FORMULATION OF INSTANT *KULFI* PREMIX INCORPORATED WITH MORINGA (*Moringa Oleifera*) LEAF POWDER

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THESIS

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Supervisor

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Submitted by

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Dear Sir,

I have great pleasure in forwarding the thesis entitled **"FORMULATION OF INSTANT *KULFI* PREMIX INCORPORATED WITH MORINGA (*Moringa oleifera*) LEAF POWDER"** submitted by **Mr. Shashank R. Kiran, ID. No. 22412MDT014, Enrolment No. 454445,** in the partial fulfilment of the requirements for the degree of **Master of Technology** in **Dairy Technology**, in the Department of Dairy Science & Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

I certify that the entire scheme of investigation presented herein was planned and carried out solely by the candidate under my guidance. To the best of my knowledge, the data in the thesis are genuine and original.

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Yours faithfully,

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by

Shashank R Kiran

Thesis submitted in partial fulfilment of the requirements for the degree of
MASTER OF TECHNOLOGY
(DAIRY TECHNOLOGY)

FROM

DEPARTMENT OF DAIRY SCIENCE AND FOOD TECHNOLOGY
INSTITUTE OF AGRICULTURAL SCIENCE
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Date: *……*
Place: Varanasi ***(Shashank R Kiran)***

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ABBREVIATIONS

%	:	Per cent
µm	:	Micrometre
^{0}C	:	Degree Celsius
^{0}K	:	Degree Kelvin
ANOVA	:	Analysis of variance
AOAC	:	Association of Official Analytical Chemists
aq.	:	Aqueous
CFU	:	Colony-forming unit
cm	:	Centimetre
Conc.	:	Concentration
DPPH	:	1,1-diphenyl-2-picrylhydrazyl
e.g.	:	(exempli gratia) for example
Eq.	:	Equation
et al.	:	et alia (and associates)
etc.	:	(et cetera) and the others
FFA	:	Free fatty acid
Fig.	:	Figure
FT-IR		Fourier transform infrared
g	:	Gram
h	:	Hour
HRAMS	:	High Resolution Atomic Mass Spectroscopy
i.e.	:	(ed est) that is
J	:	Joule
KBr	:	Potassium Bromate

Kcal	:	Kilocalorie
Kg	:	Kilogram
kJ	:	Kilo-Joule
KNO_3	:	Potassium Nitrate
log	:	Logarithm
m	:	Meter
m2	:	Square meter
Max.	:	Maximum
Mc	:	Moisture content
mg	:	Milligram
Min	:	Minutes
Min.	:	Minimum
Mix	:	Mixture
ml	:	Millilitre
MLP		Moringa Leaf Powder
mm	:	Millimetre
MPa	:	Mega pascals
MT	:	Million tonnes
N	:	Normality
NaOH	:	Sodium hydroxide
nm	:	Nanometre
NS	:	Non-significant
OAA	:	Overall acceptability
pH	:	Potential of Hydrogen

Chapter I

INTRODUCTION

The exact origins of *kulfi*, a rich and delicious Indian dessert, are unclear, but it has been enjoyed for centuries. *Kulfi* is traditionally made by slowly simmering milk with sugar and almonds until it becomes thick and flavourful, giving it a fudge-like texture, unlike regular ice cream.

Over time, different regions added their own flavours to *kulfi*. Cardamom, a popular Indian spice, adds a warm and floral taste, while saffron, the most expensive spice in the world, gives a luxurious aroma and bright colour. Pistachios and almonds are often included, adding a mild nutty flavour and a crunchy texture to the creamy dessert. These regional variations show the creativity and resourcefulness of Indian cooking. *Kulfi* is more than just a dessert; it's a cultural icon and a cherished memory for many generations (**Mehta *et al.*, 2018)**. It reminds people of warm summer evenings spent enjoying its slow-melting goodness. Each spoonful is a journey, starting with a smooth, creamy taste and ending with a burst of nutty richness and exotic spices. This dessert highlights the rich history of Indian culinary artistry and the importance of regional traditions. From street vendors to family gatherings, *kulfi* has always been a symbol of community and shared experiences, making it a beloved part of Indian culture **(Singh *et al.*, 2019)**.

