seeds. Results for *Cucurbita moschata* was reported as carbohydrate 14.01 per cent, protein 29.81 per cent, fiber 10.85 per cent, fat 45.67 per cent, ash 5.31 per cent, moisture 5.17 per cent, β -carotene 7.15 mg/kg. Among 11 varieties of seeds and nuts profile for dietary abundance, for iron content scored highest the pumpkin seeds (95.85 ± 33.01 ppm).

Pumpkin seeds contained 41.59 per cent oil, 25.4 per cent protein, 5.2 per cent Moisture, 25.19 per cent carbohydrates, 5.34 per cent fiber and 2.49 per cent total ash. Total phenolic compounds, total sterols, waxes and total tocopherols were 66.25 (mg galic acid per kg oil), 1.86 per cent, 1.56 per cent and 882.65 (mg tocopherol per kg oil), respectively (Ardabili *et al.*, 2011).

Pumpkin (*Curcurbita maxima*) is a popular vegetable in our country. This plant is locally known as "Mistikumra". The seeds of pumpkin are rich in oil and nutrients. The proximate compositions of the powdered seed were moisture 4.06 per cent, ash 3.80 per cent, crude fiber 2.91 per cent, total lipid 36.70 per cent, total protein 34.56 per cent, total soluble protein 18.10 per cent, sugar 1.08 per cent and starch 2.15 per cent.

The minerals composition of the seed were nitrogen 5.53 per cent, phosphorus 0.71 per cent, sodium 4.80 Cmol/kg, potassium 20.00 Cmol/kg, Calcium 4.40 Cmol/kg, Magnesium 348.7 ppm, iron 290.0 ppm, copper 70 ppm, zinc 39.9 ppm and manganese 17.9 ppm. It is used as a potentially attractive source of lipid, protein and crude fiber (Habib *et al.*, 2015).

Different physicochemical characteristics of pumpkin seeds were analysed for whole pumpkin seed and kernels. The proximate composition of moisture, protein, crude fat, crude fiber, ash and carbohydrate for whole pumpkin seed were 5.53 ± 0.26 per cent, 28.90 ± 1.36 per cent, 31.75 ± 0.45 per cent, 4.59 ± 1.01 per cent, 6.90 ± 0.14 per cent and 27.85 ± 1.50 per cent while the corresponding composition of kernel were 4.43 ± 0.44 per cent, 31.98 ± 1.18 per cent, 38.29 ± 1.51 per cent, 4.26 ± 0.43 per cent, 2.36 ± 0.10 per cent and 33.11 ± 2.94 per cent respectively (Devi *et al.*, 2018).

Seeds of a Tunisian variety of pumpkin (*Cucurbita maxima*) were analysed for their main chemical composition and for their oil properties. Expressed on dry weight basis, seed moisture was 8.46 per cent, whereas contents of proteins, fiber, ash, fat and total sugars established at 33.92, 3.97, 21.97, 31.57 and 0.11 per cent, respectively. Seed oil was also found to be rich in tocopherols with a predominance of δ -tocopherol (42.27 %). The sterol marker β -sitosterol accounted for 39.6 per cent of total sterols contained in seed oil of this variety. Six phenolic acids (protocatechuic, caffeic, syringic, vanillic, p-coumaric and ferulic) were detected, the syringic acid being predominant (7.96 mg/100 g). As a whole, based on its seed oil features, pumpkin may be considered as a valuable source for new multi-purpose products for industrial, cosmetic and pharmaceutical utilization (Rezig *et al.*, 2012).

The nutrient composition analysis of pumpkin seeds showed that these are very nutritious and provide many essential nutrients for health. However, the pumpkin seeds have been used for medicinal purpose and these possess also nutritional and therapeutic importance (Syed *et al.*, 2019).

Utilization of pumpkin seeds and flax seeds or pumpkin seeds and purslane seeds blend cause reduction in lipid and suggested as these seed's combinations have anti-atherogenic activity (Barakat and Mahmoud, 2011).

The pumpkin seeds have essential role in blood pressure lowering and relaxing of blood vessels. Additionally, the high magnesium (mg) content in pumpkin seeds oil is credited to lessening the risks of coronary heart attack (El-Mosallamy *et al.* 2012).

The rapidly prevailing health problem is the cancer. The diet having high amount of pumpkin seeds have been identified as lowering the cancer risks. The pumpkin seeds oil has been found to reduce the risks of cancer as it contained the high amount of the various carotenoid's pigments (Jian *et al.*, 2005). If pumpkin seeds consumed in normal food may be useful for the diabetes prevention and the complications of diabetes (Makni *et al.*, 2010).

The seeds of pumpkin provide excessive phosphorus quantity and can be used as an effective content in minimizing the threat of stone in bladder (Voranunt *et al.*, 1987).

Pumpkin seeds, often eaten as a snack, are known to have a high content of zinc, polyunsaturated fatty acids and phytosterols. They are also a good source of fiber. They contain 31.48 per cent crude fiber (Nyam *et al.*, 2013).

Park *et al.* (2010) isolated the new protein known as pr-1 protein from pumpkin seeds that have antifungal potential with no toxicity in human erythrocytes (RBCs). It is the heat stable protein and stable at 70⁰C temperature without growth inhibiting activity towards E. Coli and Staphylococcus bacteria.

The pumpkin seeds play a significant role in providing of micronutrients and also used in treatment and management of diabetes, inflammation, hyperlipidaemia, hypertension, cancer management and protect heart, etc. (Kalogropoulos, 2013).

2.8 Utilization of Pumpkin Seed in Different Products

Naghii and Mofid (2007) compared the consumption of ready-to-eat cereal fortified with iron and pumpkin seed kernels for 4 weeks. After the consumption period, level of serum iron got higher which indicates improved iron status. Children, teenagers, pregnant women and anemic women were benefited.

Norfezah *et al.* (2011) studied the effect of incorporation of flour with peel, flesh and seeds of crown pumpkin. The flour was incorporated into an extruded snack formulation at different levels and processed in an extruding machine to make 10

products. Incorporation of pumpkin seeds and peels at level of 10 per cent resulted in stiffness of product.

Nyam *et al.* (2013) determined the proximate composition, functional properties and anti-oxidant activity of pumpkin seeds and rind. Bread was prepared by incorporating pumpkin seed and rind at different levels. Result showed that crude fiber content, phenolic compound and anti-oxidant activity of pumpkin seed and rind supplemented bread was very high.

Kanwal *et al.* (2015) developed and evaluated physico-chemical properties of biscuits supplemented with pumpkin seeds to fight under nutrition in children of Pakistan. Result showed that pumpkin seed flour can be successfully used to prepare highly nutritious and wholesome biscuits without disturbing its overall acceptability.

Jerlin *et al.* (2022) formulated healthy *Cucurbita maxima* seed incorporated milkshake and concluded *Cucurbita maxima* powder in milkshake helped to enhance health benefits as well as sensory attributes of *Cucurbita maxima* incorporated milkshake were best as compared to banana milkshake.

2.9 Pumpkin Seed and Health

Pumpkin seeds play a crucial role in soothing vessels and lowering of blood pressure. El-Mosallamy *et al.* (2012) examined the effects of pumpkin seed oil treatment on chemically induced hypertension in rats. 40-100 mg/kg pumpkin seed oil was given once daily for six weeks. It was observed that the consumption of the oil considerably reduced the increased blood pressure caused by the chemical. The higher magnesium content in pumpkin seeds is credited to lower the risk of heart attack. Abuelgassim and Alshowayman (2012) stated that rats induced with atherosclerosis were fed with pumpkin seeds for 37 days. HDL cholesterol was significantly increased in rats but also a 48 per cent decrease in total cholesterol and 79 per cent reduction in LDL cholesterol were observed.

Pumpkin seeds are a good source of minerals i.e. magnesium and phosphorous which optimize the bone health and avert osteoporosis. Ryder *et al.* (2005) assessed the relationship between magnesium intake and bone mineral density, a major factor in the development of osteoporosis, in over 2000 elderly men and women aged 70-79 yrs. After taking into account confounding factors of age, calcium intake, osteoporosis

status, Body Mass Index and physical activity, they concluded that higher intakes of magnesium were correlated with greater bone mineral density, particularly for Caucasian (white people of Europe) individuals. They believed that magnesium promotes alkaline environment inside the bones, which had shown to be conducive to boost the bone mineral density.

Pumpkin seed oil has influential antioxidant properties that relieve inflammation related with arthritic symptoms. Fahim *et al.* (1995) conducted an experiment in which rats were induced with arthritis showed significant increased levels of inflammation which were reduced on the supplementation of rats with pumpkin seed oil; results that compared favourably to when the rats received the nonsteroidal anti-inflammatory drug indomethacin. Furthermore, the indomethacin supplemented rats experienced high levels of lipid peroxidation in liver i.e. an indicator of liver injury, whereas the pumpkin seed oil supplemented group of rats experienced no side effects.

Hudson *et al.* (2007) conducted a study which revealed that tryptophan was abundant in pumpkin seeds which can help to lessen anxiety. Tryptophan is converted into serotonin which is a hormone that enhances mood and promotes healthiness of brain. They investigated whether consuming a tryptophan rich food could boost serotonin levels and reduce anxiety symptoms. They discovered that subjects with anxiety disorder who consumed tryptophan rich pumpkin seeds before an anxiety test showed better improvements in subjective and objective measures on the Endler Multidimensional Anxiety Scale compared with those who didn't consume pumpkin seeds.

Makni *et al.* (2008) evaluated the effect of intake of mixture of flax and pumpkin seeds in rats fed with a 1 per cent cholesterol diet. Significant increase in monounsaturated and polyunsaturated fatty acids was observed in rats fed with pumpkin seed. Improved efficiency of antioxidant defence system indicated the antiatherogenic prospective of the seed mixture. Gossell-Williams *et al.* (2008) examined the effect of pumpkin seed oil supplementation on the cholesterol and blood pressure in rats. Both non-ovariectomized and ovariectomized rats were supplemented with corn oil or pumpkin seed oil for five days per week for twelve weeks (40mg/kg given orally). Blood analysis showed satisfactory lipid profile in the group of rats supplemented with pumpkin

seed oil. Barakat and Mahmoud (2011) examined the potential of pumpkin seeds used alongside with flax seed or purslane seed on hyperlipidaemia in rats fed with high cholesterol diet. An administration of two per cent cholesterol diet significantly increased the total cholesterol, total lipids and triacylglycerol in both liver and serum of rats. The consumption of flax and pumpkin or purslane and pumpkin seed mixtures notably decreased the lipid parameters suggesting the hypolipidaemic prospective of the seed mixture.

Makni *et al.* (2011) observed that consumption of mixture of pumpkin seeds and flax seeds reduced the increased levels of the plasma enzymes produced by the initiation of diabetes and caused a consequent revival towards normalization as compared to the control group animals. Its use in food on daily basis may be efficient in prevention of diabetes and its side effects. Teugwa *et al.* (2013) evaluated the anti-diabetic effect of proteins obtained from several species of Cucurbitaceae, including *Cucurbita moschata*. The result showed that globulin is the most abundant protein found in pumpkin seeds which measured 295.11 mg/g dry matter of extracted proteins and able to lower the blood sugar levels in rats with high blood sugar levels.

Consumption of pumpkin seeds has revealed extensive benefits in benign prostate hyperplasia i.e. enlargement of prostate gland in men. Gossell Williams *et al.* (2006) studied the efficacy of pumpkin seed oil on rats with testosterone-induced prostate hyperplasia. During the course of hyperplasia induction, 12 pumpkin seed oil and corn oil (vehicle) were orally administered for 20 days. On 21st day, rats were killed and their prostate was weighed. The induced increase in prostate size was repressed in rats fed with pumpkin seed oil i.e. 2 mg/100g. The protective effect of pumpkin seed oil was considerable at the higher dose. The results showed that utilization pumpkin seed oil can be helpful for managing benign prostatic hyperplasia. Zaineddin *et al.* (2012) given a food-frequency questionnaire to a vulnerable group of women. It was found that the eating of sunflower and pumpkin seeds was associated with extensively reduced postmenopausal breast cancer threat.

Lim (2012) reported that supplementation of pumpkin seeds in diet could be helpful to decrease the risk of bladder stone in children and adolescents in Thailand. The results revealed that the longer supplementation period of pumpkin seeds showed the

better effects on bladder stone mitigation. Pumpkin seeds lowered oxalocrystalluria i.e. occurrence of calcium-oxalate crystals and calcium level but increased phosphorous, glycosaminoglycans, pyrophosphate and potassium levels in urine as compared to orthophosphate supplementation. Pumpkin seeds provided high phosphorous levels and increased level of inhibitors of crystal formation or aggregation which would consequently reduce the formation of bladder stones.

Phytoestrogens are plant metabolites similar to 17 betaestradiol in structural and functional properties. They are renowned to lesser the risk of osteoporosis, heart disease, menopausal problems and breast cancer (Zaineddin *et al.*, 2012). Pumpkin seed oil has been discovered to have higher content of phytoestrogens as other plant sources like soybean, flax seed, sesame, sunflower seed etc. Gossell-Williams *et al.* (2011) evaluated the credible beneficial effects of pumpkin seed oil on postmenopausal women. The randomized, double-blinded and placebo-controlled study was conducted on thirty five women who had undergone menopause naturally or due to surgery. The women consuming pumpkin seed oil showed a noteworthy increase in high-density lipoprotein and considerable decrease in diastolic blood pressure. A decrease in the severity of hot flushes, frequent headaches and joint pains were reported in the women utilizing pumpkin seed oil. The placebo group administered with wheat germ oil complained of more depression and emotional anxiety. The positive reaction of pumpkin seed oil administration implies further studies to find out menopause curative properties of pumpkin seeds.

2.10 Functional Food Development using Pumpkin Seed

Naghii and Mofid (2007) compared the consumption of ready-to-eat cereal fortified with iron (30 g providing 7.1 mg iron per day) and pumpkin seed kernels (30g providing 4.0mg iron per day) for 4 weeks. After the consumption period, level of serum iron got higher 13 which indicates improved iron status. Children, teenagers, women of child bearing ages and expectant women who are often prone to anaemia caused due to iron insufficiency were benefited.

Norfezah *et al.* (2011) studied the effect of incorporation of flour with three different parts i.e. peel, flesh and seeds of crown pumpkin (*Cucurbita maxima*). The flour was incorporated into an extruded snack formulation at different levels and

processed in an extruding machine to make ten products. Incorporation of pumpkin seeds and peels at the level of 10 per cent resulted in stiffness of the product.

Ward and Ainsworth (1998) developed a low-cost weaning food enriched with protein, fat and energy for undernourished infants in Kenya. The weaning food was produced in the form of porridge which was blended with ground pumpkin seeds. The *in vitro* protein digestibility was 82.5 per cent, confirming a high-quality protein food. Free-floating water-soluble amino acids were not detected on high-performance liquid chromatography testing which indicates the better food stability. Peroxide value was also found lesser which implies the absence of rancidity in porridge.

El-Soukkary (2001) examined the effect of supplementation of pumpkin seed into wheat flour and dough properties. The results revealed that pumpkin seeds could be added to wheat flour up to a 17 per cent protein level for raw, roasted and autoclaved pumpkin flour, 19 per cent level for germinated, fermented and pumpkin protein concentrate and 21 per cent level for pumpkin protein isolate without unfavourable effect on quality of dough or loaf. The addition of pumpkin seed proteins resulted in higher content of protein, lysine and minerals as compared to the control. *In vitro* protein digestibility was also improved with the addition of pumpkin seed proteins.

Procida *et al.* (2013) studied the lutein, zeaxanthin, tocopherol and fatty acid contents of twelve samples of pumpkin seed oils along with the volatile fraction resulting from the roasting process. The roasting temperature played a vital role in the concentrations of volatile substances originating from lipid per-oxidation and Maillard reaction. The results suggested that roasting at high-temperature leads to the production of oil with strong aromatic characteristics, while roasting at mild-temperature leads to a production of oil with a minor distinctive aroma. Higher content of alpha-tocopherol, gamma-tocopherol and carotenoids 14 (lutein and zeaxanthin) was found which confirmed the nutraceutical properties in pumpkin seed oil.

Nyam *et al.* (2013) determined the proximate composition, functional properties and antioxidant activity of pumpkin seeds and rind. Bread was prepared by incorporating pumpkin seeds and rind at different levels i.e. 0, 5 and 10 per cent. Organoleptic evaluation of prepared bread samples for different attributes such as appearance, aroma, flavour, texture and overall acceptability were undertaken. The

physical properties of the bread samples like dough expansion, loaf volume, crumb, color and texture were also examined. Results showed that the crude fiber content of the pumpkin seeds and pumpkin rind was significantly high i.e. 31.48 per cent and 14.83 per cent respectively. The antioxidant activity for the pumpkin rind i.e. 69.38 per cent was higher than the pumpkin seeds. Incorporation of 5 per cent level of pumpkin rind in bread gave the higher score for overall acceptability and sensory attributes followed by bread supplemented with 5 per cent pumpkin seeds. Total dietary fiber, total phenolic compound and antioxidant activity in breads supplemented with 5 per cent pumpkin seed and 5 per cent pumpkin rind flour were higher than the control bread sample. So, pumpkin seeds and rinds can be used as fiber sources in bakery products.

Mbondo (2013) formulated and evaluated the pumpkin seed tablets. He stated that the use of herbal medicines in management of medical conditions has become popular in the recent years both for preventive and curative purposes. With the increasing prevalence of certain diseases like benign prostate hyperplasia, diabetes, arthritis and the accompanying economic burden, it is necessary to develop new safer and inexpensive medicines for their management. Pumpkin seeds have been used for the management of these and other medical condition. The seeds of pumpkin were powdered for administration or chewed whole. This mode of administration posed limitations such as inaccurate dosing due to different measuring devices used. The presence of high amount of oils in the seed powder increases the possibility of rancidity due to improper storage conditions. There is also a high potential of microbial growth. Pumpkin seed powder is subjected to high temperatures during cooking, therefore high chances of the active ingredients getting inactivated due to high temperatures used. Seed powder is also prone to adulteration hence formulation of tablets decreases this possibility. In this research work, tablets of pumpkin seed were prepared by the wet granulation technique. The purpose of this was to formulate a suitable solid dosage form of whole pumpkin seed that may address the above mentioned limitations. Pre-formulation studies indicated that the powder did not have free flowing capacity and hence wet granulation method was adopted. The tablets were evaluated for hardness, weight variation, friability and integration time. From the results obtained, it was concluded that pumpkin seed tablets

can be made from pumpkin seed powder which will improve effectiveness and patient compliance.

Hamed *et al.* (2008) determined the consequence of roasting on nutritional composition, anti-nutritional factors, protein digestibility, mineral availability and physiochemical properties of pumpkin seeds consumed in Sudan. Results showed that roasting process notably reduced the protein content. Roasting of pumpkin seeds significantly reduced tannin and phytic acid content to 125.01 and 56.1 mg/100 g which implies improvement in protein digestibility. Roasting of pumpkin seeds considerably improve mineral availability and physiochemical properties of the pumpkin seed flour.

Atuonwu and Akobundu (2010) evaluated the nutritional composition and organoleptic attributes of cookies supplemented with defatted pumpkin seed flour. Pumpkin seeds were processed into defatted flour. The potential of the pumpkin seed flour as composite with wheat flour in cookie production was also examined. The protein content of defatted pumpkin seed flour was as high as 57.50 per cent with good amino acid profile. Minerals were also found in abundance in seed flour. Defatted pumpkin seed flour had higher protein digestibility i.e. 77.91 per cent and protein efficiency ratio (PER) of 1.80. The anti-nutrients were beneath tolerable limits. The physicochemical and sensory attributes of cookies revealed that up to 10 per cent substitution of wheat flour with defatted pumpkin seed flour produced acceptable cookies similar to the control (100 per cent wheat flour).

Milovanoic *et al.* (2014) evaluated the nutritional quality of the wheat bread prepared with supplementation of pumpkin seed, buckwheat and quinoa. The principle of the research was to mix pumpkin seed, buckwheat and quinoa at the level of 40 per cent with wheat flour and to study the effect of this blend on nutritional composition and sensory parameters of the bread. Chemical composition of supplemented bread was better with an increase in protein, fat and fiber content as compared to the control wheat bread. Sensory attributes of supplemented bread like loaf volume, appearance, crust and crumb texture, aroma-odor and color were found outstanding.

Silva *et al.* (2014) developed pumpkin seed flour-based cereal bars. In this study, pumpkin seed flour (PSF) with medium granulometry i.e. PSF1 and coarse

granulometry i.e. PSF2 were used in preparation of cereal bars (CB) with different combinations with brown oats. Five bars were prepared as CB-1 (control-25 per cent brown oats and 0 per cent PSF); CB-2 (12.5 per cent PSF1 and 12.5 per cent brown oats); CB-3 (25 per cent PSF1 and 0 per cent brown oats); CB-4 (12.5 per cent PSF2 and 16 12.5 per cent brown oats); and CB-5 (25 per cent PSF2 and 0 per cent brown oats). The sensory attributes were analyzed in a conventional preference mapping which indicated that the bars CB-2 and CB-5 received maximum organoleptic acceptability score. Cereal bars with maximum score for overall acceptability i.e. CB-2 and CB-5 were further analyzed for nutritional composition and compared with the control cereal bar CB-1. The cereal bars CB-2 and CB-5 showed an increase in crude protein content with 87.5 and 62.5 per cent and dietary fiber with 77 and 44 per cent respectively. These results revealed that the CB-2 and CB-5 cereal bars as excellent source of protein and fiber.

Kanwal *et al.* (2015) developed and evaluated the physio-chemical properties of biscuits supplemented with pumpkin seeds to fight under nutrition in children of Pakistan. Pumpkin seed flour was substituted at different levels in wheat flour to produce biscuits ($T_2=5$ per cent, $T_3=10$ per cent, $T_4=15$ per cent and $T_5=20$ %) and compared with control (T_1) i.e. biscuits prepared with wheat flour only. Nutritional analysis of biscuits showed that T_5 i.e. biscuits supplemented with 20 per cent pumpkin seed flour contained moisture (1.55 %), protein (12.30 %), fat (28.29 %), ash (4.13 %), crude fiber (1.60 %), iron (2.28 mg) and zinc (3.11 mg). Sensory analysis also shown increasing trend in all sensory attributes. Results showed good acceptability at all levels but treatment T_4 with 15 per cent pumpkin seed flour scored highest (8.0) for maximum overall acceptability. From this study, it was concluded that pumpkin seed flour can be incorporated successfully to partly replace wheat flour to prepare highly nutritious and wholesome biscuits without disturbing its overall acceptability.

Bialek *et al.* (2016) studied the effect of partial replacement of wheat flour with pumpkin seed flour in muffins presented to children. In this study one control (0 per cent pumpkin seed flour) and three experimental muffins (17 per cent, 33 per cent and 50 per cent pumpkin seed flour) were prepared. Above 71 per cent of the children evaluated muffins having 33 per cent of pumpkin seed flour as tasty and very tasty. Nutritional value of control and experimental muffin with 33 per cent pumpkin seed flour per 100gm

was as energy 341 and 388 Kcal, fat 13.4 and 14.3 gm, protein 6.5 and 14.1 gm, monosaccharides 46.1 and 44.3 gm and dietary fiber 1.2 and 1.9 gm. So, as the level replacement of wheat flour with pumpkin seed flour increased, the nutritional composition of muffins enhanced. It was found suitable to incorporate pumpkin seed flour at the level of 33 per cent into muffins stored for 2 weeks without special packaging conditions. The supplemented muffin formulation resulted in a nutrient enriched product appropriate to enhance the nutritional status of undernourished children.

2.11 Microbial Count of Pumpkin Seed

Revathy and Sabitha (2013) tested the microbial contamination of pumpkin seed flour during the initial, 15th, 30th and 45th day of storage. Flour was stored in polyethylene bag and plastic container at both room temperature and refrigerator. The microbial count of pumpkin seed flour stored in plastic container at room temperature on initial, 15th, 30th and 45th day were 4×10^3 , 7×10^3 , 10×10^3 and 12×10^3 , respectively. Whereas the pumpkin seed flour stored in polyethylene bag at room temperature showed increase in bacterial count than flour stored in plastic container at room temperature. The count was 6×10^3 on initial day, 9×10^3 on 15th day, 13×10^3 on 30th day and 16×10^3 on 45th day. The bacterial count of the pumpkin seed flour stored in plastic container at refrigerated temperature on initial day was 3×10^3 , 4×10^3 on 15th, 6×10^3 on 30th day and 7×10^3 on 45th day. On the other hand, flour stored in polyethylene bag at refrigerated temperature was higher in count than flour stored in plastic container. The count was 5×10^3 on initial day, 7×10^3 on 15th day, 8×10^3 on 30th day and 10×10^3 on 45th day. It was found that microbial count of pumpkin seed flour increased when it is stored in polyethylene bag as compared to plastic container.

3. MATERIALS AND METHODS

The present work entitled, “Development of Milkshake Blended with Pumpkin Seed (*Cucurbita moschata*)” was undertaken in laboratory of Department of Animal Husbandry and Dairy Science, Post Graduate Institute, MPKV, Rahuri during the year 2022-2023. The various materials and methodology adopted during the research are presented in this chapter.

3.1 Material

3.1.1 Ingredients

3.1.1.1 Milk

The fresh, clean milk of cows was procured from Research Cum Development Project (RCDP) on cattle, Department of Animal Husbandry and Dairy Science, MPKV, Rahuri.

3.1.1.2 Pumpkin seeds

Good quality dried pumpkin seeds were procured from local market in single lot.

3.1.1.3 Sugar

Good quality, clean, crystalline, white cane sugar was purchased from local market in single lot.

3.1.1.4 Honey

Good quality honey was purchased from local market in single lot.

3.1.1.5 Stevia

Natural Sweetener Stevia (extract) was procured from local market in single lot

3.1.1.6 Stabilizer

Efficient stabilizer (CMC) was purchased from market in single lot.

3.1.2 Utensils/ Equipments

3.1.2.1 Karahi

Karahi was used for low flame roasting of pumpkin seeds and heating of milk.

3.1.2.2 Electric Mixer cum Grinder

Electric mixer cum grinder was used to grind pumpkin seeds and mix the milk, sugar and pumpkin seed powder properly.

3.1.2.3 Electronic Weighing Balance

The electronic precision balance (BT 2245, Sartorius ISO 9001) was used for weighing samples and chemicals during research work.

3.1.2.4 Hot Air Oven

The hot air oven manufactured by York Scientific Private Limited was used for sterilizing the glassware and determination of total solid and moisture content of samples throughout the study period.

3.1.2.5 Incubator

The digital temperature controlled B.O.D. incubator manufactured by M/S. Neutronic, Bombay was used for incubation of standard plate count, yeast and mould count and coliform count from the prepared products.

3.1.2.6 Autoclave

Equitron autoclave manufactured by M/S. Media Instrument Company, Bombay was used to sterilize/autoclave the microbiological media and buffer solutions, throughout the study.

3.1.2.7 pH Meter

Orion Star of Thermo Scientific make single electrode pH meter was used to measure the pH of milk, milkshake and microbial media.

3.1.2.8 Thermometer

M/S Jennsons type thermometer with temperature range of 0 to 110⁰C was used to check temperature.

3.1.2.9 Atomic Absorption Spectrophotometer

Atomic absorption spectrophotometer manufactured by Perkin Elmer was used for determination of minerals content.

3.1.2.10 Colony Counter

A colony counter with magnifying lens and hand operated tally operator was used for counting the colonies, developed by microorganisms.

3.1.2.11 Laminar Air Flow

Laminar air flow manufactured by Kirloskar Electrodyne Ltd. was used for microbiological work.

3.1.3 Chemicals

All the chemicals required for the analytical work were used of Analytical Reagent (AR) or Guaranteed reagent (GR) grade manufactured by Merk India Ltd/Glaxo India Ltd.

3.1.4 Microbial Media

Dehydrated microbiological media made by M/S Hi-media laboratories used for preparation of media and microbial examination of *milkshake*.

3.1.5 Glassware's

All the glassware's viz. petriplates, dairy microbiological pipette, test tubes, glass beakers, conical flasks, desiccator etc. of Borosil make were used to analyze *milkshake* for different parameters throughout the study. They were washed thoroughly and sterilized before they were used for the analysis.

3.2 Methodology

3.2.1 Preparation of Pumpkin Seed Powder and Paste

Pumpkin seed powder and paste was prepared by using procedure described by Kaur (2017) with slight modifications.

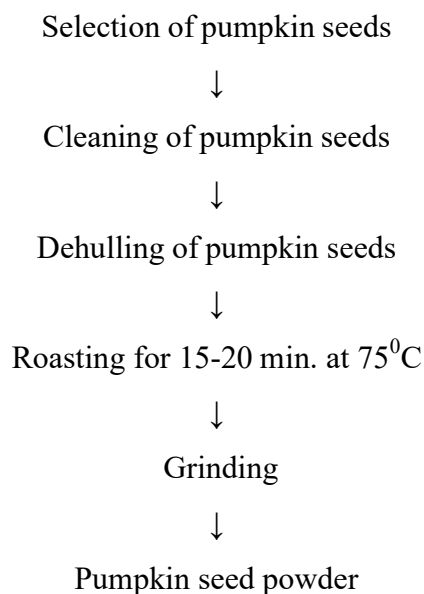


Fig. 3.1 Flow Diagram for Preparation of Roasted Pumpkin Seed Powder

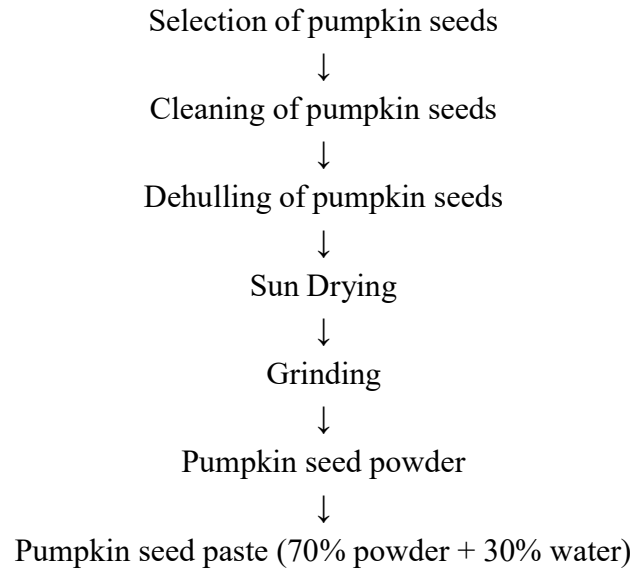


Fig. 3.2 Flow Diagram for Preparation of Sundried Pumpkin Seed Paste

3.2.2 Preparation of Sprouted Pumpkin Seed Paste

Sprouted pumpkin seed paste was prepared by using procedure described by Liu Fayuan in patent citation, China, (2015) with slight modifications.

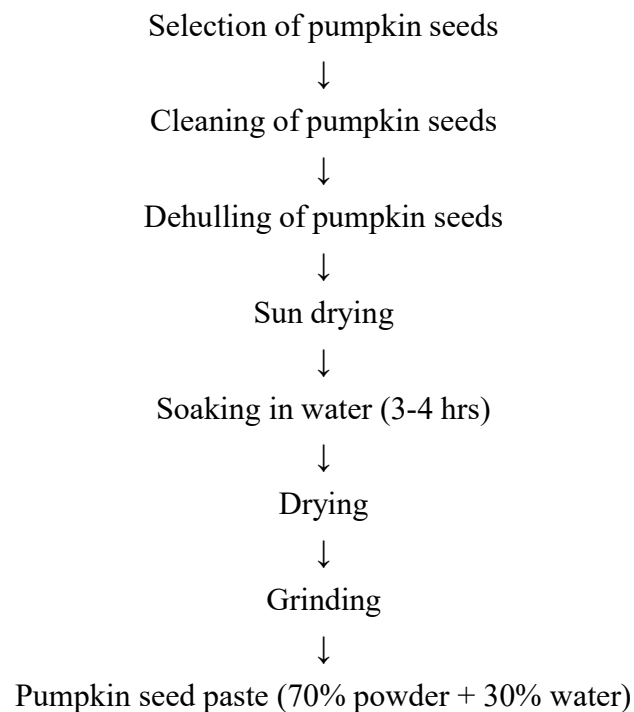


Fig.3.3 Flow Diagram for Preparation of Sprouted Pumpkin Seed Paste

3.2.3 Preparation of Milkshake Blended with Pumpkin Seed

The pumpkin seed milkshake was prepared by using procedure described by Shinde *et al.* (2018) for Date pulp milkshake with slight modifications.

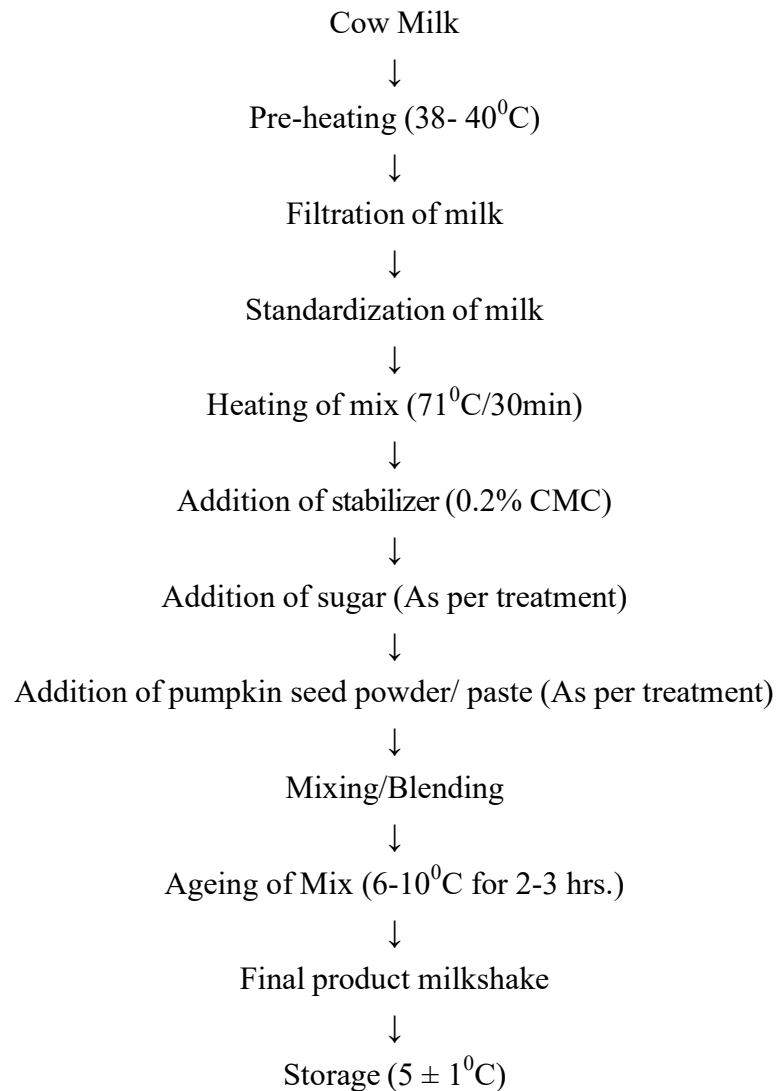


Fig. 3.4 Flow Diagram for Preparation of Pumpkin Seed Milkshake

3.3 Optimization of Ingredients

3.3.1 Selection of Form of Pumpkin Seeds

For this purpose, four forms of pumpkin seed was used viz. roasted pumpkin seed powder, sundried pumpkin seed paste, sundried pumpkin seed paste + roasted pumpkin seed powder, (1:1) and sprouted pumpkin seed paste were taken at rate of 5 per cent of milk. In all trials, sugar 10 per cent of milksake and cow milk standardized for 4.5 per cent fat was kept constant.

Treatment details as under

A₁ : Milk (4.5 % fat) + 10 % Sugar + 5 % Roasted Pumpkin Seed Powder

A₂ : Milk (4.5 % fat) + 10 % Sugar + 5 % Sundried Pumpkin Seed Paste

A₃ : Milk (4.5 % fat) + 10 % Sugar + 5 % Sundried + Roasted Pumpkin Seed Paste

A₄ : Milk (4.5 % fat) + 10 % Sugar + 5 % Sprouted Pumpkin Seed Paste

On the basis of sensory evaluation most accepted pumpkin seed form was selected for further study.

3.3.2 Selection of Type of Sweetener

For this purpose, three types of sweeteners viz. Sugar (10 per cent of milk), Natural Sweetener (Stevia-0.03 per cent of milk) and Honey (10 per cent of milk) were taken where, selected pumpkin seed form at 5 per cent of milk and cow milk standardized for 4.5 per cent fat were kept constant for all treatment combination.

Treatments details as under

B₁ : Milk (4.5% fat) + 10% Sugar + 5% Selected Form of Pumpkin Seed

B₂ : Milk (4.5% fat) + 10% Honey + 5% Selected Form of Pumpkin Seed

B₃ : Milk (4.5% fat) + 0.03% Natural Sweetener(Stevia) + 5% Selected Form of
Pumpkin Seed

On the basis of sensory evaluation most accepted sweetener was selected for further study.

3.3.3 Optimization of Pumpkin Seed and Sweetener

Optimization of selected pumpkin seed form and selected sweetener level was done on the basis of sensory evaluation, for that three levels of selected pumpkin seed form and two levels of selected forms of sweetener were considered.

Treatment details as follows

Optimization of level (3*2)

Level of Pumpkin Seed Form : K₁ (3), K₂ (5) and K₃ (7)

Level of Sweetener : S₁ (8), S₂ (10)

Treatment combinations

K ₁ S ₁ (T ₁)	K ₁ S ₂ (T ₂)
K ₂ S ₁ (T ₃)	K ₂ S ₂ (T ₄)
K ₃ S ₁ (T ₅)	K ₃ S ₂ (T ₆)

- T₀ : Controlled treatment of milk shake without addition of pumpkin seed
- T₁ (K₁S₁) : Milk (4.5 % fat) + 3 % Selected Form of Pumpkin Seed + 8 % Selected Sweetener
- T₂ (K₁S₂) : Milk (4.5 % fat) + 3 % Selected Form of Pumpkin Seed + 10 % Selected Sweetener
- T₃ (K₂S₁) : Milk (4.5 % fat) + 5 % Selected Form of Pumpkin Seed + 8 % Selected Sweetener
- T₄ (K₂S₂) : Milk (4.5 % fat) + 5 % Selected Form of Pumpkin Seed + 10 % Selected Sweetener
- T₅ (K₃S₁) : Milk (4.5 % fat) + 7 % Selected Form of Pumpkin Seed + 8 % Selected Sweetener
- T₆ (K₃S₂) : Milk (4.5 % fat) + 7 % Selected Form of Pumpkin Seed + 10 % Selected Sweetener

From the above treatment combination, one best combination was selected on the basis of sensory evaluation and all the treatments were analyzed for their physico-chemical and microbial quality.

The acceptability of milkshake blended with pumpkin seed was evaluated by the semi-trained panel of five judges from Department of Animal Husbandry and Dairy Science. The colour and appearance, body and texture, flavour and overall acceptability was evaluated by using 9-point hedonic scale (Amerine *et al.*, 1965).

3.4 Shelf Life Study of Milkshake

Optimized pumpkin seed milkshake was kept in suitable pre-sterilized glass bottle and stored at $5 \pm 1^{\circ}\text{C}$ for assessment of shelf life and product was analyzed for sensory, physico-chemical and microbial qualities at 3 days interval till the product sensorially unacceptable.

3.5 Analytical Methods

Fresh milk, pumpkin seeds and pumpkin seed milkshake were analyzed by adopting standard procedure as under

3.5.1 Physico-chemical Analysis of Milk

3.5.1.1 Fat

Determined by using Standard Gerber method as described in IS: 1224 (Part II), 1977.

3.5.1.2 Protein

Determined by using method as described in SP:18 (Part-XI), 1981.

3.5.1.3 Lactose

Determined by using method as described in SP:18 (Part-XI), 1981.

3.5.1.4 Ash

Determined by using method as described in SP: 18 (Part-XI), 1981.

3.5.1.5 Total solid

Determined by gravimetric method as described in IS: 1479 (Part-II), 1961.

3.5.1.6 Acidity

Determined as described in IS: 1479 (Part-I), 1960.

3.5.1.7 pH

Determined as per IS: 1465 (1967).

3.5.2 Physico-Chemical Analysis of Pumpkin Seeds

Acidity, protein, fibre, fat and mineral content were estimated by using Near Infrared (NIR) Spectrometer at Dept. of Agriculture Process Engineering, MPKV, Rahuri.

3.5.3 Physico-Chemical Analysis of Milkshake

3.5.3.1 Fat

Fat in milkshake was determined by gerber method as described in SP: 18 (Part XI), 1981 with some modifications.

Clean and dry butyrometer were kept in butyrometer stand with mouth upward. 10 ml of sulphuric acid by automatic tilt measure was poured into the milk butyrometer, with the help of 10.75 ml graduated pipette. 10.75 ml of sample was poured by the side of butyrometer followed by 1ml of amyl alcohol by using 1ml automatic tilt measure. The butyrometer were stopped by the rubber stopper using guiding pin. The content of the butyrometer was mixed thoroughly till mahogany colour was obtained.

When mixing completed butyrometer was placed into the gerber centrifugal machine for 5 min, in upright position with stopper end downward. After 5 min reading on the column of butyrometer (appeared clear yellowish liquid at the stem portion) was recorded.

3.5.3.2 Protein

Total protein content in milkshake samples was determined by Micro-Kjeldahl method (IS: SP: 18 (Part XI), 1981).

Three grams of the milkshake sample was accurately weighed and transferred carefully to the Kjeldahl flask, taking precaution to see that particles of sample do not stick to neck of the flask. 10 g of potassium sulphate, 0.5 g of mercuric oxide and 20 ml of sulphuric acid were added to the flask. The content was mixed and boiling chips added in it and heated the flask carefully on the digestion apparatus until foaming stops and the contents have become liquid. The digestion was continued with more vigorous heating until the contents of the Kjeldahl flask became completely clear and colourless. During heating, the content was mixed from time to time. The liquid was boiled vigorously for 1 ½ hours after it has become clear. Local over heating was avoided. The contents of the flask were cooled to room temperature and made up volume to 100 ml in volumetric flask with distilled water. Ten ml of aliquot of the solution was pipette out to the Micro-Kjeldahl distillation apparatus and the solution was made alkaline by 8 ml of sodium hydroxy-sodium thiosulphate solution and steam distilled. The evolved ammonia was absorbed in 5 ml of boric acid solution containing 2 to 3 drops of indicator. About 25 ml of distillate was collected and titrated against hydrochloric acid (0.02 N) till violet colour appeared. Blank was carried out using all reagents except the sample material.

$$\text{Protein (\% by weight)} = \frac{89.32 (V_2 - V_1) N}{W}$$

Where,

V₂ = Number of ml of hydrochloric acid used in distillation

V₁ = Number of ml of hydrochloric acid used in the blank test

N = Normality of the hydrochloric acid

W = Weight of sample taken for analysis

3.5.3.3 Carbohydrate

Carbohydrate content i.e. total sugar of pumpkin seed added milkshake was determined as per the method described as per SP: 18 (Part XI) 1981.

The total sugar content of milkshake was determined by difference i.e. by subtracting the sum of per cent values of protein, fat and ash from total solids. The resultant figure represented per cent sugar in the sample.

$$\text{Carbohydrate (\%)} = \text{Total solid} - (\text{Protein} + \text{Fat} + \text{Ash})$$

3.5.3.4 Crude Fiber

Crude fiber content of pumpkin seed added milkshake was determined by using standard method, A.O.A.C. (2000).

About 2 g of moisture and fat free samples were weighted into 500 ml beaker and 200 ml boiled 0.255N (1.25 W/V) H₂SO₄ was added in it. The mixture was boiled for 30 minutes keeping the volume constant by addition of water at frequent intervals. At the end of this period, the mixture was filtered through a filter paper and the residue washed with hot water till free from acid. The material then transferred to the same beaker and 200ml of boiled 0.313N NaOH solution added in it. After boiling for 30 minute, the mix residue was washed with hot water till free from alkali followed with same alcohol. It was then transferred to a crucible, dried over night at 80-100⁰C for 2-3 hours, then cooled and weighted again. The difference in the weights represented the weight of crude fiber.

$$\text{Crude fiber (\%)} = \frac{[(W_2 - W_1) - (W_3 - W_1)]}{\text{Weight of sample}} \times 100$$

Where,

W₁ = Weight of empty silica crucible

W₂ = Weight of crucible with residue

W₃ = Weight of crucible with residue after ashing

3.5.3.5 Ash

The ash content of milkshake was determined as per method described in IS: 1479 (Part-II), 1961.