Kulfi is a creamy, frozen treat with a rich history and is one of the most popular desserts in India. The process starts with gently simmering milk to bring out its creamy essence. Sugar adds sweetness, but *kulfi* needs more than just sweetness to be complete. Finely chopped nuts like almonds and pistachios are mixed in, adding subtle flavours and a delightful texture. Regional variations further enhance this blend. Saffron, the world's most expensive spice, adds a touch of luxury with its bright colour and honeyed aroma, while cardamom, the queen of Indian spices, brings warm and floral notes. These elements together create a dessert that is both flavourful and rich in tradition. *Kulfi* is more than just a treat; it's a cultural symbol and a lasting memory. It evokes

warm summer nights and the joy of savouring its slowly melting sweetness. Each bite offers a creamy, nutty, and spicy experience that leaves a lasting impression. This dessert showcases the creativity and tradition of Indian cuisine **(Adugna *et al.*, 2021)**.

Research on *kulfi* has the potential to change how people enjoy this traditional dessert, adding a healthy twist while maintaining its rich heritage. This blend of tradition and innovation offers a convenient and delicious treat, ensuring that the cultural legacy of *kulfi* continues for generations. This approach promises to create a product that appeals to modern tastes while honouring its cultural roots **(Fahey *et al.*, 2005)**.

Moringa oleifera, commonly known as the drumstick tree or horseradish tree, is native to India and has been used for centuries in traditional medicine. Its leaves, in particular, are known for their high nutritional value, being rich in vitamins A, C, and E, calcium, potassium, and protein. Moringa leaf powder has gained popularity in recent years as a superfood, thanks to its numerous health benefits, including anti-inflammatory and antioxidant properties **(Singh *et al.*, 2011)**.

Incorporating moringa leaf powder into instant *kulfi* premix offers an opportunity to enhance the nutritional profile of this beloved dessert. By adding moringa, the *kulfi* can provide additional vitamins and minerals, making it a healthier alternative to traditional recipes. This combination of traditional flavours with modern nutritional benefits is an innovative approach to *kulfi* formulation. The modern consumer demands convenience without compromising on taste and quality. Instant food products have become increasingly popular, as they save time and effort while providing a satisfying culinary experience. An instant *kulfi* premix incorporated with moringa leaf powder addresses this demand by offering a quick and easy way to prepare a nutritious dessert. This convenience factor can make *kulfi* more accessible to a broader audience, including those who may not have the time or skills to prepare traditional *kulfi* from scratch.

Innovation in Traditional Foods: Innovation in traditional foods involves blending time-honoured recipes with modern ingredients and techniques to create new

and exciting products. This approach helps preserve cultural heritage while meeting the evolving preferences of consumers. By incorporating moringa leaf powder into *kulfi*, we are honouring the traditional recipe while introducing a modern twist that enhances its nutritional value. This innovation can also spark interest in traditional foods among younger generations, ensuring that these cultural treasures are passed down and appreciated **(Brown *et al.,* 2018)**.

Challenges and Considerations: Developing an instant *kulfi* premix with moringa leaf powder presents several challenges. The primary challenge is achieving the right balance of flavours, ensuring that the moringa does not dominate the traditional taste of *kulfi*. Additionally, the texture and consistency of the premix must be carefully formulated to match the creamy, dense texture that is characteristic of *kulfi*. There are also considerations related to the shelf life and stability of the premix, which must be addressed to ensure the product remains fresh and palatable over time **(Pareek *et al.,* 2019)**.