Weighed exact 5 g of sample into a silica dish and ignited on a laboratory bunsen burner. The final incineration was done in a muffle furnace at not more than

550⁰C temperature for 2 hrs until the ash was free from carbons, cooled into desiccators and weighed. Constant weight of ash considered to have reached when the difference in two consecutive weighing after repeated ignition was less than 0.5 mg. The ash content was expressed as per cent of the gross product.

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

Where,

W_1 = Weight of silica dish

W_2 = Weight of silica dish + total ash (after final ignition)

W = Weight of sample taken

3.5.3.6 Total Solid

Total solid content of pumpkin seed added milkshake was determined as per the method described in IS 1479 (Part- II) 1961.

A clean dry empty porcelain crucible was kept in hot air oven maintained 100°C for one and half hour, cooled and weighed. Then 5 g of sample was weighed in the crucible. Put the crucible in hot air oven adjusted to 100°C for 3 to 4 hours. Remove the crucible from oven and cool in a desiccator and weigh. Again put the crucible for half hour in oven. Remove the crucible from oven and cooled in desiccator and weigh. This process repeated till getting the constant weight or difference in last two weights should not exceed 0.01 gm.

The per cent total solid in milkshake was calculated by using formula

$$\text{Total solid (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

3.5.3.7 Acidity

A.O.A.C. (1995) method was adopted for determining acidity in terms of per cent lactic acid of milkshake.

Burette was filled with 0.1N NaOH solution. Simultaneously 10 ml sample was transferred to the 100 ml conical flask added with equal quantity of distilled water. Then 3-4 drops of phenolphthalein indicator was mixed. Content was titrated with 0.1N NaOH until the colour changes to faint pink colour. The titration is completed

within 10- 15 sec. and final burette reading was recorded. The acidity was determined using following formula,

$$\text{Acidity (\% LA)} = \frac{\text{ml of 0.1 N NaOH required for titration} \times 0.009}{\text{Weight of sample (gm)}} \times 100$$

Where,

$$\text{Weight of the sample (gm)} = \text{volume of sample (ml)} \times \text{specific gravity}$$

3.5.3.8 pH

The pH of sample was measured by using Oroion 3 star pH benchtop pH meter.

Firstly the pH meter was standardized at 25°C by using standard buffer solution. The pH of the sample was observed on the dial and recorded.

3.5.3.9 Minerals

The following minerals content of milkshake was estimated as per the procedure given by Mani (2011).

3.5.3.9.1 Calcium

3.5.3.9.2 Magnesium

3.5.3.9.3 Zinc

3.5.3.9.4 Iron

3.5.3.9.5 Phosphorous

Weighed one gram of the milkshake sample was transferred to digestion tube in which 30 ml of diacid mixture was added. Thereafter, it was kept for predigestion for overnight. Suspension was filtered through Whatman No. 42 filter paper. Filtrate was analyzed for Ca, P, Mg and Fe using Atomic Absorption spectrophotometer (AAS) and appropriate standards of each element.

$$\text{Minerals (mg/100g)} = \frac{\text{Reading (Conc.}\mu\text{g/ml)} \times \text{volume made}}{\text{Weight of sample (g)} \times 1000} \times 100$$

3.5.3.9.6 Potassium

The content of potassium in milkshake was determined by the method of Chapman and Pratt (1961).

Standard Stock Solution of Potassium : Dissolved 1.907g of potassium chloride in distilled water and made up to 1000ml. This solution contained 1000mg potassium. 100ml of this stock solution was diluted to 1000ml. This working solution contain 100mg of potassium.

Preparation of Standard Curve : Pipetted 0, 0.5, 2, 3, 4 and 5ml of stock solution of 100mg per liter separately into 100ml volumetric flask. The volume was made with distilled water and shaken well. This contained 0, 0.5, 2, 3, 4 and 5 mg potassium per liter, respectively and read on flame photometer. A standard curve plotting flame photometer reading on Y-axis and concentration of potassium mg per liter on X-axis was then prepared.

Procedure

1. Pipette out 5.0 ml of sample into a 25.0 ml volumetric flask and dilute with distilled water.
2. Aspirate the standard and then milkshake sample to flame, note the meter reading and calculate the potassium content by referring to the standard curve prepared.
3. Concentration of potassium is expressed as mg/100gm.

3.5.4 Microbial Analysis

3.5.4.1 Standard Plate Count

Standard plate count (SPC) was determined by using standard procedure using Standard Plate Count Agar (SPCA) media as mentioned by Amin, (1997).

Plate count agar (Hi-media) was used to enumerate the standard plate count in the milk sample. 23.5g of the dry medium was suspended in 1000 ml distilled water. The mixture was then boiled to dissolve the medium completely. It was then filled in conical flask and the mouth of the conical were closed with cotton plugs. The conical flasks were then sterilized by autoclaving at 15 psi pressure (121°C) for 15 min. One ml of the diluted sample (suitable dilution) was transferred in each of the duplicate petridishes. Ten to fifteen ml of the melted agar (at 45°C) was then poured and the contents were mixed well by rotating in a horizontal position. The contents were allowed to solidify. The plates were then inverted and incubated at 37°C for 48 hrs.

3.5.4.2 Yeast and Mould Count

Yeast and mould count (YMC) was determined as per procedure described in IS: 5403 (1969) using Potato Dextrose Agar (PDA).

Ten fold serial dilution of the bacterial suspension was prepared. Normal saline was used as diluents for the organism. Accurate 9 ml of the diluents was pipette into several sterile test tubes. The bacterial suspensions were uniformly mixed. Using a sterile 1 ml pipetted, 1 ml of the suspension was transferred into the first tube of diluents and mixed thoroughly. From this mixed dilution, 1 ml was transferred to the next diluents. Similar dilutions were made in the same way using fresh pipettes. 1 ml of each dilution was pipetted into sterile petri plates and 15 ml of melted agar medium which was cooled around 45°C was poured into plates containing diluted samples. The agar medium was immediately distributed by gently mixing the petridish in circular movements both clock wise and anti clock wise on a flat bench and then allowed to set evenly and the inverted plates were incubated for 3-5 days at 22°C for yeast and mould count.

3.5.4.3 Coliform Count

Coliform count of samples was determined as per procedure described in IS: 1981 by using violet red bile agar (VRBA).

To rehydrate this medium, 41.5 g of VRBA powder was suspended in 1000 ml distilled water. Then the mixture was brought to boil to dissolve completely. The media was then cooled to 45⁰C and poured into conical flask (150 ml). This media was not autoclaved.

1 ml (1:10 dilution) of the sample was added to each of the duplicate sterile petridishes. To each of these petridishes 10-15 ml of the melted (45⁰ C) VRBA was added and the contents of the petridishes were mixed well by rotating the plates by placing them horizontally on a table. The media was then allowed to solidify and then a second layer of agar was made by adding 5-10 ml of melted agar. The media was allowed to solidify and then incubated after inverting the plates at 37 ± 0.5⁰ C for 48 hrs.

3.6 Consumer study

The developed pumpkin seed milkshake was also subjected to consumer acceptance. The tasting was carried out with 100 respondents from local area. The information for the same was collected on prescribed format (Appendix II).

3.7 Cost structure

The cost of the newly formulated pumpkin seed milkshake was worked out by taking into consideration the prevailing market rates of the ingredients used in addition to fuel and labour charges etc. during experimental period. Procedure followed for estimation of cost was being on the lines of the standard economic procedure.

3.8 Statistical design

The experiment was laid down in the Completely Randomized Design with 4 replications for optimization process and Factorial Completely Randomized Design with 3 replications for storage study. The data was tabulated and analyze as per Snedecor and Cochran (1967).

4. RESULTS AND DISCUSSION

The present study was undertaken with the prime objective to develop milkshake blended with pumpkin seed (*Cucurbita moschata*). During the studies form of pumpkin seed and form of sweetener were selected and later on studies were conducted to optimize level of selected pumpkin seed form and sweetener form. The optimized product was also assessed for sensory, physico-chemical and microbiological qualities, storage stability, consumer acceptance and cost of production. The results obtained on different aspects during this investigation are presented and discussed under the following headings.

- 4.1 Chemical Composition of Standardized Milk
- 4.2 Chemical Composition of Roasted Pumpkin Seed
- 4.3 Optimization of Ingredients
- 4.4 Shelf Life Study of Milkshake
- 4.5 Consumer Acceptance Study of Milkshake
- 4.6 Cost of Production of Milkshake

4.1 Chemical Composition of Standardized Milk

The average chemical composition of milk used for preparation of milkshake is presented in the Table 4.1. The mean value of fat, protein, lactose, ash, total solid, acidity and pH were 4.50, 3.45, 4.47, 0.69, 13.11, 0.14 per cent and 6.28, respectively. The figures obtained were resembles to the study of Ramteke (2017) and Patil (2018).

Table 4.1. Chemical composition of standardized milk

Sr. No.	Constituent (%)	Content
1	Water	86.88
2	Fat	4.50
3	Protein	3.45
4	Lactose	4.47
5	Ash	0.69
6	Total solids	13.11
7	Acidity	0.14
8	pH	6.28

4.2 Chemical Composition of Roasted Pumpkin Seed

The average chemical composition of roasted pumpkin seed used for preparation of milkshake is presented in the Table 4.2 and had an average 31.89 per cent fat, 23.46 per cent protein, 3.01 per cent moisture, 26.03 per cent carbohydrate, 8.01 per cent, crude fiber, 7.59 per cent ash, 25.6 mg/100g Calcium, 335.7 mg/100g Magnesium, 907.0 mg/100g Zinc, 16.4 mg/100g Iron, 404.8 mg/100g Potassium, 848.6 mg/100g Phosphorous. The figures obtained were resembles to the study of Kaur (2017) and Devi (2018).

Table 4.2. Chemical composition of roasted pumpkin seed

Sr. No.	Constituent (%)	Content
1.	Fat	31.89
2.	Protein	23.46
3.	Moisture	3.01
4.	Carbohydrate	26.03
5.	Crude fiber	8.01
6.	Total Ash	7.59
7.	Minerals (mg/100g)	
	Calcium	25.6
	Magnesium	335.7
	Zinc	907.0
	Iron	16.4
	Phosphorous	848.6
	Potassium	404.8

4.3 Optimization of Ingredients

During the phase of investigation, optimization of ingredients viz. selection of form of pumpkin seed and selection of form of sweetener and level of pumpkin seed and sweetener for preparation of pumpkin seed added milkshake were carried out.

4.3.1 Selection of Form of Pumpkin Seeds

For this purpose, four forms of pumpkin seed were used viz. roasted pumpkin seed powder, sundried pumpkin seed paste, sun dried pumpkin seed paste + roasted pumpkin seed powder, (1:1) and sprouted pumpkin seed paste were taken at rate of 5 per cent of milkshake. In this trial sugar 10 per cent and cow milk standardized for 4.5 per cent fat were kept constant.

The treatment details were as under

A₁ : Milk (4.5 % fat) + 10 % Sugar + 5 % Roasted Pumpkin Seed Powder

A₂ : Milk (4.5 % fat) + 10 % Sugar + 5 % Sundried Pumpkin Seed Paste

A₃ : Milk (4.5 % fat) + 10 % Sugar + 5 % Sundried + Roasted Pumpkin Seed Paste

A₄ : Milk (4.5 % fat) + 10 % Sugar + 5 % Sprouted Pumpkin Seed Paste

On the basis of sensory evaluation most accepted pumpkin seed form was selected for further study.

4.3.1.1 Effect of Form of Pumpkin Seed on Sensory Score of Milkshake

4.3.1.1.1 Colour and Appearance

The colour and appearance of milkshake blended with pumpkin seed are illustrated in Table 4.3. The colour and appearance of milkshake significantly ($P < 0.05$) influenced by addition of different forms of pumpkin seed in milkshake. The score for colour and appearance for A₁, A₂, A₃ and A₄ were 8.82, 8.06, 8.21 and 7.78, respectively. Treatment A₁ had maximum score for colour and appearance as compared to other treatments while, A₄ had lowest colour and appearance score. The score for colour and appearance for form of milkshake blended with pumpkin seed was categorised as “like moderately” to “liked very much” by the panel of semi-trained judges.

Table 4.3. Effect of form of pumpkin seed on sensory score of milkshake

Treatments	Sensory Attributes			
	Colour and Appearance	Flavour	Body and texture	Overall Acceptability
A ₁	8.82 ^a	8.77 ^a	8.80 ^a	8.80 ^a
A ₂	8.06 ^c	8.54 ^b	8.50 ^b	8.35 ^b
A ₃	8.21 ^b	8.19 ^c	8.16 ^c	8.19 ^c
A ₄	7.78 ^d	7.90 ^d	7.99 ^d	7.89 ^d
S.E. \pm	0.01	0.01	0.01	0.01
CD at 5 %	0.04	0.02	0.03	0.03

Mean of 4 replications

The colour of pumpkin seed milkshake ranged from olive-greenish to yellowish which was combined effect of protochlorophyll pigment in pumpkin seed and carotene in cow milk. Olive-greenish colour of treatment A₁ was most acceptable while yellowish colour of A₄ was less acceptable by the panel.

4.3.1.1.2 Flavour

The score for flavour for milkshake blended with pumpkin seed is presented in Table 4.3. The score for flavour for milkshake significantly ($P < 0.05$) influenced by addition of different forms of pumpkin seed in milkshake. The score for flavour for the forms A₁, A₂, A₃ and A₄ were 8.77, 8.54, 8.19 and 7.90, respectively. The treatment A₁ had maximum score for flavour while A₄ had minimum flavour score. The score for flavour for milkshake categorised as “like moderately” to “liked very much” by the panel of semi-trained judges.

The unique nutty aromatic flavour developed during roasting process made sample A₁ most acceptable. Compounds alkylated pyrazines and 2-acetylpyrrole were responsible for nutty flavour in the milkshake (Barbara *et al.* 2004).

4.3.1.1.3 Body and Texture

The score for body and texture for milkshake blended with pumpkin seed revealed in Table 4.3. The body and texture of milkshake significantly ($P < 0.05$) influenced by addition of different forms of pumpkin seed in milkshake. The score for body and texture for the treatments ranged from 7.99 to 8.80. Treatment A₁ secured maximum score for body and texture as compared to other treatments while A₄ had minimum body and texture score. The score for body and texture for milkshake blended with pumpkin seed was categorised as “like moderately” to “liked very much” by the panel of semi-trained judges.

The observed behavior of treatment could be explained in term that buttery and creamy texture was liked by the judges. However, treatment A₃ had lower score followed by A₄ for body and texture might be due to pumpkin seed paste had slightly watery body which made it less acceptable by the panel.

4.3.1.1.4 Overall Acceptability

The overall acceptability is sum of combination of colour and appearance, body and texture and flavour of the product. Score for overall acceptability for milkshake

blended with pumpkin seed are mentioned in Table 4.3. It is clear that overall acceptability for milkshake significantly ($P < 0.05$) influenced by different forms of pumpkin seed in milkshake. The score for overall acceptability for A₁, A₂, A₃ and A₄ were 8.80, 8.35, 8.19 and 7.89, respectively. Treatment A₁ had maximum score for overall acceptability as compared to other treatments while, the sample A₄ had lowest overall acceptability score. Score for overall acceptability for form of milkshake blended with pumpkin seed was categorised as “like moderately” to “liked very much” by the panel of semi-trained judges.

Addition of roasted pumpkin seed powder (A₁) was found suitable in this study. Similar result reported by Mengyao *et al.* (2021) who observed roasting at certain temperature can increase the sensory and nutritional attributes of pumpkin seed. After roasting, the concentrations of total phenols, total flavonoids and antioxidant properties of pumpkin seeds were improved, which indicated that roasting enhanced the biological activity and antioxidant properties as well as sensory attributes of pumpkin seeds. Also, roasting of pumpkin seed reduces tannin and phytic acid contents with improvement in digestibility (Nkosi *et al.*, 2006).

Based on above discussion of Table 4.3 it is concluded that, use of roasted pumpkin seed powder for preparation of milkshake resulted in the maximum sensory perception. Hence, this form was selected for further study.

4.3.2 Selection of Type of Sweetener

The attempt was made to prepare pumpkin seed milkshake by using different type of sweeteners. viz. Sugar (10 per cent), Natural Sweetener (Stevia-0.03 per cent i.e. 30mg/100ml) and Honey (10 %) where, selected pumpkin seed form i.e. roasted pumpkin seed powder at 5 per cent of milk and milk having 4.5 per cent fat were kept constant.

The treatment details were as under

B₁ : Milk (4.5 % fat) + 10 % Sugar + 5 % Roasted Pumpkin Seed Powder

B₂ : Milk (4.5 % fat) + 10 % Honey + 5 % Roasted Pumpkin Seed Powder

B₃ : Milk (4.5 % fat) + 0.03 % Natural Sweetener (Stevia) + 5 % Roasted Pumpkin Seed Powder

On the basis of sensory evaluation most accepted sweetener was selected for further study.

4.3.2.1 Effect of Type of Sweetener on Sensory Score of Milkshake

4.3.2.1.1 Colour and Appearance

The score for colour and appearance for milkshake blended with pumpkin seed are depicted in Table 4.4. The colour and appearance for milkshake significantly ($P < 0.05$) influenced by addition of different types of sweeteners in milkshake. The score for colour and appearance for treatment B₁, B₂ and B₃ ranged from 8.05 to 8.11. The score for colour and appearance for sweetener selection of milkshake blended with pumpkin seed was categorised as “liked very much” by the panel of semi-trained judges.

The highest score for treatment B₁ followed by B₃ was observed, because addition of sugar and stevia had little or no interference with olive-green colour of milkshake while lowest score for B₂ might be because of amber colour imparted by honey in the milkshake. Mahuya *et al.* (2008) also observed carotene retention of honey in fortified milk which was responsible for change in colour of the product.

Table 4.4. Effect of type of sweetener on sensory score of milkshake

Treatments	Sensory Attributes			
	Colour and Appearance	Flavour	Body and texture	Overall Acceptability
B ₁	8.11 ^a	8.21 ^a	8.04 ^a	8.12 ^a
B ₂	8.05 ^c	7.78 ^b	7.59 ^c	7.81 ^b
B ₃	8.08 ^b	7.04 ^c	7.82 ^b	7.65 ^c
S.E. \pm	0.003	0.008	0.004	0.005
CD at 5 %	0.011	0.023	0.012	0.015

Mean of 4 replications

4.3.2.1.2 Flavour

The score for flavour for milkshake blended with pumpkin seed are revealed in Table 4.4. The flavour for milkshake significantly ($P < 0.05$) influenced by addition of different types of sweetener in milkshake. The score for flavour for B₁, B₂ and B₃ were 8.21, 7.78 and 7.04, respectively. Treatment B₁ had maximum score for flavour while B₃ had minimum flavour score. The score for flavour for sweetener selection of

milkshake blended with pumpkin seed was categorised as “liked moderately” to “liked very much” by the panel of semi-trained judges.

The lower score for flavour for B₂ was given by the judges because honey masked the nutty flavour of pumpkin seed however, B₃ had lowest score because stevia had bitter after taste. Anne Brockhoff (2012) also observed steviol glycosides in stevia activate two bitter receptors on the human tongue and this elicits a bitter after taste in the mouth which in accordance with present study.

4.3.2.1.3 Body and Texture

The score for body and texture for milkshake blended with pumpkin seed are illustrated in Table 4.4. The score for body and texture for the sweetener B₁, B₂ and B₃ were 8.04, 7.59 and 7.82, respectively. The score for body and texture was significantly ($P < 0.05$) influenced by addition of different types of sweetener in milkshake. From data it looks that, B₂ had lowest score for body and texture as it gives gumminess to the milkshake which made it less acceptable by the judges. The score for body and texture for sweetener selection of milkshake blended with pumpkin seed was categorised as “liked moderately” to “liked very much” by the panel of semi-trained judges.

4.3.2.1.4 Overall Acceptability

The overall acceptability for milkshake blended with pumpkin seed are illustrated in Table 4.4. The overall acceptability for milkshake significantly ($P < 0.05$) influenced by addition of different types of sweeteners in milkshake. The score for overall acceptability for the sweetener B₁, B₂ and B₃ were 8.12, 7.81 and 7.65, respectively. Treatment B₁ had maximum score for overall acceptability while B₃ had lowest overall acceptability score. The score for overall acceptability for sweetener selection of milkshake blended with pumpkin seed was categorised as “liked moderately” to “liked very much” by the panel of semi-trained judges.

The specific behaviour of treatment combinations with regard to this particular character could be understood with the fact that the overall acceptability is sum of combinations of colour and appearance, flavour and body and texture of the product. It was observed that treatment B₁ i.e. Sugar match well to govern sensory attributes to desired level. Hence, it could be inferred that use of sugar for preparation of pumpkin seed milkshake resulted in the maximum sensory perception and selected for further study.

4.3.3 Optimization of Roasted Pumpkin Seed Powder and Sugar Level

For optimization of roasted pumpkin seed powder and sugar level, three levels of selected pumpkin seed form and two levels of selected type of sweetener were considered.

Treatment details were as follows

Level of Roasted Pumpkin Seed Powder : K_1, K_2 and K_3

Level of Sugar : S_1, S_2

Treatment combinations

K_1S_1 (T_1) K_1S_2 (T_2)

K_2S_1 (T_3) K_2S_2 (T_4)

K_3S_1 (T_5) K_3S_2 (T_6)

T_0 : Controlled treatment of milk shake without addition of pumpkin seed

T_1 (K_1S_1) : Milk (4.5 % fat) + 3 % Selected Form of Pumpkin Seed + 8 % Selected Sweetener

T_2 (K_1S_2) : Milk (4.5 % fat) + 3 % Selected Form of Pumpkin Seed + 10 % Selected Sweetener

T_3 (K_2S_1) : Milk (4.5 % fat) + 5 % Selected Form of Pumpkin Seed + 8 % Selected Sweetener

T_4 (K_2S_2) : Milk (4.5 % fat) + 5 % Selected Form of Pumpkin Seed + 10 % Selected Sweetener

T_5 (K_3S_1) : Milk (4.5 % fat) + 7 % Selected Form of Pumpkin Seed + 8 % Selected Sweetener

T_6 (K_3S_2) : Milk (4.5 % fat) + 7 % Selected Form of Pumpkin Seed + 10 % Selected Sweetener

On the basis of sensory evaluation, one best treatment combination was selected for further study.

4.3.3.1 Effect of Levels of Roasted Pumpkin Seed Powder and Sugar on Sensory Score of Milkshake

The sensory evaluation of freshly prepared milkshake blended with roasted pumpkin seed powder was summarised in Table 4.5.

Table 4.5. Effect of levels of roasted pumpkin seed powder and sugar on sensory score of milkshake

Treatments	Sensory Attributes			
	Colour and Appearance	Flavour	Body and texture	Overall Acceptability
T ₀	7.61 ^g	7.92 ^g	7.96 ^d	7.83 ^f
T ₁ (K ₁ S ₁)	8.10 ^f	8.15 ^c	8.08 ^c	8.11 ^c
T ₂ (K ₁ S ₂)	8.13 ^e	8.11 ^f	8.07 ^c	8.10 ^e
T ₃ (K ₂ S ₁)	8.26 ^c	8.23 ^c	8.32 ^b	8.27 ^c
T ₄ (K ₂ S ₂)	8.23 ^d	8.19 ^d	8.30 ^b	8.24 ^d
T ₅ (K ₃ S ₁)	8.38 ^b	8.53 ^a	8.40 ^a	8.45 ^a
T ₆ (K ₃ S ₂)	8.41 ^a	8.31 ^b	8.42 ^a	8.39 ^b
S.E. ±	0.008	0.008	0.007	0.009
CD at 5 %	0.023	0.024	0.021	0.027

Mean of 4 replications

4.3.3.1.1 Colour and Appearance

The colour of pumpkin seed milkshake ranged from olive-greenish to yellowish which was combined effect of protochlorophyll pigment in pumpkin seed and carotene in cow milk. The colour and appearance score of milkshake are presented in Table 4.5. The colour and appearance of milkshake significantly ($P < 0.05$) influenced by addition of different levels of roasted pumpkin seed powder and sugar in milkshake. The mean maximum score for colour and appearance was obtained to the treatment T₆ i.e. K₃S₂ (8.41) followed by T₅ i.e. K₃S₁ (8.38). The score for colour and appearance of milkshake samples was categorised as “liked moderately” to “liked very much” by the panel of semi-trained judges.

It is observed that, with increased level of pumpkin seed the colour of finished product became dark which was more acceptable by the judges. Such trend with respect to addition carrot puree in carrot milkshake was noticed by Patil *et al.* (2019). Neeta Kumari (2020) also recorded the similar trend for pumpkin seed added *ladoo* and biscuit.

4.3.3.1.2 Flavour

The flavour score of milkshake prepared using different levels of roasted pumpkin seed powder and sugar are presented in Table 4.5. The mean flavour scores for milkshake varied from 7.92 to 8.53 for different treatment combinations. The flavour of milkshake significantly ($P < 0.05$) affected by addition of different levels of roasted pumpkin seed powder and sugar in the milkshake. The treatment T₅ (7 per cent roasted pumpkin seed powder and 8 per cent sugar) was superior over other treatment combinations. Development of typical nutty flavour to milkshake is because of roasting which improved compounds like alkylated pyrazines and 2-acetylpyrrole that responsible for nutty flavour (Barbara *et al.* 2004). Further, level of 10 per cent sugar in milkshake was not as much liked by the judges and commented that milkshake had slightly unpleasant flavour due to increasing sugar level.

It was noticed that score for flavour increases with increasing level of roasted pumpkin seed powder in milkshake and sugar level at certain point. The findings are in accordance with Bhosale, (2013) that increasing level of *ramphal* pulp improved score for flavour of milkshake.

4.3.3.1.3 Body and Texture

In the Table 4.5, the score for body and texture of developed milkshake is highlighted. The average scores for body and texture attribute of pumpkin seed milkshake prepared under each treatment ranged between 7.96 to 8.42 which were significantly ($P < 0.05$) changed by addition of different levels of roasted pumpkin seed powder in milkshake. The treatment T₅ and T₆ had better body and texture score as compared to the other treatments. T₁ and T₂, T₃ and T₄ as well as T₅ and T₆ were at par with other. The score for body and texture of milkshake was categorised as “liked moderately” to “liked very much” by the panel of semi-trained judges.

It was observed that buttery/creamy texture and thick consistency improved with increasing level of roasted pumpkin seed powder in the milkshake. Similar findings have been reported by Bhosale (2013) for *ramphal* milkshake and Kuchekar (2011) for almond milkshake.

4.3.3.1.4 Overall Acceptability

The overall acceptability score of milkshake prepared using different levels of roasted pumpkin seed powder and sugar are tabulated in Table 4.5. The score for overall acceptability of milkshake for treatments T₀, T₁, T₂, T₃, T₄, T₅ and T₆ were 7.83, 8.11, 8.10, 8.27, 8.24, 8.47 and 8.29, respectively. The effect of roasted pumpkin seed powder level and sugar on overall acceptability was significant (P<0.05). The treatment T₅ had better overall acceptability as compared to other treatments.

The specific behaviour of treatment combinations with regard to this particular character could be understood with the fact that the overall acceptability is sum of combinations of colour and appearance, flavour and body and texture of the product. There seemed to have significant improvement in all the characters which might have enhanced the judge's preference for overall acceptability of all the six treatment combinations of milkshake. Moreover, it is stressed that the treatment T₅ (K₃S₁) had meet all the sensory attributes to desired level. Treatment T₅ had 7 per cent roasted pumpkin seed powder which meets dietary allowances as well as economically feasible for milkshake preparation. Hence, it could be inferred that addition of 7 per cent roasted pumpkin seed powder and 8 per cent sugar to milkshake were most optimum to prepare pumpkin seed milkshake.

Patil (1984) also studied the bio-chemical, nutritional and keeping quality aspects of banana milk shake. He reported that 1:10, banana : milk combination had maximum score for overall acceptability as 7.50 as compared to 1:5 banana : milk combination score as 7.00. Similarly, Poul *et al.* (2009) advocated 10 per cent custard apple pulp in milk for preparation of custard apple milkshake. The results recorded in the present study are quite agreeable with the above research findings.

4.3.3.2 Effect of Levels of Roasted Pumpkin Seed Powder and Sugar on Physico-chemical Composition of Milkshake

The samples of pumpkin seed milkshake prepared under each treatment were analyzed for fat, protein, carbohydrates, crude fiber, ash, total solid, acidity, pH and mineral content. The observations and statistical analysis of which are given in table 4.6.

Table 4.6. Effect of levels of roasted pumpkin seed powder and sugar on physico-chemical composition of milkshake

Treatments	Physico-chemical Constituents							
	Fat (%)	Protein (%)	Carbohydrates (%)	Crude Fiber (%)	Ash (%)	Total Solid (%)	Acidity (% LA)	pH
T ₀	3.40 ^g	3.02 ^f	10.37 ^g	ND	0.77 ^d	17.56 ^c	0.14	6.28 ^d
T ₁	3.50 ^c	3.08 ^c	11.06 ^f	0.65 ^c	1.05 ^c	19.34 ^d	0.14	6.30 ^c
T ₂	3.46 ^f	3.06 ^c	11.15 ^e	0.64 ^c	1.07 ^c	19.38 ^c	0.14	6.32 ^c
T ₃	3.97 ^c	3.13 ^c	11.26 ^d	1.01 ^b	1.11 ^b	20.48 ^b	0.13	6.43 ^b
T ₄	3.92 ^d	3.09 ^d	11.34 ^c	1.00 ^b	1.12 ^b	20.47 ^b	0.12	6.44 ^b
T ₅	4.12 ^a	3.22 ^a	11.48 ^b	1.45 ^a	1.15 ^a	21.42 ^a	0.12	6.49 ^a
T ₆	4.03 ^b	3.16 ^b	11.62 ^a	1.46 ^a	1.16 ^a	21.43 ^a	0.12	6.50 ^a
S.E. ±	0.006	0.006	0.016	0.035	0.006	0.01	0.01	0.007
CD at 5%	0.019	0.018	0.047	0.103	0.019	0.02	NS	0.020

Mean of 4 replications

4.3.3.2.1 Fat

The results presented in Table 4.6 show that average fat content of pumpkin seed milkshake were 3.40, 3.50, 3.46, 3.97, 3.92, 4.12 and 4.03 per cent for treatment T₀, T₁, T₂, T₃, T₄, T₅ and T₆, respectively. The fat content in milkshake were significantly ($P < 0.05$) differed by addition of roasted pumpkin seed powder at different per cent level. The treatment T₀ had the lowest fat content (3.40 %) whereas the treatment T₅ (K₃S₁) had maximum fat content (4.12 %). These observations indicated that as pumpkin seed powder increased, the fat content in final product was increased because pumpkin seeds also rich in fat content but then as sugar content increased, the fat content in final product decreased.

These findings are in accordance with Dhiman *et al.* (2018) who reported increasing fat content with increasing pumpkin seed level during preparation of pumpkin seed supplemented weaning mix. However, More (2016) reported fat in the range of present finding for aloe vera added custard apple milkshake. On the other hand, Kashid (2005) recorded slightly higher fat content (4.5 %) in golden milkshake.

4.3.3.2.2 Protein

The protein content in pumpkin seed milkshake was one of the important constituent from nutritional point of view which provides valuable animal and plant

protein. The values of protein content of milkshake are presented in Table 4.6. Protein values of milkshake ranged from 3.02 to 3.22 per cent for treatment T₀ to T₅, respectively. While, treatment T₅ (K₃S₁) had highest protein content (3.22 %). Though variation in protein content was in narrow range but interaction effect of roasted pumpkin seed powder and sugar were significant (P<0.05). The protein content of milkshake increased due to increase in pumpkin seed powder which is good source of protein and decreased with increase in sugar level might be due to increased volume of the final product. Surve (2017) found that protein increased with increase in level of date pulp and decreased with increase in level of jaggery. Kuchekar (2011) have reported increasing trend of protein content with increasing levels of almond in almond milkshake. Kashid (2005) and Bhosale (2013) reported that the protein content in range similar to present finding for safflower milkshake and *ramphal* milkshake, respectively.

4.3.3.2.3 Carbohydrates

The Table 4.6 represents average carbohydrate content of pumpkin seed milkshake. It is clear that there was increase in carbohydrate content of pumpkin seed milkshake from T₀ to T₆. This was due to increase proportion of roasted pumpkin seed powder and sugar in different treatment combinations. The carbohydrate content in milkshake was significantly (P<0.05) increased as it is sum total of carbohydrate in pumpkin seed, lactose in milk and added sugar. The treatment T₀ had the lowest carbohydrate content (10.37 %) whereas value of carbohydrate content of T₆ i.e. K₃S₂ (11.62 %) is superior over other treatments.

These findings are in accordance with Kuchekar (2011) who reported carbohydrate in the range of present finding for almond milkshake. He also noticed increasing trend of carbohydrate content with increase in almond and added sugar. However, Bhosale (2013) observed high carbohydrate content (14.35 to 17.37 %) in *ramphal* milkshake.

4.3.3.2.4 Crude Fiber

The crude fiber content of pumpkin seed milkshake samples are depicted in Table 4.6. The crude fiber content of milkshake was significantly (P<0.05) highest in T₆ (1.46 %) however, it was at par with T₅. It significantly (P<0.05) differed by addition of roasted pumpkin seed powder and sugar at different level. The minimum value of

crude fiber content observed in T₂ (0.64) and T₁ (0.65). Both were at par with each other. Crude Fiber content was not detected in the controlled sample. Hemant (2021) reported high percentage of (2.25 %) crude fiber in milkshake prepared using psyllium husk. It is cleared that increase in roasted pumpkin seed powder level resulted in increased crude fiber content. This increasing trend supported by reports of Dhiman *et al.* (2018) for pumpkin seed supplemented weaning mix.

4.3.3.2.5 Ash

Ash is predominantly mineral compound in the product. It contributes mainly through milk and pumpkin seed. The ash values for treatments T₀ to T₆ ranged from 0.77 to 1.16 per cent and significantly (P<0.05) inclined by addition of pumpkin seed. Treatment T₆ i.e. K₃S₂ found significantly superior value of ash over other treatments. Treatments T₅ and T₆ were at par with each other. It indicated that there was increasing trend in the ash content of finished product, this might be due to increasing proportion of pumpkin seed. The observations in respect to ash content of present investigation are more or less similar to the values reported by Surve (2017) for date milkshake, Ubale *et al.* (2014) for sapota milkshake and Poul *et al.* (2009) for custard apple milkshake.

4.3.3.2.6 Total Solid

The total solid content of the product is counterpart of moisture content and contributed by the milk solid, sugar and pumpkin seed powder. From table 4.6 it can be observed that total solids in pumpkin seed milkshake ranged between 17.56 to 21.43 per cent. The lowest value recorded for T₀ (17.56) and that of highest value for T₆ (K₃S₂) was observed as 21.43 per cent. T₅ and T₆ were at par with each other.

It was noticed that as percentage of pumpkin seed powder and sugar increased the total solid content of product was also increased significantly (P<0.05). Sharma and Gupta (1978), Varpe (1992), Kshirsagar (1996) and Kadav (2001) found 17.00, 26.55, 24.32 and 25.27 per cent total solids, respectively in plain milk shake. The values are more or less comparable with the results obtained in present study. Similarly, Jadhav (1999) also noticed the increase in total solid with increasing level of sapota pulp in milkshake.

4.3.3.2.7 Acidity

The acidity of pumpkin seed milk shake is illustrated in Table 4.6. The values of acidity for treatment T₀, T₁, T₂, T₃, T₄, T₅ and T₆ were 0.14, 0.14, 0.14, 0.13, 0.12, 0.12, 0.12 per cent, respectively. The intermediate value for acidity (0.13 per cent LA) was observed in the treatment T₃. The acidity of milkshake affected non-significantly (P<0.05). This obtained result indicated that titrable acidity of milkshake decreases slightly as the per cent level of pumpkin seed powder increases might be due to alkaline nature of pumpkin seeds.

Similar decreasing trend for acidity was noticed by Dhiman *et al.* (2018) for pumpkin based ready to serve beverage. This observation also supported by Kashid (2005) who evaluated that acidity of milkshake decreased along with addition of safflower in golden milkshake.

4.3.3.2.8 pH

pH of milkshake prepared with addition of roasted pumpkin seed powder are presented in Table 4.6. The pH values for the treatment T₀, T₁, T₂, T₃, T₄, T₅ and T₆ were 6.28, 6.30, 6.32, 6.43, 6.44, 6.49 and 6.50, respectively. The pH values of milkshake significantly (P<0.05) inclined by addition of roasted pumpkin seed powder and sugar. The maximum incline in pH value (6.50) was observed in milkshake prepared using 7 per cent roasted pumpkin seed powder due to decrease in acidity. Kashid (2005) evaluated that pH of milkshake increased along with addition of safflower in golden milkshake which resembles with above finding.

4.3.3.2.9 Minerals

The Calcium content in milkshake samples varied significantly (P<0.05) from 9.40 to 10.38 mg/100g. Treatment T₆ (10.38) had highest calcium content which was at par with treatment T₅ (10.37). The magnesium content in pumpkin seed milkshake significantly (P<0.05) increases with increase in level of roasted pumpkin seed powder. Treatment T₆ (5.50) had highest magnesium content followed by T₅ (5.47). Controlled milkshake T₀ (3.02) had minimum zinc content while T₆ (5.45) had maximum zinc content. Treatments T₅ and T₆ were at par with each other. The iron content in milkshake samples ranged between 0.80 to 1.14 mg/100g. Treatments T₅ and T₆ as well as T₃ and T₄ were at par with each other. Highest potassium content observed in T₆ (6.28) while lowest

in controlled sample T₀ (5.26). The phosphorous content varied significantly (P<0.05) from 11.31 to 11.96 mg/100g. Treatments T₅ and T₆ were at par with each other. The maximum mineral content found in treatment T₆ followed by T₅ that had 7 per cent roasted pumpkin seed powder as compared to other treatments as mentioned in table 4.7.

Table 4.7. Effect of levels of roasted pumpkin seed powder and sugar on mineral content of milkshake

Treatments	Calcium	Magnesium	Zinc	Iron	Potassium	Phosphorus
T ₀	9.40 ^d	4.12 ^f	3.02 ^e	0.80 ^e	5.26 ^d	11.31 ^d
T ₁	10.12 ^c	5.24 ^e	4.21 ^d	0.93 ^d	6.12 ^c	11.51 ^c
T ₂	10.13 ^c	5.26 ^d	4.21 ^d	0.95 ^c	6.13 ^c	11.53 ^c
T ₃	10.25 ^b	5.38 ^c	5.34 ^c	1.06 ^b	6.18 ^b	11.72 ^b
T ₄	10.26 ^b	5.39 ^c	5.36 ^b	1.07 ^b	6.19 ^b	11.74 ^b
T ₅	10.37 ^a	5.47 ^b	5.44 ^a	1.13 ^a	6.27 ^a	11.95 ^a
T ₆	10.38 ^a	5.50 ^a	5.45 ^a	1.14 ^a	6.28 ^a	11.96 ^a
S.E. ±	0.0097	0.0025	0.0025	0.0026	0.0028	0.0099
CD at 5%	0.0286	0.0073	0.0073	0.0075	0.0083	0.0288

Mean of 4 replications

The average values of mineral content observed in the present study in close proximation with values reported by Neeta Kumari (2020) evaluated that mineral content of pumpkin powder added bakery products were increased from treatment T₁ to T₄. As the level of pumpkin seed powder added as per treatment the mineral score also increased. Denka *et al.* (2022) also noticed increase in mineral content with increasing pumpkin seed flour in bread.

4.3.3.3 Effect of Levels of Roasted Pumpkin Seed Powder and Sugar on Microbial Quality of Milkshake

4.3.3.3.1 Standard Plate Count (SPC)

The Standard Plate Count is a bacterial load of milkshake blended with roasted pumpkin seed powder is showed in Table 4.8. The SPC count for the milkshake samples T₀, T₁, T₂, T₃, T₄, T₅ and T₆ were 1.12 1.14, 1.16, 1.17, 1.17, 1.19 and 1.21 × 10⁵ cfu/ml, respectively.

More (2016) noticed SPC ranged from 1.75 to 4.25×10^6 cfu/g for different treatments in aloe vera added custard apple milkshake. Hemant *et al.* (2021) found TPC 1.6×10^4 for psyllium husk milkshake.

Table 4.8. Effect of levels of roasted pumpkin seed powder and sugar on microbial quality of milkshake

Treatments	Microbial Attributes		
	Standard Plate Count (10^5 cfu/ml)	Yeast And Mould Count	Coliform Count
T ₀	1.12	ND	ND
T ₁	1.14	ND	ND
T ₂	1.16	ND	ND
T ₃	1.17	ND	ND
T ₄	1.17	ND	ND
T ₅	1.19	ND	ND
T ₆	1.21	ND	ND
S.E. \pm	0.0053	ND	ND
CD at 5%	0.0157	ND	ND

Mean of 4 replications

ND = Not- detected

4.3.3.3.2 Yeast and Mould Count (YMC)

The Yeast and Mould Count of milkshake mentioned in Table 4.8. No growth of Yeast and Mould was seen in the samples of freshly prepared milkshake blended with pumpkin seed powder.

4.3.3.3.3 Coliform Count

The coliform count of milkshake showed in Table 4.8. No growth of *E. coli* was seen in the freshly prepared samples of milkshake blended with pumpkin seed powder.

Devi (2018) observed that coliforms, yeast and mold counts were absent and the standard plate count was found to be 2×10^3 cfu/g in pumpkin seed spread indicating that the product was safe and had good quality which resembles with present study.

4.3.3.4 Optimized Flow Diagram for Preparation of Milkshake Blended with Roasted Pumpkin Seed Powder

On the basis of results obtained on the optimization of the ingredients for preparation of milkshake blended with roasted pumpkin seed powder (section 4.3 and 4.4) following steps were finalized and found most satisfactory for preparation of good quality milkshake blended with pumpkin seed.

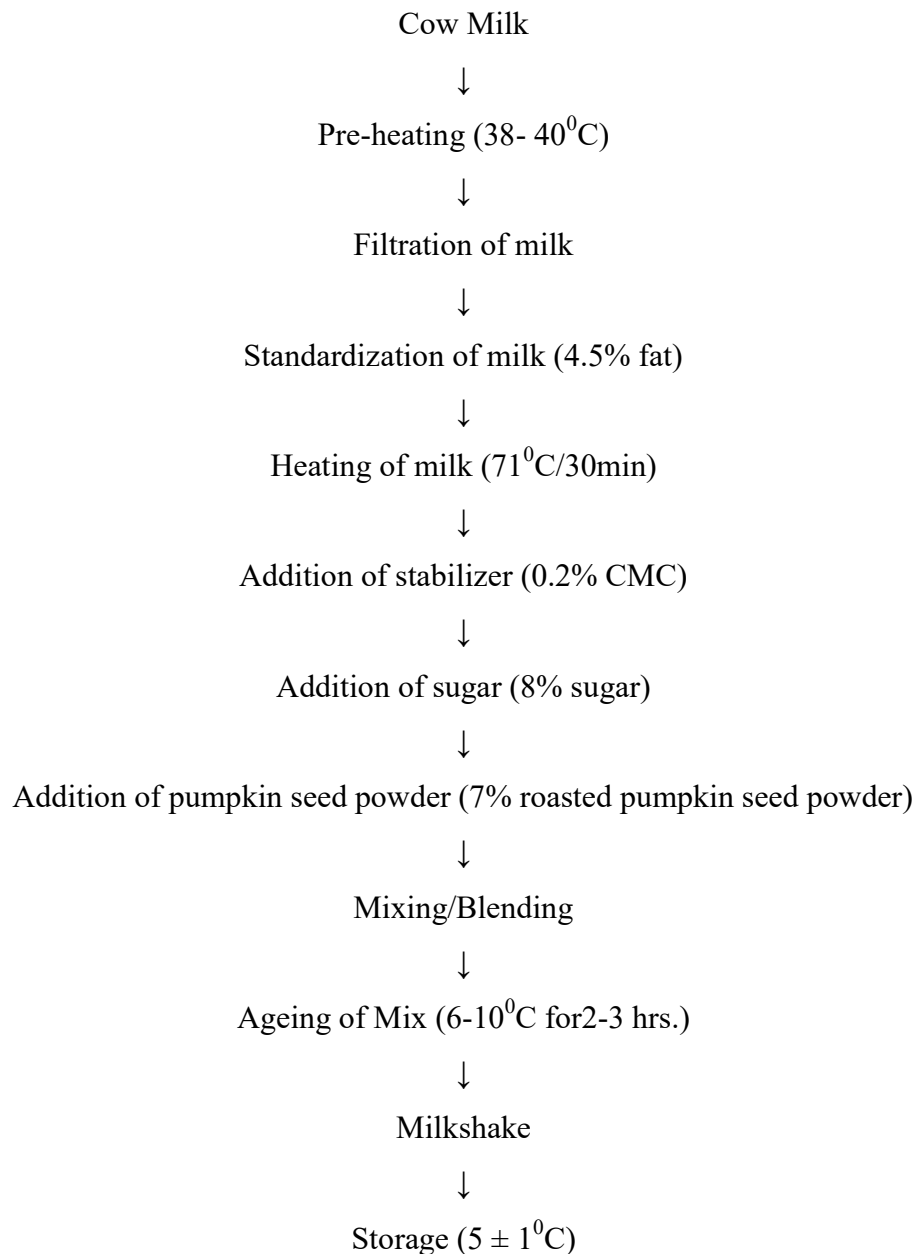


Fig. 4.1. Flow Diagram for Optimized Milkshake Blended with Pumpkin Seed

4.3.3.5 Comparison of Physico-chemical Composition of Controlled and Optimized Milkshake Blended with Roasted Pumpkin Seed Powder

The physico-chemical composition of optimized pumpkin seed milkshake and control sample are presented in Table 4.9 for comparison. The pumpkin seed milkshake prepared by using 7 per cent roasted pumpkin seed powder and 8 per cent sugar of milkshake was analyzed for fat, protein, carbohydrate, crude fiber, ash, total solid, acidity, pH and mineral content.

Table 4.9. Physico-chemical composition of control and optimized milkshake blended with roasted pumpkin seed powder

Physico-chemical Constituents	Values (%)	
	Control Milkshake	Optimized Pumpkin Seed Milkshake
Fat (%)	3.40	4.12
Protein (%)	3.02	3.22
Carbohydrate (%)	10.37	11.48
Crude fiber (%)	ND	1.45
Ash (%)	0.77	1.15
Total Solid (%)	17.56	21.42
Acidity (% LA)	0.14	0.12
pH (%)	6.28	6.49
Minerals (mg/100g)		
Calcium	9.40	10.37
Magnesium	4.12	5.47
Zinc	3.02	5.44
Iron	0.80	1.13
Potassium	5.26	6.27
Phosphorous	11.31	11.95

From the Table 4.9, it is observed that the controlled treatment had 3.40 per cent fat, 3.02 per cent protein, 10.37 per cent carbohydrate, 0.77 per cent ash, 17.56 per cent total solid, 0.14 per cent acidity and 6.28 pH and Calcium 9.40, Magnesium 4.12, Zinc 3.02, Iron 0.80, Potassium 5.26, Phosphorous 11.31 mg/100g. While milkshake prepared by using 7 per cent roasted pumpkin seed powder and 8 per cent sugar had 4.12 per cent fat, 3.22 per cent protein, 11.48 per cent carbohydrate, 1.45 per cent crude fiber, 1.15 per cent ash, 21.42 per cent total solid, 0.12 per cent acidity and

6.49 pH and Calcium 10.37, Magnesium 5.47, Zinc 5.44, Iron 1.13, Potassium 6.27, Phosphorous 11.95 mg/100g. It showed that there was increase in all the constituents of optimized milkshake than control milkshake.

4.4 Shelf Life Study of Milkshake

Shelf life is defined as the number of days a product can be stored at certain temperature and be consumed safely, without deterioration of sensory characteristics such as colour, taste and texture. The shelf life is therefore determined by organoleptic, physico-chemical and microbial quality (Aneja *et al.* 2002).

In present investigation, an attempt has been made to examine the shelf life of pumpkin seed milkshake. Accordingly, pumpkin seed milkshake was prepared by standardized method as under 4.3.3.4 to conduct storage study. The optimized sample was kept in pre-sterilized glass bottle at refrigeration temperature ($5 \pm 1^{\circ}\text{C}$) and compared with controlled sample of milkshake. The samples were evaluated for sensory, chemical and microbiological qualities during storage at an interval of 3 days. The analysis of stored sample was discontinued when product unacceptable mainly due to increased acidity, spore forming bacterial growth and gelation of product because of redistribution of protein.

4.4.1 Changes in Sensory Score of Milkshake Stored at $5 \pm 1^{\circ}\text{C}$

Milkshake prepared with 7 per cent roasted pumpkin seed powder and 8 per cent sugar was used to conduct storage study on the basis on sensory evaluation with the help of 9-point hedonic scale.

4.4.1.1 Colour and Appearance

The data pertaining to change in score for colour and appearance of stored milkshake are given in Table 4.10 and 4.11. On first day, score for optimized pumpkin seed milkshake was higher than control and gave an indication that samples were highly acceptable with respect to colour and appearance.

As storage progressed, the colour and appearance scores were decreased from 7.61 to 6.54 and 8.38 to 6.92 in T_0 and T_1 , respectively because they lacked the smooth and whipped appearance on the surface of product desired. Overall, the given storage period and treatment was significant and their interaction showed a significant ($P < 0.05$) effect on colour and appearance scores. Mhetre (2019) was observed that score

for colour and appearance of gulkand flavoured milk was declined from 0 day to 12th day of storage which resembles with present study.

Table 4.10. Changes in score for colour and appearance of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Treatments	Storage Period (days)				
	0	3	6	9	12
T ₀	7.61	7.30	6.81	6.54	--
T ₁	8.38	8.04	7.60	6.92	--

Mean score of 3 replications

(--) Indicates that product was unacceptable and no further analysis was carried out.

Table 4.11. ANOVA for Changes in score for colour and appearance of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Source of variation	DF	MSS	F value	CD
Between treatment	1	2.700104	29455.68	0.01
Between period	3	1.825904	19918.95	0.01
Interaction	3	0.056938	621.14	0.02
Error	16	0.000938	--	--

4.4.1.2 Flavour

Flavour is an important criterion for deciding the quality of the product, which in terms determines its acceptability. The sensory score for flavour of milkshake samples during storage are presented in Table 4.12 and 4.13. The score for flavour for stored milkshake blended with roasted pumpkin seed powder were decreased significantly ($P < 0.05$) during storage at $5 \pm 1^{\circ}\text{C}$ for 9 days.

Table 4.12. Changes in score for flavour of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Treatments	Storage Period (days)				
	0	3	6	9	12
T ₀	7.92	7.38	7.11	6.87	--
T ₁	8.53	8.09	7.86	6.98	--

Mean score of 3 replications

(--) Indicates that product was unacceptable and no further analysis was carried out.

Table 4.13. ANOVA for Changes in score for flavour of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Source of variation	DF	MSS	F value	CD
Between treatment	1	1.78215	17821.50	0.01
Between period	3	1.75495	17549.50	0.01
Interaction	3	0.13135	1313.50	0.02
Error	16	0.0001	--	--

The score for flavour was found to be decreased from 7.92 to 6.87 and 8.53 to 6.98 in T_0 and T_1 , respectively. The given storage period and treatment was significant and their interaction showed a significant ($P < 0.05$) effect on flavour score. The decrease in score for flavour may be attributed to slight loss of freshness and flavour become stale. Also, fat hydrolysis caused by lipase can result in undesirable flavours as well as loss of functional properties of product like foaming during storage (Shelley *et al.* 1987). Mhetre (2019) was observed that score for flavour of *gulkand* flavoured milk was declined from 7.75 to 6.0 during 0 day to 12th day of storage which resembles with present study.

4.4.1.3 Body and Texture

The score for body and texture of milkshake are illustrated in Table 4.14 and 4.15. The Score for body and texture of milkshake samples were significantly (< 0.05) influenced during storage of 9 days period at $5 \pm 1^{\circ}\text{C}$. The data showed that score for body and texture were found to be decreased from 7.96 to 6.60 and 8.40 to 7.03 in T_0 and T_1 , respectively. The given storage period and treatment was significant and their interaction showed a significant ($P < 0.05$) effect on body and texture score. The rate of decline in body and texture scores for control milkshake (T_0) is slightly higher than pumpkin seed milkshake (T_1). Verma (2021) also observed decline in body and texture parameters of turmeric fortified milkshake during storage which was in proximation with present study.

Table 4.14. Changes in score for body and texture of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Treatments	Storage Period (days)				
	0	3	6	9	12
T ₀	7.96	7.62	7.14	6.60	--
T ₁	8.40	8.05	7.63	7.03	--

Mean score of 3 replications

(--) Indicates that product was unacceptable and no further analysis was carried out

Table 4.15. ANOVA for Changes in score for body and texture of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Source of variation	DF	MSS	F value	CD
Between treatment	1	1.201538	12015.37	0.01
Between period	3	2.091038	20910.37	0.01
Interaction	3	0.001237	12.37	0.02
Error	16	0.0001	--	--

4.4.1.4 Overall Acceptability

The score for overall acceptability of milkshake are illustrated in Table 4.16 and 4.17. The overall acceptability of milkshake samples were significantly (<0.05) decrease during storage period of 9 days at $5 \pm 1^{\circ}\text{C}$, respectively. During storage the score for overall acceptability of finished milkshake were acceptable up to 9th days when stored at freezing temperature. The data showed that score for overall acceptability were found to be decreased from 7.83 to 6.70 and 8.45 to 6.98 in T₀ and T₁, respectively.

Table 4.16. Changes in score for overall acceptability of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Treatments	Storage Period (days)				
	0	3	6	9	12
T ₀	7.83	7.43	7.03	6.70	--
T ₁	8.45	8.06	7.70	6.98	--

Mean score of 3 replications

(--) Indicates that product was unacceptable and no further analysis was carried out

Table 4.17. ANOVA for Changes in score for overall acceptability of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Source of variation	DF	MSS	F value	CD
Between treatment	1	1.815	18150.00	0.01
Between period	3	1.84285	18428.50	0.01
Interaction	3	0.0493	493.00	0.02
Error	16	0.0001	--	--

The given storage period and treatment was significant and their interaction showed a significant ($P < 0.05$) effect on overall acceptability score. The decreased score of overall acceptability with advancement of storage period might be attributed to the declining colour and appearance, body and texture and flavour of the product. All deteriorative changes like appearance and textural changes are collectively reflected in sensory quality and thus led to unacceptability of product after definite period. Decrease in score for overall acceptability at end of storage period also observed by Devi (2018) for pumpkin seed spread. Verma (2021) also reported decline in sensory parameters of turmeric fortified milkshake during storage which was in proximation with present study.

4.4.2 Changes in Physico-chemical Composition of Milkshake Stored at $5 \pm 1^{\circ}\text{C}$

4.4.2.1 Fat

The changes in fat during storage with different treatment (T_0 and T_1) are depicted in the Table 4.18 and 4.19. The fat content of milkshake significantly (< 0.05) declined during storage of 9th days at $5 \pm 1^{\circ}\text{C}$. The data showed that fat were found to be decreased from 3.40 to 3.16 per cent and 4.12 to 3.85 per cent in T_0 and T_1 , respectively.

Table 4.18. Changes in fat content of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Treatments	Storage Period (days)				
	0	3	6	9	12
T_0	3.40	3.37	3.29	3.16	--
T_1	4.12	4.08	3.97	3.85	--

Mean score of 3 replications

(--) Indicates that product was unacceptable and no further analysis was carried out

Table 4.19. ANOVA for Changes in fat content of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Source of variation	DF	MSS	F value	CD
Between treatment	1	2.94	29400.00	0.01
Between period	3	0.0781	781.00	0.01
Interaction	3	0.0005	5.00	0.02
Error	16	0.0001	--	--

Decrease in fat content of milkshake during storage period might be due to conversion of fats and lipids to free fatty acids i.e. hydrolysis of fat due to microflora. Patel (2020) observed similar trend of decreasing fat content during storage period for pumpkin flavoured buffalo milk during storage of 15 days.

4.4.2.2 Protein

The protein content of milkshake samples are presented in Table 4.20 and 4.21. The protein content of milkshake samples which were stored significantly (<0.05) decline during storage period of 9 days at $5 \pm 1^{\circ}\text{C}$. The data showed that protein were found to be decreased from 3.02 to 2.68 per cent and 3.22 to 2.94 per cent in T_0 and T_1 , respectively. The slight decrease in protein content might be due to combined effect of proteolysis, degradation of amino acids and aggregation of protein. Redistribution of protein in colloidal and serum phase responsible for aggregation of protein in milk products was evident by Hilton *et al.* (2016). Patel (2020) noticed that protein remain stable during 15 days storage while decrease in protein content during storage period of 30 days for pumpkin flavoured buffalo milk.

Table 4.20. Changes in protein content of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Treatments	Storage Period (days)				
	0	3	6	9	12
T_0	3.02	2.96	2.83	2.68	--
T_1	3.22	3.17	3.05	2.94	--

Mean score of 3 replications

(--) Indicates that product was unacceptable and no further analysis was carried out

Table 4.21. ANOVA for Changes in protein content of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Source of variation	DF	MSS	F value	CD
Between treatment	1	0.297037	1080.14	0.01
Between period	3	0.114538	416.50	0.02
Interaction	3	0.001038	3.77	0.03
Error	16	0.000275	--	--

4.4.2.3 Carbohydrate

Carbohydrate i.e. Total sugar contributed by both reducing and non reducing sugar showed decreasing trend during period of storage. The carbohydrate content of pumpkin seed milkshake are depicted in Table 4.22 and 4.23. It is observed that carbohydrate content of milkshake samples significantly (<0.05) decreased during storage period of 9 days at $5 \pm 1^{\circ}\text{C}$. The data showed that carbohydrate was found to be decreased slightly from 10.37 to 10.02 per cent and 11.48 to 11.10 per cent in T_0 and T_1 , respectively.

Table 4.22. Changes in carbohydrate content of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Treatments	Storage Period (days)				
	0	3	6	9	12
T_0	10.37	10.29	10.17	10.02	--
T_1	11.48	11.41	11.27	11.10	--

Mean score of 3 replications

(--) Indicates that product was unacceptable and no further analysis was carried out

Decline in rate of carbohydrate content of optimized sample was high as compare to controlled sample on 6th day of storage. Reducing sugar (lactose) is utilized by bacteria and converted to lactic acid while non reducing sugar (sucrose) also converted to acid by microbial development. Overall there is decrease in carbohydrate content of milkshake by action of developed microflora during storage. During storage, decrease in carbohydrate also reported by Patel *et al.* (2020) whereas Poul (2009) noticed increase in total sugar during storage for custard apple milkshake.

Table 4.23. ANOVA for Changes in carbohydrate content of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Source of variation	DF	MSS	F value	CD
Between treatment	1	7.293037	72930.37	0.01
Between period	3	0.153737	1537.37	0.01
Interaction	3	0.000438	4.37	0.02
Error	16	0.0001	--	--

4.4.2.4 Crude Fiber

The values of crude fiber content of milkshake blended with roasted pumpkin seed powder summarized in the Table 4.24 and 4.25. For T_1 Significant (<0.05) differences were found towards quantum of crude fiber degradation. Decrease in crude fiber content might be because of decreased carbohydrate content due to microbial growth. The data showed that crude fiber was not detected in controlled sample (T_0) while decreased from 1.45 to 1.35 per cent in optimized sample (T_1) during 0, 3, 6 and 9th days of storage.

Table 4.24. Changes in crude fiber content of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Treatments	Storage Period (days)				
	0	3	6	9	12
T_0	ND	ND	ND	ND	--
T_1	1.45	1.41	1.38	1.35	--

Mean score of 3 replications

ND = Not- detected

(--) Indicates that product was unacceptable and no further analysis was carried out.

Table 4.25. ANOVA for Changes in crude fiber content of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Source of variation	DF	MSS	F value	CD
Between treatment	1	0.75615	3024.60	0.01
Between period	3	0.0223	89.20	0.02
Interaction	3	0.00245	9.80	0.03
Error	16	0.00025	--	--

4.4.2.5 Ash

Ash is the name given to all non-aqueous residue that remains after sample is burned and consist mostly of minerals. The ash content of milkshake blended with pumpkin seed powder summarized in the Table 4.26 and 4.27. The effect of storage period and treatment was significant (<0.05) while their interaction effect is non-significant (<0.05). There was slightly decrease in ash content of samples from 0.78 to 0.71 per cent in T_0 and 1.15 to 1.08 per cent in T_1 during 0 to 9th days of storage. Decrease in ash might be due to loss of minerals during storage.

Table 4.26. Changes in score for ash of milkshake samples during storage at $5 \pm 1^\circ\text{C}$

Treatments	Storage Period (days)				
	0	3	6	9	12
T_0	0.78	0.77	0.74	0.71	--
T_1	1.15	1.13	1.10	1.08	--

Mean score of 3 replications

(--) Indicates that product was unacceptable and no further analysis was carried out

Table 4.27. ANOVA for Changes in ash content of milkshake samples during storage at $5 \pm 1^\circ\text{C}$

Source of variation	DF	MSS	F value	CD
Between treatment	1	0.79935	1184.22	0.02
Between period	3	0.00585	8.67	0.03
Interaction	3	0.000067	0.07	NS
Error	16	0.000675	--	--

NS = Non-significant

4.4.2.6 Total Solid

Total solid is the sum total of constituents like fat, protein, carbohydrate, fiber and ash in the product. The values for total solid of milkshake are depicted in the Table 4.28 and 4.29. The total solids of milkshake blended with pumpkin seed powder significantly (<0.05) declined during storage at $5 \pm 1^\circ\text{C}$.

The data showed that total solid were found to be decreased from 17.56 to 16.57 per cent and 21.42 to 20.32 per cent in T_0 and T_1 , respectively. Decrease in total solid might be due to decrease in other constituents i.e. fat, protein, carbohydrate, crude

fiber and ash in milkshake during 9 days period of storage. Present study is supported by findings of Mittal and Bajwa (2014) who reported decline in total solids of cardamom flavoured milk drinks during storage. Similarly, Shukla *et al.* (2017) reported that the total solid content reduced significantly from 12.79 to 11.94 and 15.91 to 14.84 during storage of mango flavoured milk beverage.

Table 4.28. Changes in total solid content of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Treatments	Storage Period (days)				
	0	3	6	9	12
T ₀	17.56	17.39	17.02	16.57	--
T ₁	21.42	21.20	20.76	20.32	--

Mean score of 3 replications

(--) Indicates that product was unacceptable and no further analysis was carried out

Table 4.29. ANOVA for Changes in total solid content of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Source of variation	DF	MSS	F value	CD
Between treatment	1	86.1846	916857.44	0.01
Between period	3	1.2873	13694	0.01
Interaction	3	0.0047	50.00.68	0.02
Error	16	0.000094	--	--

4.4.2.7 Acidity

It is one of the important chemical parameters of any dairy product which affects the acceptability to the consumers. The acidity (% LA) of milkshake samples which were stored at $5 \pm 1^{\circ}\text{C}$ are presented in Table 4.30 and 4.31. The effect of storage period and treatment was statistically significant (<0.05) but interaction was non-significant (<0.05) for acidity of milkshake. The data revealed that acidity was found to be increased from 0.14 to 0.18 and 0.12 to 0.17 in T₀ and T₁, respectively. The acidity increased in all samples with increase in storage period might be due to increasing microbial growth.

Table 4.30. Changes in acidity (% LA) of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Treatments	Storage Period (days)				
	0	3	6	9	12
T ₀	0.14	0.15	0.16	0.18	--
T ₁	0.12	0.13	0.15	0.17	--

Mean score of 3 replications

(--) Indicates that product was unacceptable and no further analysis was carried out

Verma (2021) reported increase in acidity of turmeric fortified milkshake from 0.13 to 0.23 during 7 days storage. Mittal and Bajwa (2014) observed increase in titrable acidity of cardamom flavoured milk drinks during storage. Findings of above researchers support the observations in present study.

Table 4.31. ANOVA for Changes in acidity of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Source of variation	DF	MSS	F value	CD
Between treatment	1	0.00135	13.50	0.01
Between period	3	0.0023	23.00	0.01
Interaction	3	0.0001	1.00	NS
Error	16	0.0001	--	--

NS = Non-significant

4.4.2.8 pH

The pH of the product is inversely proportional to acidity of product. The pH of milkshake blended with pumpkin seed powder samples which stored at $5 \pm 1^{\circ}\text{C}$ are presented in Table 4.32 and 4.33. The effect of storage period and treatment was statistically significant (<0.05) but their interaction was non-significant (<0.05).

Table 4.32. Changes in pH of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Treatments	Storage Period (days)				
	0	3	6	9	12
T ₀	6.28	6.23	6.14	6.02	--
T ₁	6.49	6.48	6.37	6.24	--

Mean score of 3 replications

(--) Indicates that product was unacceptable and no further analysis was carried out

Table 4.33. ANOVA for Changes in pH of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Source of variation	DF	MSS	F value	CD
Between treatment	1	0.312817	605.45	0.02
Between period	3	0.080033	154.90	0.03
Interaction	3	0.000517	1.00	NS
Error	16	0.000517	--	--

NS = Non-significant

Initially, values for pH of samples T_0 and T_1 were 6.28 and 6.49, respectively but later on pH decreased to 6.02 and 6.24 for T_0 and T_1 , respectively on 9th day of storage. Increase in acidity resulted in decreasing pH during storage. The finding is in accordance with reports of Mohammad *et. al.* (2018) for raw milk, Mittal and Bajwa (2014) for cardamom flavoured milk and Verma (2021) for turmeric milkshake.

4.4.2.9 Minerals

Table 4.34. Changes in mineral content (mg/100g) of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Minerals	Treatments	Storage Period (days)				
		0	3	6	9	12
Calcium	T_0	9.41	9.37	9.34	8.65	--
	T_1	10.37	10.35	10.28	9.82	--
Magnesium	T_0	4.12	4.10	4.02	3.95	--
	T_1	5.47	5.44	5.38	5.15	--
Zinc	T_0	3.02	2.97	2.88	2.69	--
	T_1	5.44	5.38	5.32	4.88	--
Iron	T_0	2.56	2.54	2.43	2.34	--
	T_1	3.88	3.82	3.74	3.53	--
Potassium	T_0	5.26	5.22	5.20	4.47	--
	T_1	6.27	6.24	6.15	5.36	--
Phosphorous	T_0	11.31	11.26	11.18	10.74	--
	T_1	11.94	11.90	11.74	10.45	--

Mean score of 3 replications

(--) Indicates that product was unacceptable and no further analysis was carried out.

Table 4.35. ANOVA for Changes in mineral content of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Minerals	Source of variation	DF	MSS	F value	CD
Calcium	Between treatment	1	6.150938	44734.09	0.01
	Between period	3	0.580037	4218.45	0.01
	Interaction	3	0.016938	123.18	0.02
	Error	16	0.000137	--	--
Magnesium	Between treatment	1	10.32282	11416.94	0.03
	Between period	3	0.072717	80.42	0.04
	Interaction	3	0.008383	9.27	0.05
	Error	16	0.000904	--	--
Zinc	Between treatment	1	33.583	71963.58	0.02
	Between period	3	0.239371	512.94	0.03
	Interaction	3	0.020071	43.01	0.04
	Error	16	0.000467	--	--
Iron	Between treatment	1	9.75375	13300.57	0.02
	Between period	3	0.095039	129.60	0.03
	Interaction	3	0.004861	6.63	0.05
	Error	16	0.000733	--	--
Potassium	Between treatment	1	5.626017	29353.13	0.01
	Between period	3	0.989967	5165.04	0.02
	Interaction	3	0.005583	29.13	0.02
	Error	16	0.000192	--	--
Phosphorous	Between treatment	1	0.88935	1226.69	0.02
	Between period	3	1.4115	1946.90	0.03
	Interaction	3	0.30565	421.59	0.05
	Error	16	0.000725	--	--

The mineral content of milkshake samples are illustrated in Tables 4.34 and 4.35. The mineral content (mg/100g) of milkshake samples was significantly (<0.05) influenced during storage of 9 days period at $5 \pm 1^{\circ}\text{C}$. During storage the Calcium, Magnesium, Zinc, Iron, Potassium and Phosphorus content for T_0 and T_1 decreased from 9.41 to 8.65, 4.12 to 3.95, 3.02 to 2.69, 2.56 to 2.34, 5.26 to 4.47 and 11.31 to 10.74 and 10.37 to 9.82, 5.47 to 5.15, 5.44 to 4.88, 3.88 to 3.53, 6.27 to 5.36 and 11.94 to 10.45 at $5 \pm 1^{\circ}\text{C}$, respectively.

The mineral content of milkshake samples was significantly declined might be because of loss of minerals due to oxidation and microbial growth during storage stages (0th, 3rd, 6th and 9th days) at $5 \pm 1^{\circ}\text{C}$.

4.4.3 Effect of Level of Roasted Pumpkin Seed Powder and Sugar on Microbial Quality of Milkshake Stored at $5 \pm 1^{\circ}\text{C}$

4.4.3.1 Standard Plate Count (SPC)

The SPC of milkshake samples are presented in the Table 4.36 and 4.37. The SPC was significantly ($P < 0.05$) increased during storage at $5 \pm 1^{\circ}\text{C}$. It was 1.12 and 1.19×10^5 cfu/ml initially while 2.16 and 2.35×10^5 cfu/ml at 9th day for T₀ and T₁, respectively.

Devi (2018) identified the marginal increase in standard plate count in pumpkin seed spread during storage. Also, Hemant *et al.* (2021) noticed increasing SPC in psyllium husk milkshake from 1.6 to 4.2×10^4 cfu/g during 3 weeks of storage which supports with present study.

Table 4.36. Changes in SPC (10^5 cfu/ml) of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Treatments	Storage Period (days)				
	0	3	6	9	12
T ₀	1.12	1.40	1.84	2.16	--
T ₁	1.19	1.60	1.98	2.35	--

Mean score of 3 replications

(--) Indicates that product was unacceptable and no further analysis was carried out

Table 4.37. ANOVA for Changes in SPC (10^5 cfu/ml) of milkshake samples during storage at $5 \pm 1^{\circ}\text{C}$

Source of variation	DF	MSS	F value	CD
Between treatment	1	0.135	1350.00	0.01
Between period	3	1.3781	13781.00	0.01
Interaction	3	0.0053	53.00	0.02
Error	16	0.0001	--	--

4.4.3.2 Yeast and Mould Count (YMC)

The YMC is major problem and limiting factor for shelf life of milk products. The YMC of milkshake samples during storage can be visualized from the Table 4.38 and 4.39. There were no growth observed in the fresh product upto 3rd day both in controlled and optimized milkshake while, it was 1.27 and 1.52×10^2 cfu/ml on 6th day and 1.51 and 1.74×10^2 cfu/ml on 9th day for T₀ and T₁, respectively. Interaction was statistically significant ($P < 0.05$).

Hemant *et al.* (2021) noticed increase in YMC from 2.1 to 2.9×10^4 cfu/g during 2nd and 3rd week of storage while YMC not detected in 1st week during storage period of psyllium husk milkshke.

Table 4.38. Changes in YMC (10^2 cfu/ml) of milkshake samples during storage at $5 \pm 1^\circ\text{C}$

Treatments	Storage Period (days)				
	0	3	6	9	12
T ₀	ND	ND	1.27	1.51	--
T ₁	ND	ND	1.52	1.74	--

Mean score of 3 replications

ND = Not-Detected

(--) Indicates that product was unacceptable and no further analysis was carried out.

Table 4.39. ANOVA for Changes in YMC (10^2 cfu/ml) of milkshake samples during storage at $5 \pm 1^\circ\text{C}$

Source of variation	DF	MSS	F value	CD
Between treatment	1	0.091267	706.58	0.01
Between period	3	4.167483	32264.39	0.01
Interaction	3	0.030467	235.87	0.02
Error	16	0.000129	--	--

4.4.3.3 Coliform Count

During storage of 9th day period no coliform growth was observed. It indicates that the proper hygienic conditions were followed throughout the manufacture of milkshake samples.

Hemant *et al.* (2021) reported that there was no coliform growth in psyllium husk milkshake during storage. Kaur (2017) found that roasted pumpkin seed

flour has longer shelf life than raw as roasting kill some microorganisms and increase the shelf life.

4.5 Consumer Acceptance Study of Milkshake

Consumer Acceptance Study of “Developed milkshake blended with roasted pumpkin seed powder (*Cucurbita moschata*)” provided valuable information and encouraging results which indicate that the developed product was better. However, it would be of commercial significance only if the consumers also have same feeling as was judged by the laboratory panel. Hence, an attempt was made to assess acceptability of milkshake blended with roasted pumpkin seed powder by offering it to 100 consumers belonging to different classes in the society for seeking their opinion. The optimized milkshake was offered to consumers and requested them to indicate their observations about the product on the given Performa (Appendix II).

Table 4.40. Profile of the consumers (N=100)

Profile	Particular	No. of respondents	Percentage
Age	a. Young (up to 35 yrs.)	48	48
	b. Middle (35 to 50 yrs.)	42	42
	c. Old (Above 50 yrs.)	10	10
Education	a. Illiterate	3	3
	b. Primary	7	7
	c. Secondary	15	15
	d. Higher education	10	10
	e. Graduate	30	30
	f. Post graduate	35	35
Occupation	a. Agricultural labour	9	9
	b. Agriculture	38	38
	c. Student	25	25
	d. Service	28	28
Family income	a. Upto Rs. 1,00,000/-	30	30
	b. Rs.1,00,000 to 1,50,000/-	18	18
	c. Rs.1,50,000/- to 2,00,000/-	47	47
	d. Above Rs. 2,00,000/-	5	5

4.5.1 Profile of the consumers

In the present study, efforts were made to know the profile of consumers (N = 100) in terms of their age, education, occupation and family income. From table 4.40, it is evident that 48 per cent of the consumers belonged to the young age group (Up to 35 years) followed by middle (42 %) and old (10 %). It was observed that 30 per cent and 35 per cent of the consumers had completed their graduation and post-graduation, respectively, followed by secondary (15 %), higher secondary (10 %) and primary level (7 %) of education. Only 3 per cent of consumers were illiterates. Considering the occupation 38 per cent of the consumers having agriculture as an occupation followed by service (28 %), student (25 %) and agricultural labour (9 %). As regards family income of the consumers, it was found that nearly half the consumers (47 %) had their family income in between Rs. 1,50,000 to 2,00,000/-, remaining 30 per cent of consumers belonged to the income category of Rs. 1,00,000/- while, 18 per cent of the consumers had their family income in between Rs. 1,00,000 to Rs. 1,50,000/- and only 5 per cent of the consumers had income 2,00,000 and above.

4.5.2 Willingness of Purchasing Milkshake

The information was also collected for willingness of consumers to purchase milkshake blended with roasted pumpkin seed powder and presented in Table 4.41. It is observed from table that more than half of consumer (52 %) rarely purchase milkshake and 30 per cent of the consumers purchase milkshake monthly. Only 10 per cent of the consumers purchasing milkshake fortnightly and 3 per cent consumers purchasing weekly.

Table 4.41. Willingness of purchasing milkshake

Particulars	Number of consumers (N=100)	Percentage
Weekly	3	3
Fortnightly	10	10
Monthly	30	30
Rarely	52	52
Never	5	5

4.5.3 Acceptance of Milkshake

The frequency distribution of the consumer perception of the milkshake blended with roasted pumpkin seed powder are given in Table 4.42 and found that 62 per cent of the consumers reported excellent quality of milkshake and 28 per cent reported very good remark about quality of pumpkin seed added milkshake. Further 9 and 1 per cent of the consumer expressed good and fair status of milkshake, respectively.

Table 4.42. Acceptance of milkshake

Preferences	Score	Number of respondents	Percentage
Excellent	04	62	62
Very good	03	28	28
Good	02	9	9
Fair	01	1	1

4.6 Cost of Production of Milkshake Blended with Roasted Pumpkin Seed Powder

The prevailing price/rate of each ingredient and services were taken into consideration. The labour charges considered on the basis of actual hours of work of the preparation of 100 lit. of Milkshake. The list of expenditure and their contribution in deciding unit cost of production of milkshake blended with roasted pumpkin seed powder are depicted in Table 4.43. It is revealed from the data that the cost of production of 100 lit. pumpkin seed added milkshake was estimated to Rs. 11932.95 which comes to Rs. 119.32/lit.

Table 4.43. Cost model for production of 100 lit. pumpkin seed milkshake

Sr. No.	Cost items	Unit price (Rs)	Quantity	Amount (Rs)
1	Milk	40/lit.	88 lit.	3520
2	Pumpkin Seed	75/100 gm	7.2 kg	5400
3	Sugar	42/kg	8.3 kg	348.6
4	CMC	---	---	10
5	Electricity	---	---	65
6	Water	---	---	40
7	Gas	---	---	175
	Working capital	---	---	9558.6
8	Interest on working capital @ 6 per cent	---	---	573.51
9	Depreciation charges	---	---	32.5
	Cost A	---	---	10164.61
10	Rental value of land	---	---	609.87
11	Interest on fixed capital	---	---	1016.46
	Cost B	---	---	11790.94
12	Labour	---	---	500
	Cost C	---	---	12290.94
	Cost per 100 Lit	---	---	11932.95
	Cost per Lit	---	---	119.32
	B:C ratio			1.25:1

The benefit-cost ratio was used to determine the viability of cash flow from the asset and ensured economic feasibility of the product. From above table, it is resulted that pumpkin seed milkshake has B:C ratio 1.25:1. It means benefit is 1.25 times of the cost.

5. SUMMERY AND CONCLUSION

5.1 Summary

A milkshake is a dairy product with lower fat and sugar contents and higher milk solids not fat (MSNF) than ice cream. It is a product obtained by preparing mix containing milk, skim milk powder, stabilizer and sugar and speed mixing the product in mixer to make it pourable and generate foam in it.

The pumpkin seeds are unique in flavor and nutty in taste and rich in iron, protein, manganese, magnesium, zinc, potassium, copper, phosphorous, polyunsaturated fatty acids and carotenoids thus consumed in various regions. Pumpkin seeds play important role in relaxing vessels and lowering blood pressure, anti-diabetic effect, cancer management, Gynaecological effect and also for natural treatment of parasites. Blending of pumpkin seeds in milkshake is beneficial for malnourished children, pregnant women and aged people due to its nutraceutical importance.

Present investigation “Development of milkshake blended with pumpkin seed (*Cucurbita moschata*)” was conducted during the year 2022-23. Initially, the preliminary trials were conducted to finalize form of pumpkin seed and type of sweetener. On the basis of sensory score, three levels of pumpkin seed (3, 5 and 7 %) and two levels of sugar (8 and 10 %) were chosen for experimental trials. The experiment was laid out in Completely Randomized Design (CRD) with four replications for treatment combinations and Factorial Completely Randomized Design (FCRD) with three replications for storage study. The experimental treatments were without pumpkin seed in milkshake (T_0), 3 per cent roasted pumpkin seed powder + 8 per cent sugar (T_1), 3 per cent roasted pumpkin seed powder + 10 per cent sugar (T_2), 5 per cent roasted pumpkin seed powder + 8 per cent sugar (T_3), 5 per cent roasted pumpkin seed powder + 10 per cent sugar (T_4), 7 per cent roasted pumpkin seed powder + 8 per cent sugar (T_5) and 7 per cent roasted pumpkin seed powder + 10 per cent sugar (T_6). Among the all treatment combinations, one best combination was selected. Afterwards the optimized samples were used for storage studies at $5 \pm 1^\circ\text{C}$ for storage period of 12 days. The experimental samples were analyzed for physico-chemical and microbiological qualities at intervals of 3 days.

5.1.1 Chemical Composition of Standardized Milk

The average chemical composition of milk used for preparation of milkshake had mean value of fat, protein, lactose, ash, total solid, acidity and pH was 4.50 per cent, 3.45 per cent, 4.47 per cent, 0.69 per cent, 13.11 per cent, 0.14 per cent and 6.28, respectively.

5.1.2 Chemical Composition of Roasted Pumpkin Seed

The average chemical composition of roasted pumpkin seed used for preparation of milkshake had an average 31.89 per cent fat, 23.46 per cent protein, 3.01 per cent moisture, 26.03 per cent carbohydrate, 8.01 per cent crude fiber, 7.59 per cent ash, 25.6 mg/100g Calcium, 335.7 mg/100g Magnesium, 907.0 mg/100g Zinc, 16.4 mg/100g Iron, 404.8 mg/100g Potassium, 848.6 mg/100g Phosphorous.

5.1.3 Optimization of Ingredients

5.1.3.1 Selection of Form of Pumpkin Seeds

For this purpose, four forms of pumpkin seed viz., roasted pumpkin seed powder (A_1), sundried pumpkin seed paste(A_2), sun dried pumpkin seed + roasted pumpkin seed paste (1:1) i.e.(A_3) and sprouted pumpkin seed paste(A_4) were taken at rate of 5 per cent of milk. In this trial sugar 10 per cent and cow milk standardized for 4.5 per cent fat was taken with constant rate. On the basis of sensory evaluation most accepted pumpkin seed form was selected for further study.

The colour and appearance of milkshake was significantly ($P<0.05$) influenced by addition of different forms of pumpkin seed in milkshake. The score for colour and appearance for A_1 , A_2 , A_3 and A_4 were 8.82, 8.06, 8.21 and 7.78, respectively. Treatment A_1 had maximum score for colour and appearance as compared to other treatments while A_4 had lowest colour and appearance score. The score for flavour for the forms A_1 , A_2 , A_3 and A_4 were 8.77, 8.54, 8.19 and 7.90, respectively. The treatment A_1 had maximum score for flavour while A_4 had minimum flavour score. The body and texture of milkshake significantly ($P<0.05$) influenced by addition of different forms of pumpkin seed in milkshake. The score for body and texture for the treatments ranged from 7.99 to 8.80. The overall acceptability for milkshake significantly ($P<0.05$) influenced by different forms of pumpkin seed in milkshake. The score for overall acceptability for A_1 , A_2 , A_3 and A_4 were 8.80, 8.35, 8.19 and 7.89, respectively.

Treatment A₁ had maximum score for overall acceptability as compared to other treatments. The sample A₄ had lowest overall acceptability score.

Based on score, use of roasted pumpkin seed powder for preparation of milkshake resulted in the maximum sensory perception. Hence, this form was selected for further study.

5.1.3.2 Selection of type of sweetener

For this purpose, three forms of sweeteners viz., 10 per cent Sugar (B₁), 0.03 per cent Natural Sweetener i.e. Stevia (B₂) and 10 per cent Honey (B₃) were taken where, selected pumpkin seed form i.e. roasted pumpkin seed powder had been kept constant at 5 per cent. In this trial, cow milk standardized for 4.5 per cent fat was taken with constant rate. On the basis of sensory evaluation most accepted sweetener was selected for further study.

The colour and appearance for milkshake significantly ($P < 0.05$) influenced by addition of different types of sweeteners in milkshake. The score for colour and appearance for treatment B₁, B₂ and B₃ ranged from 8.05 to 8.11. The score for flavour for B₁, B₂ and B₃ were 8.21, 7.78 and 7.04, respectively. Treatment B₁ had maximum score for flavour followed by treatment B₃. The score for body and texture for the sweetener B₁, B₂ and B₃ were 8.04, 7.59 and 7.82, respectively. The score for body and texture was significantly ($P < 0.05$) influenced by addition of different types of sweetener in milkshake. The overall acceptability for milkshake significantly ($P < 0.05$) influenced by addition of different types of sweeteners in milkshake. The score for overall acceptability for the sweetener B₁, B₂ and B₃ were 8.12, 7.81 and 7.65, respectively. Treatment B₁ had maximum score for overall acceptability while B₃ had lowest overall acceptability score.

Hence, it could be inferred that use of sugar for preparation of pumpkin seed milkshake resulted in the maximum sensory perception and selected for further study.

5.1.3.3 Optimization of Roasted Pumpkin Seed Powder and Sugar Level

Optimization of pumpkin seed powder and sugar level was done on the basis of sensory evaluation for that three levels of selected pumpkin seed form and two levels of selected forms of sweetener were considered. The experimental treatments were

without pumpkin seed powder in milkshake (T_0), 3 per cent roasted pumpkin seed powder + 8 per cent sugar (T_1), 3 per cent roasted pumpkin seed powder + 10 per cent sugar (T_2), 5 per cent roasted pumpkin seed powder + 8 per cent sugar (T_3), 5 per cent roasted pumpkin seed powder + 10 per cent sugar (T_4), 7 per cent roasted pumpkin seed powder + 8 per cent sugar (T_5) and 7 per cent roasted pumpkin seed powder + 10 per cent sugar (T_6).

5.1.3.3.1 Effect of Levels of Roasted Pumpkin Seed Powder and Sugar on Sensory Score of Milkshake

The mean sensory scores for colour and appearance, flavour, body and texture and overall acceptability of fresh product was significant ($P < 0.05$) due to blending of roasted pumpkin seed powder in the milkshake. The mean sensory scores for colour and appearance, flavour, body and texture and overall acceptability for most acceptable milkshake sample were 8.35, 8.53, 8.40 and 8.45, respectively.

The colour and appearance of milkshake significantly ($P < 0.05$) influenced by addition of different levels of roasted pumpkin seed powder in milkshake. The mean maximum score for colour and appearance was obtained to the treatment T_6 i.e. K_3S_2 (8.41) followed by T_5 i.e. K_3S_1 (8.38).

The mean flavour scores of milkshake were as 7.92, 5.15, 8.11, 8.23, 8.19, 8.53 and 8.31 for treatments T_0 , T_1 , T_2 , T_3 , T_4 , T_5 and T_6 , respectively. The flavour of milkshake significantly ($P < 0.05$) affected by addition of different levels of roasted pumpkin seed powder and sugar in the milkshake. The treatment T_5 with 7 per cent roasted pumpkin seed powder and 8 per cent sugar was superior over other treatment combinations.

The average scores for body and texture attribute of pumpkin seed milkshake prepared under each treatment ranged between 7.96 to 8.42 which were significantly ($P < 0.05$) changed by addition of different levels of roasted pumpkin seed powder in milkshake. The treatment T_5 and T_6 had better body and texture as compared to the other treatments.

The score for overall acceptability of milkshake prepared using different levels of roasted pumpkin seed powder for treatments T_0 , T_1 , T_2 , T_3 , T_4 , T_5 and T_6 were 7.83, 8.11, 8.10, 8.27, 8.24, 8.47 and 8.29, respectively. The effect of level of pumpkin

seed powder and sugar on overall acceptability was significant ($P < 0.05$). The treatment T_5 had better overall acceptability as compared to other treatments.

5.1.3.4 Effect of Levels of Roasted Pumpkin Seed Powder and Sugar on Physico-chemical Composition of Milkshake

The average fat content of pumpkin seed milkshake were 3.40, 3.50, 3.46, 3.97, 3.92, 4.12 and 4.03 per cent for treatment T_0 , T_1 , T_2 , T_3 , T_4 , T_5 and T_6 , respectively. The fat content in milkshake were significantly ($P < 0.05$) differed by addition of roasted pumpkin seed powder at different level. The treatment T_0 had the lowest fat content (3.40 %) whereas the treatment T_5 (K_3S_1) had maximum fat content (4.12 %).

The values of protein content of milkshake ranged from 3.02 to 3.22 per cent for treatment T_0 to T_6 , respectively. While, Treatment T_5 (K_3S_1) had highest protein content (3.22 %). Though variation in protein content was in narrow range however, interaction effect of pumpkin seed powder and sugar were significant ($P < 0.05$).

The carbohydrate content of pumpkin seed milkshake increased from T_0 to T_6 . The carbohydrate content in obtained milkshake was significantly ($P < 0.05$) increased. The treatment T_0 had the lowest carbohydrate content (10.37 %) whereas value of carbohydrate content of T_6 i.e. K_3S_2 (11.62 %) is superior over other treatments.

The crude fiber content of milkshake was 0.65, 0.64, 1.01, 1.00, 1.45 and 1.46 per cent for the treatments T_1 , T_2 , T_3 , T_4 , T_5 and T_6 , respectively. Crude fiber was not detected in T_0 while minimum value of crude fiber content observed in T_2 (0.64 %) while maximum in T_6 (1.46 %) which was at par with T_5 (1.45 %).

The ash values for treatments T_0 to T_6 ranged from 0.77 to 1.16 per cent and significantly ($P < 0.05$) inclined by addition of pumpkin seed powder. Treatments T_1 and T_2 , T_3 and T_4 as well as T_5 and T_6 were at par with each other. Treatment T_6 i.e. K_3S_2 found significantly superior over other treatments.

The total solids in pumpkin seed milkshake ranged between 17.56 to 21.43 per cent. The intermediate value for T_3 and T_4 was recorded as 20.48 and 20.47 per cent. The lowest value recorded for T_0 and that of highest value for T_6 (K_3S_2) was observed as 21.43 per cent. T_5 and T_6 were at par with each other.

The values for acidity of milkshake were 0.14, 0.14, 0.14, 0.13, 0.12, 0.12 and 0.12 per cent for treatments T_0 , T_1 , T_2 , T_3 , T_4 , T_5 and T_6 , respectively. The

intermediate value for acidity (0.13 % LA) was observed in the treatment T₃. The acidity of milkshake affected non-significantly (P<0.05).

The pH values for the treatment T₀, T₁, T₂, T₃, T₄, T₅ and T₆ were 6.28, 6.30, 6.32, 6.43, 6.44, 6.49 and 6.50, respectively. The pH values of milkshake significantly (P<0.05) inclined by addition of pumpkin seed powder and sugar. The maximum incline in pH value (6.50) was observed in milkshake prepared using 7 per cent pumpkin seed powder.

The control milkshake had mineral content 9.4 mg/100g calcium, 4.12mg/100g magnesium, 3.02 mg/100g zinc, 0.8 mg/100g iron, 5.26 mg/100g potassium and 11.31 mg/100g phosphorus. All the minerals were found to improved with addition of roasted pumpkin seed powder. Respective mineral composition for calcium, magnesium, zinc, iron, potassium as well as phosphorus were 10.37, 5.47, 5.44, 1.13, 6.27, 11.95 mg/100g for treatment T₅ and 10.38, 5.50, 5.45, 1.14, 6.28 and 11.96 mg/100g for treatment T₆ which had 7 per cent roasted pumpkin seed powder. The maximum mineral content found in treatment T₅ and T₆ which having 7 per cent roasted pumpkin seed powder and 8 per cent sugar.

5.1.3.5 Effect of Levels of Roasted Pumpkin Seed Powder and Sugar on Microbial Quality of Milkshake

The SPC for the milkshake samples T₀, T₁, T₂, T₃, T₄, T₅ and T₆ were 1.12, 1.14, 1.16, 1.17, 1.17, 1.19 and 1.21×10^5 cfu/ml, respectively. No yeast and mould and coliform count were observed in the fresh samples of pumpkin seed powder added milkshake.

5.1.3.6 Comparison of Physico-chemical Composition of Optimized Milkshake Blended with Roasted Pumpkin Seed Powder

The optimized pumpkin seed milkshake prepared by using 7 per cent roasted pumpkin seed powder and 8 per cent sugar had 4.12 per cent fat, 3.22 per cent protein, 11.48 per cent carbohydrate, 1.45 per cent crude fiber, 1.15 per cent ash, 21.42 per cent total solid, 0.12 per cent acidity and 6.49 pH and Calcium 10.37, Magnesium 5.47, Zinc 5.44, Iron 1.13, Potassium 6.27 and Phosphorous 11.95 mg/100g.

5.1.4 Shelf Life Study of Milkshake

The optimized sample was kept at refrigeration temperature ($5 \pm 1^{\circ}\text{C}$) and compared with controlled sample of milkshake. The samples were evaluated for sensory, chemical and microbiological qualities during storage at an interval of 3 days till the product was sensorily accepted.

5.1.4.1 Changes in Sensory Score of Milkshake Stored at $5 \pm 1^{\circ}\text{C}$

On first day, score for colour and appearance were 7.61 for control and 8.38 for optimized pumpkin seed milkshake. As storage progressed, the colour and appearance scores were decreased from 7.61 to 6.54 and 8.38 to 6.92 in T_0 and T_1 , respectively.

The score for flavour for stored milkshake sample was decreased significantly ($P < 0.05$) during storage at $5 \pm 1^{\circ}\text{C}$ for 9 days. The score for flavour was found to be decreased from 7.92 to 6.87 and 8.53 to 6.98 in T_0 and T_1 , respectively. Despite decreasing trends, the samples had score rated between 6 to 7 was “liked slightly” to “liked moderately” on 9th days of storage.

The score for body and texture of obtained milkshake samples were significantly (< 0.05) influenced during storage of 9 days period at $5 \pm 1^{\circ}\text{C}$, respectively. The data showed that score for body and texture were found to be decreased from 7.96 to 6.60 and 8.40 to 7.03 in T_0 and T_1 , respectively. The rate of decline in body and texture scores for control milkshake (T_0) is slightly higher than pumpkin seed milkshake (T_1) on 9th day of storage.

The overall acceptability of milkshake samples were significantly (< 0.05) decrease during storage period of 9 days at $5 \pm 1^{\circ}\text{C}$, respectively. During storage the score for overall acceptability of obtained milk shake were acceptable up to 9th days when stored at $5 \pm 1^{\circ}\text{C}$ temperature. The data showed that score for overall acceptability were found to be decreased from 7.83 to 6.70 and 8.45 to 6.98 in T_0 and T_1 , respectively.

5.1.4.2 Changes in Physico-chemical Composition of Milkshake stored at $5 \pm 1^{\circ}\text{C}$

The fat content of milkshake samples significantly (< 0.05) declined during storage of 9th days at $5 \pm 1^{\circ}\text{C}$. The data showed that fat were found to be decreased from 3.40 to 3.16 per cent and 4.12 to 3.85 per cent in T_0 and T_1 , respectively.

The protein content of milkshake samples which were stored significantly (<0.05) decline during storage period of 9 days at $5 \pm 1^{\circ}\text{C}$. The data showed that protein were found to be decreased from 3.02 to 2.68 per cent and 3.22 to 2.94 per cent in T_0 and T_1 , respectively.

The carbohydrate content of milkshake blended with pumpkin seed significantly (<0.05) decreased during storage period of 9 days at $5 \pm 1^{\circ}\text{C}$. The data showed that carbohydrate was found to be decreased slightly from 10.37 to 10.02 per cent and 11.48 to 11.10 per cent in T_0 and T_1 , respectively.

Crude fiber was not detected in controlled sample (T_0) while decreased from 1.45 to 1.35 per cent in optimized sample (T_1) during 3, 6 and 9 days of storage. Significant (<0.05) differences were found towards quantum of crude fiber degradation.

The effect of storage period and treatment was significant (<0.05) while their interaction effect is non-significant (<0.05). There was slightly decrease in ash content of samples from 0.78 to 0.71 per cent in T_0 and 1.15 to 1.08 per cent in T_1 during 9 days of storage.

The total solid content of milkshake samples were significantly (<0.05) declined during storage at $5 \pm 1^{\circ}\text{C}$. The total solid were found to be decreased from 17.56 to 16.57 per cent and 21.42 to 20.32 per cent in T_0 and T_1 , respectively.

The data revealed that acidity was found to be increased from 0.14 to 0.18 and 0.12 to 0.17 in T_0 and T_1 respectively. The effect of storage period and treatment was statistically significant (<0.05) but interaction was non-significant (<0.05) for acidity of milk shake.

Initially, values for pH of samples T_0 and T_1 were 6.28 and 6.49, respectively but later on pH decreased to 6.02 and 6.24 for T_0 and T_1 , respectively on 9th day of storage. The effect of storage period and treatment was statistically significant (<0.05) but their interaction was non-significant (<0.05).

The mineral content (mg/100g) of milkshake samples was significantly (<0.05) influenced during storage of 9 days period at $5 \pm 1^{\circ}\text{C}$. During storage the Calcium, Magnesium, Zinc, Iron, Potassium and Phosphorus content decreased from 9.41 to 8.65, 4.12 to 3.95, 3.02 to 2.69, 2.56 to 2.34, 5.26 to 4.47 and 11.31 to 10.74, respectively for controlled treatment T_0 at $5 \pm 1^{\circ}\text{C}$ while, from 10.37 to 9.82, 5.47 to

5.15, 5.44 to 4.88, 3.88 to 3.53, 6.27 to 5.36 and 11.94 to 10.45 for treatment T₁ at 5 ± 1⁰C.

5.1.4.3 Changes in Microbial Quality of Milkshake Stored at 5 ± 1⁰C

The SPC of milkshake samples were significantly (P<0.05) increased during storage at 5 ± 1⁰C. It was 1.12 and 1.19 × 10⁵ cfu/ml initially while 2.16 and 2.35 × 10⁵ cfu/ml at 9th day for T₀ and T₁, respectively.

There were no growth of yeast and mould in the fresh product upto 3rd day both in controlled and optimized milkshake while, it was 1.27 and 1.52 × 10² cfu/ml on 6th day and 1.51 and 1.74 × 10² cfu/ml on 9th day for T₀ and T₁, respectively. Interaction was statistically significant (P<0.05).

During storage period of 9th days no coliform growth was observed. It indicates that the proper hygienic conditions were followed throughout the manufacture of milkshake samples.

5.1.5 Consumer Acceptance Study of Milkshake

Consumer Acceptance Study of “Development of milkshake blended with pumpkin seed (*Cucurbita moschata*)” provided valuable information and encouraging results which indicate that the developed product was better. The optimized milkshake blended with roasted pumpkin seed powder was offered to consumers of different age, education, occupation and family income and requested them to indicate their observations about the product. They rated the product between “very good” to “excellent”.

5.1.6 Cost of Production of Milkshake

The cost of production of experimental milkshake blended with roasted pumpkin seed powder was found Rs. 119.32/lit.

5.2 Conclusion

From the present study it was concluded that

1. Among the different compositional variables tried, the best quality milkshake blended with pumpkin seed can be prepared by using 7 per cent roasted pumpkin seed powder and 8 per cent sugar of milk and it was liked very much by the panel of semi-trained judges.

2. The milkshake blended with pumpkin seed (7 per cent roasted pumpkin seed powder and 8 per cent sugar) had physico-chemical composition as 4.12 per cent fat, 3.22 per cent protein, 11.48 per cent carbohydrate, 1.45 per cent crude fiber, 1.15 per cent ash, 21.42 per cent total solid, 0.12 per cent acidity and 6.49 pH and Calcium 10.37, Magnesium 5.47, Zinc 5.44, Iron 1.13, Potassium 6.27, Phosphorous 11.95 mg/100g. The mean SPC was 1.19×10^5 cfu/ml for optimized milkshake. YMC and CC was not detected in the fresh sample of optimized milkshake.
3. The milkshake blended with 7 per cent roasted pumpkin seed powder and 8 per cent sugar had shelf life of 9 days at $5 \pm 1^{\circ}\text{C}$. During storage the fat, protein, carbohydrate, crude fiber, ash and total solid and mineral content viz., calcium, magnesium, zinc, iron, potassium and phosphorous decreased with increase in storage period. While, acidity increased with decreased pH. The SPC and YMC increased as storage period progressed while, no colliform growth was observed during 9th days of storage.
4. Consumer as a whole rated the product as 'very good' to 'excellent'.
5. The cost of production of experimental milkshake blended with roasted pumpkin seed powder was found Rs. 119.32/lit.

6. LITERATURE CITED

- Abd El-Ghany, M. A., Dalia, A. Hafez and Soha, M. Sameh El-Safty 2010. Biological study on the effect of pumpkin seeds and zinc on reproductive potential of male rats. *Proc 5th Arab and 2nd International Annual Scientific Conf.* Mansoura University, Egypt. pp 2384-2404.
- Abuelgassim, O. and Al-Showayman, I.A. 2012. The effect of pumpkin seeds (*Cucurbita pepo L.*) and L-arginine supplementation on serum lipid concentrations in atherogenic rats. *Afr Journal Tradit Complement Altern Med.* **9**: 131-37.
- Adegboye, A.R., Christensen, L.B., Holm.Pedersen, P., Avlund, K., Boucher, B.J. and Heitmann, B.L. 2012. Intake of dairy products in relation to periodontitis in older Danish adults. *Nutrients.* **4**: 1219-1229.
- Ajmal, M., Nadeem, M. and Imran, M. 2018. Impact of immediate and delayed chilling of raw milk on chemical changes in lipid fraction of pasteurized milk. *Lipids Health Dis.* **17**:190.
- Al-Haq, M.I. and Mottyuddin, G. 1996. Commercial manufacture of pasteurized mango fruit flavoured milk-based beverages. *Dairy Sci. Abstr.* **57**(4):1958.
- Amerine, M. A., Pangborn, R. M. and Roesster, E. B. 1975. Principles of Sensory Evaluation of Food. Academic press, INC, New York, USA.
- Amin, A.I. and Ahmed 1997. Effect of hot dry summer tropical climate on forage intake and milk yield in Holstein–Friesian and indigenous zebu cows in Sudan. *Journal of Arid Environments.* **35**: 737–745.
- Aneja, R.P., Mathur, B.N., Chandan, R.C., Banerjee, A.K. 2002. Technology of Indian Milk Products. A Dairy India Publication, Delhi, India. 126-127.
- Anne Brockhoff, Caroline, H., Frauke, S., Wolfgang, M. 2012. Human psychometric and taste receptor responses to steviol glycosides, *Journal of Agricultural and Food Chemistry.* **60**(27):157-170.
- A.O.A.C. 1995. Official methods of analysis, 16th Edition. Association of Official Analytical Chemists; Washington, D.C., U.S.A.
- A.O.A.C. 2000. Official methods of Analysis of the Association of Official Analytical Chemists; Washington, D.C., U.S.A.
- A.O.A.C. 2016. Official methods of Analysis of the Association of Official Analytical

Chemists; Washington, D.C., U.S.A.

- Atuonwu, A.C. and Akobundu, E.N.T. 2010. Nutritional and sensory quality of cookies supplemented with defatted pumpkin (*Cucubita pepo*) seed flour. *Pak Journal Nutr.* **9**:672- 77.
- Bakane, P.H., Khakare., M.M., Gajabe, M.H. and Khedkar, M.B. 2016. Standardization of process for custard apple milkshake. *Inter. J. of Env. and Agril. and Biotech.* **1**(4):708 712.
- Barakat, L.A. and Mahmoud, R.H. 2011. The anti-atherogenic, renal protective and immunomodulatory effects of purslane, pumpkin and flax seeds on hypercholesterolemic rats. *North American Journal of Medical Sciences.* **3**(9):411-417.
- Barbara, S. and Michael, M. 2004.Changes in chemical composition of pumpkin seeds during the roasting process for production of pumpkin seed oil. *Food Chemistry.* **84** (3):367-374.
- Barnard, S.E., Smeltz, R., Miller, B. and Pipenhagen, A. 1995. Vanilla and other flavoured milk shakes purchased in consumer containers from fast food outlets in pennsylvania. *Dairy Food and Environmental Sanitation.* **16**(2):81-83.
- Bhandari, V., Ladkani, B.G. and Muley, M.G. 1976. Utilization of filled milk in flavoured milk production. *J. Food Sci. Tech.* **13**(4):199.
- Bhosale, K.S. 2013. Utilization of ramphal pulp in preparation of milkshake. M.Sc (Agri.) Thesis submitted to M.K.V., Parbhani.
- Bialek, M., Rutkowska, J., Adamska, A. and Bajdalow, E. 2016. Partial replacement of wheat flour with pumpkin seed flour in muffins offered to children. *CyTA- Journal Food.* **14**:391- 98.
- Bonjour, J.P. 2005. Dietary protein: an essential nutrient for bone health. *Journal Am. Coll. Nutr.* **24**:526S–536S.
- Burrington, K.J. 1999. Dairy Ingredients for health. *Food Product Design.* pp. 1-7.
- Chapman, H.D. and Pratt, P.F. 1961. Methods of analysis for soils, plants and waters. University of California, Los Angeles. **60-61** : 150-179.

- Comi, G., Meillani, M., Cittario, B., Manzeno, M. and Bolani, L. 1993. Bacteriological quality of milkshakes. *Microbiologic Eliminetals Nutrition*. **51**(50) : 37-42.
- Davies, J.E., Freed, V. and Whittemore F.W. 1986. An agro medical approach to pesticide management. Coral Gables, USA: University of Miami School of Medicine Washington DC.
- Denka, Z., Stefanova, D., Chochkov, R. and Ivanova, P. 2022. Study on the impact of pumpkin seed flour on mineral content of wheat bread. *Food Science and Applied Biotechnology*. **5**:131-139.
- Devi Ningthoujam Manda. 2018. Production technologies for value added products from pumpkin seeds. Doctoral Thesis submitted to College of Food Processing Technology and Bioenergy, Anand Agricultural University, Anand.
- Dhiman, A., Bavita, K., Attri, S. and Ramachandran, P. 2018. Preparation of pumpkin powder and pumpkin seed kernel powder for supplementation in weaning mix and cookies. *International Journal of Chemical Studies*. **6**:167-175.
- El-Adawy, T.A. and Taha, K.M. 2001. Characteristics and composition of different seed oils and flours. *Food Chem*. **74**:47-54.
- El-Deremy, M. E. 2011. Evaluation of physico–chemical properties of toast breads fortified with pumpkin (*Curcubita moschata*) flour. *The 6th Arab and 3rd Int. Annual Scientif Conf., Egypt*.
- Elinge, C.M., Muhammad A., Atiku, F.A., Itodu, A.U., Peni, I. J., Sanni, O.M. and Mbongo, A.N. 2012. Proximate, mineral and anti-nutrient composition of pumpkin (*Cucurbita pepo L.*) seeds extract. *Int Journal Plant Res*. **2** : 146-50.
- El-Mosallamy, A.E., Sleem, A.A., Abdel Salam, O.M., Shaffie, N. and Kenawy, S.A. 2012. Antihypertensive and cardioprotective effects of pumpkin seed oil. *Journal of Medicinal Food*. **15**(2) : 180-189.
- El-Soukkary, F. A. 2001. Evaluation of pumpkin seed products for bread fortification. *Plants Foods Hum Nutr*. **56** : 365-84.
- Enayat, U., Cittariojhabib, J.M., Rinthaler, F.F., Mascher, F., Pfeiffer, K. and Sirel, W. 1990. Outpatient treatment of children with protein energy malnutrition

- (pem) in the area of cairo. *Mitteilungen Der Asterreichischen Gessellschaft For Tropenmedizin and Parasitologie*. **12** : 43-50.
- Fagemi, T.N., Oshodi, A.A. and Ipinmoroti, K.O. 2005. Processing effects on some antinutritional factors and in vitro multi enzyme protein digestibility (ivpd) of three tropical seeds: breadnut, cashue nut and fluted pumpkin. *Pakistan Journal Nutr*. **4** : 250-56.
- Fahim, A.T., Abd-el Fattah, A.A., Agha, A.M. and Gad, M.Z. 1995. Effect of pumpkin seed oil on the levels of free radical scavengers induced during adjuvant-arthritis in rats. *Pharmacol Res*. **31**:73-79.
- Franzoi, M., Niero, G., Penasa, M., Cassandro, M. and De Marchi, M. 2017. Development and validation of a new method for the quantification of soluble and micellar calcium, magnesium and potassium in milk. *J. Dairy Sci*. **101**:1883–1888.
- Giami, S.Y., Achinewhu, S.C. and Ibaakee, C. 2005. The quality and sensory attributes of cookies supplemented with fluted pumpkin (*Telfairia occidentalis* Hook) seed flour. *Int Journal Food Sci Technol*. **40** : 613-20.
- GOI, Annual Report 2022-23, Department of Animal Husbandry and Dairying, GOI.
- Gonzalez, M.A., Ferrero, B. and Cabezudo, M.D. 2000. Perfecting vanilla milk shakes using the response surface methodology (rsm), *Alimentacion, Equipos, Y. Tecnologia*. **19**(6) : 77-80.
- Gossell-Williams, M., Davis, A. and O'Connor, N. 2006. Inhibition of testosterone-induced hyperplasia of the prostate of Sprague-Dawley rats by pumpkin seed oil. *J. Med. Food*. **9** : 284-86.
- Gossell-Williams, M., Hyde, C., Hunter, T., Simms-Stewart, D., Fletcher, H., Mcgrowder, D. and Walters, C.A. 2011. Improvement in HDL cholesterol in postmenopausal women supplemented with pumpkin seed oil: pilot study. *Climacteric*. **14** : 558-64.
- Gossell-Williams, M., Lyttle, K., Clarke, T., Gardner, M. and Simon, O. 2008. Supplementation with pumpkin seed oil improves plasma lipid profile and cardiovascular outcomes of female non-ovariectomized and ovariectomized sprague-dawley rats. *Phytother Res*. **22** : 873-77.

- Guetouache, M., Guessas, B. and Medjekal, S. 2014. Composition and nutritional value of raw milk. *Journal Issues*. **2**(10) : 115-122.
- Habib, A., Biswas, S., Siddique, A. H., Manirujjaman, M., Uddin, B., Hasan, S., Khan, M. M. H., Uddin, M., Islam, M. and Hasan, M. 2015. Nutritional and lipid composition analysis of pumpkin seed (*Cucurbita maxima* Linn.). *Journal of Nutritional Food Science*. **5** : 374-379.
- Hamed, S.Y., El-Hassan, N.M., Hassan, A.B, Eltayed, M.M. and Babikar, E.E. 2008. Nutritional evaluation and physiochemical properties of processed pumpkin (*telfairia occidentalis* hook) seed flour. *Pak Journal Nutr*. **7** : 330-34.
- Haug, A., Høstmark, A.T. and Harstad, O.M. 2007. Bovine milk in human nutrition- A review. *Lipids Health Dis*. **6** : 1-16.
- Hemanth, M., Deshpande, H.W., Katke, S.D., Machewad, G.M. and Gangakhedkar, P.S. 2021. Studies on process standardization of milkshake by using acid modified Psyllium husk. *The Pharma Innovation Journal*. **10**(7) : 1215-1218
- Hilton, D. and Lewis, M. 2016. Protein stability in sterilised milk and milk products. *Advance Dairy Chemistry*. pp. 247-286.
- Holsinger, V.H., Smith, P.W., Talley, F.B., Edmundson, L.E. Tobas, J. 1987. Preparation and evaluation of chocolate flavoured shakes of reduced sweetener content. *J. Dairy. Sci*. **70**(6) : 115-167.
- Hudson, C., Hudson, S. and Mackenzie, J. 2007. Protein-source tryptophan as an efficacious treatment for social anxiety disorder: a pilot study. *Can Journal Physiol Pharmacol*. **85** : 928- 932.
- IS: 1165. 1967. Specification for condensed milk. Indian Standard Institution, Manak Bhawan, New Delhi.
- IS: 1166. (Part-II). 1973. Specification for condensed milk. Indian Standards Institute, Manak Bhavan, New Delhi.
- IS: 1224. (Part-II). 1977. Determination of Fat by Gerber Method. Indian Standard Institution, Manak Bhavan, New Delhi, India.

- IS: 1465. 1967. Specification for condensed milk. Indian Standard Institution, Manak Bhawan, New Delhi.
- IS: 1479 (Part-II). 1961. Methods of tests for dairy industry. Chemical analysis of milk.
- IS: 1479. (Part-I) 1960. Method for test in dairy industry. Rapid estimation of milk. Indian Standards Institute, Manak Bhawan, New Delhi.
- IS: 1479. (Part-II). 1961. Methods of tests for dairy industry. Chemical analysis of milk. Indian Standards Institute, Manak Bhawan, New Delhi.
- IS: 5403. 1969. Indian Standard method for yeast and mould count in food stuff. Indian Standards Institution, Manak Bhawan, New Delhi.
- IS: 5404. 1969. Indian Standard method for Coliform count in food stuff. Indian Standards Institution, Manak Bhawan, New Delhi.
- Jadhav, V.S., Awaz, H.B., Patil, G.R. and Thombre, B.M. 2003. Studies on preparation of sapota milk shake. *Journal Maharashtra Agric. Univ.* **27** (3) :306-308
- Jian, L., Du, C.J., Lee, A.H. and Binns, C.W. 2005. Do dietary lycopene and other carotenoids protect against prostate cancer? *International Journal of Cancer.* **113**(6) : 1010-1014.
- Kadav, V.B. 2001. Preparation of fruit flavoured milk shake. M.sc. (agri.) Thesis submitted to Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli and Dist. Ratnagiri.
- Kalogeropoulos, N., Chiou, A., Ioannou, M. and Karathanos, V. 2013. Nutritional evaluation and health promoting activities of nuts and seeds cultivated in Greece. *International Journal of Food Sciences and Nutrition.* **64**(10) : 3109.
- Kanwal, S., Raza, S., Naseem, K., Amjad, M., Naseem, B. and Gillani, M. 2015. Development physico-chemical and sensory properties of biscuits supplemented with pumpkin seeds to combat malnutrition in Pakistan. *Pakistan Journal Agric. Res.* **28** : 400-405.
- Karki, A., Yadav, R.S. and Banerjee, J. 2015. Development of fibre enriched milkshakes and its quality evaluation. *Inter. J. of advanced Engg. And Tech. and Innovative Sci.* **1**(2):14-20.

- Kashid, U.B. 2005. Preparation of golden milk shake from cow milk blended with safflower milk. M.Sc. (Agri.) Thesis submitted to M.K.V. Parbhani, India.
- Kaur, M. 2017. Development and nutritional evaluation of pumpkin seed (*Cucurbita moschata*) supplemented products. M.Sc. Thesis, Punjab Agriculture University, Ludhiana.
- Khan, M.L. and Lynch, R.J. 1985. Freezer staple whipped ice-cream and milk shake milk products, United States Patent US, 4:552- 773.
- Kholif, A.M., Abo El-Nor, S.A.H. and Abou-Arab, A.A.K. 1994. Effect of spraying diazinon to control the external parasites on the productive performance of dairy animals. I. Yield and composition of buffalo's and Friesian cow's milks. *Egypt Journal Dairy Sci.* **22**:145–154.
- Kim, M.Y., Kim, E.J., Kim, Y.J., Choi, C. and Lee, B.H. 2012. Comparison of the chemical compositions and nutritive values of various pumpkin (Cucurbitaceae) species and parts. *Nutrition Research Practices.* **6**(1): 21-27.
- Kshirsagar, D.V. 1996. Studies on blending of milk shake with different fruit flavouring agents. M.Sc. (Agri.) Thesis, submitted to Dr. BSKKV, Dapoli, Dist. Ratnagiri.
- Kuchekar, D.D. 2011. Studies on preparation of almond milkshake. M.Sc (Agri.) Thesis submitted to M.K.V. Parbhani.
- Laxminarayana, G.S., Bikash, C.G. and Kulkarni, S. 1997. Technology of ready to use banana milk shake powder. *J. Food Sci. Tech.* **34**(1): 41-45.
- Lee, Y.K., Chung, W.I. and Ezura, H. 2003. Efficient Plant Regeneration via Organogenesis in Winter Squash (*Cucurbita maxima Duch*). *Plant Sciences.* **164**(3) : 413-418.
- Lim, T.K. 2012. Edible medicinal and non-medicinal plants. Springer Dordrecht Heidelberg, New York. **67** : 272-76.
- Liu Fayuan, 2015. Patent citation on pumpkin seed oil, china, CN105231385A.
- Mahuya, B., Chakraborty, R. and Utpal, R. 2008. Effect of beet and honey on quality improvement and carotene retention in a carrot fortified milk product. *Innovative Food Science & Emerging Technologies.* **9** : 9-17.

- Makni, M., Fetoui, H., Gargouri, N. K., El-Garoui, M. and Zeghal, N. 2011. Antidiabetic effect of flax and pumpkin seed mixture powder: effect on hyperlipidemia and antioxidant status in alloxan diabetic rats. *J. Diabetes Complicat.* **25**: 339-45.
- Makni, M., Sefi, M., Fetoui, H., El Garoui, M. and Garouri, M.K. 2010. Flax and pumpkin seeds mixture ameliorates diabetic nephropathy in rats. *Food Chemistry and Toxicology.* **48**(8-9) : 2407-2412.
- Makni, M., Sefi, M., Fetoui, H., Garoui, E.M., Gargouri, N.K., Boudawara, T. and Zeghal, N. 2010. Flax and Pumpkin seeds mixture ameliorates diabetic nephropathy in rats. *Food Chem Toxicol.* **48** : 2407–2412.
- Mani, V. 2011. Compendium of lectures winter school on chemical analysis of value added dairy products and their quality assurance. pp. 225-229.
- Mbondi, J.K. 2013. Formulation and evaluation of pumpkin seed (*Cucurbita pepo*) tablets. (retrieved from <http://hdl.handle.net/11295/94920>).
- Mengyao Peng, Dan Lu, Jie Liu, Bo Jiang and Jingjing Chen. 2021. Effect of Roasting on the Antioxidant Activity, Phenolic Composition and Nutritional Quality of Pumpkin (*Cucurbita pepo L.*) Seeds. *Front. Nutr., Sec. Nutrition and Food Science Technology.* **8** : 647354.
- Mhetre, S. 2019. Preparation of gulkand flavoured milk. M.Sc. (Agri.) Thesis submitted to M.P.K.V., Rahuri.
- Milovanoic, M.M., Demin, M.A., Vucelic-Radovic, B.V., Zarkovic, B.M. and Stikic, R.I. 2014. Evaluation of the nutritional quality of wheat bread prepared with quinoa, buckwheat and pumpkin seed blends. *J Agric Sci.* **59**: 319-28.
- Mittal, S. and Bajwa, U. 2014. Effect of heat treatment on the storage stability of low calorie milk drinks. *Journal Food Sci Technol.* **51**(9):1875-83.
- More, D.D. 2016. Preoation of aloevera added custard apple milkshake. M.Sc. Thesis submitted to M.P.K.V., Rahuri.
- Muehlhoff, E., Bennett, A. and McMahon, D. 2013. Milk and Dairy Products in Human Nutrition; Food and Agriculture Organization of the United Nations: Rome, Italy.

- Mule, P.R. 2012. Preparation of *milkshake* blended with fig. M.Sc (Agri.) Thesis submitted to M.K.V., Parbhani.
- Murphy, M.M., Douglass, J.S., Johnson, R.K. and Spence, L.A. 2008. Drinking flavored or plain milk is positively associated with nutrient intake and is not associated with adverse effects on weight status in US children and adolescents. *J.American Dietetic Association*. **108** (4): 631-639.
- Naghii, M.R. and Mofid, M. 2007. Impact of daily consumption of iron fortified ready-to-eat cereal and pumpkin seed kernels (*Cucurbita pepo*) on serum iron in adult women. *Biofactors*. **30**(1): 19-26.
- Neeta Kumari. 2020. Processing of Pumpkin seeds and their utilization in product development. Doctoral thesis submitted to CCS Haryana Agricultural University, Hissar.
- NHB, 2022. Third advance estimate of area and production of horticultural crops, Horticultural statistics at glance, National Horticulture Board, India.
- Nkosi, C.Z, Opoku, A.R. and Terblanche, S.E. 2006. Antioxidative effects of pumpkin seed (*Cucurbita pepo*) protein isolate in CCl₄-induced liver injury in low-protein fed rats. *Phytother Res*. **20**(11):935-40.
- Nolan, A.L. 1982. Great shakes from dry mix. *Food Engineering*. **54**(7):63.
- Norfezah, M.N., Hardacre, A. and Brennan, C.S. 2011. Comparison of waste pumpkin material and its potential use in extruded snack foods. *Food Sci Technol Int*. **17**: 367-73.
- Nyam, K.L., Lau, M. and Tan, C.P. 2013. Fibre from pumpkin (*Cucurbita pepo* L.) Seeds and rinds: PHYSICO-chemical properties, antioxidant capacity and application as bakery product ingredients. *Mal Journal Nutr*. **19**: 99-109.
- Paklwad, S. T., Awaz, H. B., Pawar, S. L. and Paul, S. P.2010. Preparation and sensory evaluation of papaya milkshake. *Veterinary World*. **3**(4): 185-187.
- Park, Y.W. 2009. Introduction: Overview of bioactive components in milk and dairy products. In *Bioactive Components in Milk and Dairy Products*; Park, Y.W., Ed.; Publishers, Wiley- Blackwell: Ames, Iowa; Oxford, UK. pp. 3–14.

- Patel, A.S., Bariya, A.R., Ghodasara, S.N., Chavda, J.A. and Patil, S.S. 2020. Total carotene content and quality characteristics of pumpkin flavoured buffalo milk. *Heliyon*. **6(7)**: e04509.
- Patil, A.M. 1984. Studies on biochemical, nutritional and keeping quality of banana milk shake. M.Sc. (Agri.) Thesis submitted to M.K.V., Parbhani.
- Patil, D.T. 1983. Studies on physico-chemical and rheological properties of banana milk beverage. M.Sc. (Agri.) Thesis submitted to M.K.V., Parbhani.
- Patil, S.R., Patil, N.J. and Sahoo, A.K. 2019. Process optimization, sensory evaluation and physico-chemical analysis of carrot milkshake. *Journal of Emerging Technologies and Innovative Research*. **6 (6)** : 248-255.
- Patil, V.P. 2018. Preparation of mango herbal quarg type cheese. M.Sc. (Agri.) thesis submitted to M.P.K.V., Rahuri.
- Pereira, P.C. 2014. Milk Nutritional Composition and Its Role in Human Health. *Nutrition*. **30**: 619-627.
- Pfeuffer, M. and Watzl, B. 2017. Nutrition and health aspects of milk and dairy products and their ingredients. *Ernahr. Umschau Sci. Res.* **65**: 22–33.
- Planovskii, A.A. and Golovach, V.P. 1988. Manufacture of dried skim milk with apple juice. *Journal Molochnayai Myasnaya. Promyslennost, USSR*. **3**:20-21.
- Pongjanta, J., Naulbunrang, A., Kawngdang, S., Manon, T. and Thepjaikat, T. 2006. Utilization of pumpkin powder in bakery products. *Songklanakarinn J. Sci. Technol.* **28(1)**:71-79.
- Poul, S.P., Sontakke, A.T., Munde, S.S., Adangale., A.B and Jabhav, P.B. 2009. Manufacture of custard apple milk shake. *J. Dairying, Foods and Home Set.* **28 (3/4)**: 202-205.
- Ramteke, S.P. 2017. Studies on preparation of quarg type cheese from cow milk and goat milk. M.Sc. Thesis submitted to M.P.K.V., Rahuri.
- Repate, K.C., Kamble, V.J., Hasan Bin Awaz and Thombre, B.M. 2010. Preparation of flavoured milk from cow milk blended with safflower milk. *J. Dairying, Foods and Home Sci.* **29(2)**: 92- 96.
- Revathy, M.N. and Sabitha, N. 2013. Development, quality evaluation and popularization of pumpkin seed flour incorporated bakery products. *Int Journal Food*

Nutr Sci. **2**: 40-41.

- Rezig, L., Chouaibi, M., Msaada, K. and Hamdi, S. 2012. Chemical composition and profile characterisation of pumpkin (*Cucurbita maxima*) seed oil. *Industrial Crops and Products*. **37** (1): 82-87.
- Rizzoli, R. 2014. Dairy products, yogurts and bone health. *Am. J. Clin. Nutr.* **99**: 1256S-1262S.
- Rochas, C., Metayer, M.M. and Granier, F. 1989. Ready to eat breakfast pack. French Patent Application. Fr 2 632 828 A1. pp 15.
- Rodriguez-Mirand, A. J., Hernandez-Santos, B., Herman-Lara, E., Vivar-Vera, M. A., Carmonagarcia, R., Gomez-Aldapa, C.A. and Martinez-Sanchez, C.E. 2012. Physicochemical and functional properties of whole and defatted meals from mexican (*Cucurbita pepo*) pumpkin seeds. *Int Journal Food Sci Technol.* **47**: 2297-2303.
- Ryan, E., Galvin, K., Connor, T.P., Maguire, A.R. and Brien N.M. 2007. Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains and legumes. *Plant Foods and Human Nutrition.* **62**(3): 85-919.
- Ryder, K. M., Shorr, R.I. and Bush, A.J. 2005. Magnesium intake from food and supplements is associated with bone mineral density in healthy older white subjects. *Journal Am Geriatr Soc.* **53**: 1875-80.
- Saeleaw, M. and Schleining, G. 2011. Composition, physicochemical and morphological characterization of pumpkin flour. *ICEF11- 11th International Congress on Engineering and Food "Food Process Engineering In a Changing World"*, Athens.
- Sankhla, S. and Bhatnager, S. 2006. Production of instant anjeer shake. *J. Dairying, Foods and Home Sci.* **25**(3/4): 212-217.
- See, E., Noor Aziah A. and Wan, N.W. 2007. Physico-chemical and sensory evaluation of breads supplemented with pumpkin flour. *SEAN Food Journal.* **14**(2):123-130.
- Sharma, A.K. and Gupta, S.K. 1978. Manufacture of milk shake. *Indian dairyman.* **30**(8):585.

- Shinde, D.V., Gawali, A.S., Manohar, A.B., Inamke, N.D. and Kedare, V.S. 2018. Physico chemical analysis of milkshake blending with date pulp. *The Pharma Innovation Journal*. **7**(5): 673-674.
- Shukla, P., Bajwal, U. and Bhise, S. 2017. Effect of storage on quality characteristics of sterilized mango based dairy beverage. *Int. Journal Curr. Microbiology and Applied Science*. **7**(4):1173-1182.
- Silva, J.S., Marques, T.R., Simao, A.A., Correa, A.D., Pinheiro, A.N. and Silva, R.L. 2014. Development and chemical and sensory characterization of pumpkin seed flour-based cereal bars. *Food Sci Technol*. **34**(2).
- Singh, W., Kapoor, C.M. and Srivastava, D.N. 1999. Standardization of technology for the manufacture of guava whey beverage. *Indian J. Dairy sci*. **52**(5):268
- Snedecor, G.W. and Cochran, W.G. 1967. Statistical Method. 6th Ed. Oxford and I.D.B. Pub. Co., Calcutta, India.
- SP: 18 (Part XI) 1981. Determination of Proteins by Micro-Kjeldahl method. Handbook of Food Analysis. pp. 181-182.
- SP: 18 (Part XI) 1981. Determination of total sugar. Handbook of Food Analysis. pp. 181-182.
- Srivastava, A. and Verma, A. 2014. Nutritional composition of sunflower seeds flour and nutritive value of products prepared by incorporating sunflower seeds flour. *Int. J. Pharm. Res. Allied Sci*. **3**: 45- 49.
- Surve, S. 2017. Process standardization for preparation of milk shake by incorporation of date (*Phoenix dactylifera L.*) and jiggery. Doctoral Dissertation submitted to D.B.S.K.K.V., Dapoli.
- Syed, Q. A., Akram, M. and Shukat, R. 2019. Nutritional and therapeutic importance of the pumpkin seeds. *Seed*. **21**(2): 15798–15803.
- Taware, M.G. 2002. Preparation of custard apple milkshake. M.Sc (Agri) Thesis submitted to M.P.K.V. Rahuri.
- Teugwa C.M., Boudjeko, T., Tchinda, B.T., Majiata, P.C. and Zofou, D. 2013. Antihyperglycaemic globulins from selected cucurbitaceae seeds used as antidiabetic medicinal plants in africa. *Bmc Complement Altern Med*. **13**: 63.

- Ubale, P.J., Hembade, A.S. and Choudhari, D.M. 2014. Sensory and Chemical Quality of Sapota Milkshake. *Research J. of Animal Husbandary and Dairy Sci.* **5**(2):116-121.
- Usha, R., Lakshmi, M. and Ranjani, M. 2010. Nutritional, sensory and physical analysis of pumpkin flour incorporated into weaning mix. *Malaysian Journal of Nutrition.* **16**(3): 379–387.
- Varpe, N.K. 1992. Studies on blending of milk shake with different fruit pulp. M.Sc. (Agri.) Thesis submitted to Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri.
- Verma, V. 2021. Process optimization and quality characteristics of turmeric fortified milkshake with honey as sugar replacer. M.Sc. (Veterinary) thesis submitted to A.N.D. University of Agriculture and Technology, U.P.
- Voranunt, S.S., Yarnnon, C. and Ngunboonsri, P. 1987. The effects of pumpkin seeds on oxalicytafluria and urinary compositions of children in hyperendemic area. *American Journal of Clinical Nutrition.* **45**(1): 115- 121.
- Ward, D. and Ainsworth, P. 1998. The development of a nutritious low cost weaning food for kenya infants. *Afr Journal Health Sci.* **5**: 89-95.
- Wheeler, R.J. and Gillies, A.J. 1973. Banana milk a milk fruit food. *Australian Journal Dairy Technol.* **28**(3):96-99.
- Yadav, M., Jain, S., Tomar, S., Prasad and Yadav, H. 2010. Medicinal and biological potential of pumpkin. *Nutrition Research Review.* **23**(2): 184- 190.
- Zaineddin, A.K., Buck, K., Vrieling, A., Heinz, J., Flesch-Janys, D., Linseisen, J. and Chang-Claude, J. 2012. The association between dietary lignans, phytoestrogen-rich foods and fiber intake and postmenopausal breast cancer risk: A German case-control study. *Nutr Cancer.* **64**: 652-65.

7. APPENDICES

APPENDIX - I

Evaluation of Milkshake Blended with Pumpkin Seed on 9-point hedonic scale (Evaluation Score Card)

Name of product:

Date:

Name of the evaluator:

Please Score the sample

Sample Code	Colour and Appearance	Flavour	Body and Texture	Overall Acceptability
1				
2				
3				
4				
5				
6				
7				

Signature of Judge

Maximum score 9 for each parameter

Like extremely	:	9
Like very much	:	8
Like moderately	:	7
Like slightly	:	6
Neither like or dislike	:	5
Dislike slightly	:	4
Dislike moderately	:	3
Dislike very much	:	2
Dislike extremely	:	1

Remarks:

APPENDIX-II

DEPARTMENT OF ANIMAL HUSBANDRY AND DAIRY SCIENCE, MAHATMA PHULE KRISHI VIDYAPEETH, RAHURI

Consumer Study of Milkshake Blended with Pumpkin Seed

You are requested to give your honest opinion about the product that will help us to decide its acceptability amongst randomly selected consumers across widely distributed population.

1. Name of consumer :
2. Address :
3. Age :
4. Education :
5. Occupation :
6. Annual income of family :
7. Did you like this Milkshake : Yes/ No
8. Degree of liking : i) Excellent
ii) Very good
iii) Good
iv) Fair
9. Time of *milkshake* purchasing : i) Weekly
ii) Fortnightly
iii) Monthly
iv) Rarely
v) Never
10. Would you like to drink this *milkshake* in place of conventional products , if it available at reasonable cost : Yes/No

APPENDIX-III

Composition and method for preparation of the culture media

1. Nutrient Agar (NA)

The media (Nutrient agar media) was prepared by using following ingredients in their respective quantities as follows:

Tryptophan (g)	: 5.0
Yeast extract (g)	: 2.5
Glucose (g)	: 1.0
water (ml)	: 1000
Agar (g)	: 15.0
pH (at 25 ⁰ C)	: 7

To rehydrate this medium, 23.5 g of the dry medium was suspended in 1000ml distilled water. The mixture was then boiled to dissolve the medium completely. It was then fill in the conical flask were closed with cotton plugs. The conical flask was then sterilized by autoclaving at 15 psi pressure (121⁰C) for 15 min.

2. Potato Dextrose Agar (PDA)

Infusion from white potatoes	: 200 g
Dextrose	: 20 g Distilled
Agar	: 15 g
Distilled water	: 1000 ml

Steam potato cubes for 1 hour in 500 ml water, mesh well, strain through muslin and make a filtrate to 1000 ml. pH is adjusted to 3.5 after sterilization with 10 % tartaric acid, at the time of plating.

3. Violet Red Bile Agar (VRBA)

The following ingredient were weighed accurately and dissolved in distilled water and made final volume 1000 ml and pH was adjusted to 7.4 ± 0.1 . The care was exercised not to overhead the media which would otherwise affect the selectivity of medium. Freshly prepare medium was used without sterilization.

Yeast extract	: 3 g
Peptone	: 7 g
Bile salt	: 1.5 g
Lactose	: 10 g
Sodium chloride	: 5 g
Neutral Red	: 0.03 g
Crystal Violet	: 0.002 g
Distilled water	: 1000 ml
Agar	: 15 g

8. VITAE

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MASTER OF SCIENCE (AGRICULTURE)
in
DAIRY SCIENCE
2024

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