This research was necessary for innovation within traditional Indian desserts, specifically *kulfi*, by improving its nutritional value without losing its familiar taste and texture. Adding moringa (*Moringa oleifera*) leaf powder in *kulfi* premix was chosen due to its high levels of antioxidants (quercetin, chlorogenic acid), minerals (iron, calcium, phosphorus), vitamins. The main goal of this study was to prepare an instant *kulfi* premix with incorporation of moringa leaf powder that aligned with modern health trends while keeping the traditional sensory qualities of *kulfi*. Preparation of instant *kulfi* premix incorporated with Moringa Leaf Powder (MLP) has never been considered by the workers as major part of their research in the country as done in present case and the impact of this product is also not known.

In this thesis entitled **"FORMULATION OF INSTANT *KULFI* PREMIX INCORPORATED WITH MORINGA *(Moringa oleifera)* LEAF POWDER"** which was performed at Department of Dairy Science and Food Technology, Banaras Hindu University, Varanasi, Uttar Pradesh, India in collaboration with Shyam Dairy Products, Prayagraj, Uttar Pradesh, India with the following objectives.

1. To formulate and optimise an instant *kulfi* premix incorporated with moringa leaf powder using sensory properties and melting rate.
2. To evaluate the physico chemical, mineral analysis, textural profile and microbial analysis of the formulated *kulfi* premix.
3. Characterization of the optimised *kulfi* premix by colour hunter, FT-IR and HRMS analysis.

Chapter II

REVIEW OF LITERATURE

Several studies have explored the formulation and production of *kulfi* using various methods. Research by **Sharma *et al.*, (2018**) investigated the use of unconventional ingredients like khoya (dried milk solids) and maltodextrin in *kulfi* making. However, incorporating fresh ingredients into a dry premix poses a challenge due to their perishable nature. Studies on incorporating flavour into other food products, such as ice cream by **Singh *et al.*, (2021)**, can provide valuable insights.

One of the most widespread adaptations involves the use of condensed milk or khoa (dried milk solids) in place of simmering full-fat milk. Condensed milk, with its pre-thickened consistency and readily available sweetness, offers a significant reduction in preparation time. Similarly, khoa, a shelf-stable product made from evaporated whole milk, allows for quicker preparation compared to the traditional method. Studies by **Sharma *et al.*, (2018)** explored the use of unconventional ingredients like khoa in *kulfi* making, highlighting its potential to achieve a similar dense texture while streamlining the process. The slow simmering process in traditional *kulfi* making not only concentrates the milk solids but also allows for complex flavour development through caramelization and Maillard reactions. These reactions contribute to the depth and richness of flavour associated with traditional *kulfi*. Condensed milk and khoa, while offering convenience, may not fully replicate this intricate flavour profile **(Bennett *et al.*, 2021).**

2.1 Frozen dairy foods

Frozen dairy products are a delightful and well-researched sector within the dairy industry, offering a vast array of textures, flavours, and functionalities. The beloved ice cream, a classic enjoyed by all ages, is formed by churning a base of milk, cream, sugar, and flavourings while incorporating air to create its light and scoopable texture. Variations on this theme include frozen custard, boasting a denser texture due to the addition of egg yolks (**Marshall & Goff, 2019**).

Frozen yogurt incorporates live and active cultures similar to yogurt, although freezing temperatures typically halt their probiotic activity. Even health-conscious consumers can find a place within the frozen dairy world. Innovative products feature reduced sugar content or incorporate protein-rich yogurt as a base (**Marshall & Goff, 2019**). This category extends beyond just sweet treats; frozen dairy can also serve as a functional ingredient. Frozen yogurt cultures are used in the production of frozen dairy products with potential probiotic benefits.

Ice-cream: Ice-Cream, Kulfi, Chocolate Ice Cream or Softy Ice-Cream means the frozen milk product conforming to the composition specified in table 2.1, obtained by freezing a pasteurized mix prepared from milk or other products derived from milk, or both, with or without addition of nutritive sweeteners and other permitted non-dairy ingredients. The said product may contain incorporated air and shall be frozen hard except in case of softy ice-cream where it can be frozen to a soft consistency (**Food Safety and Standards Authority of India, 2023**).

Table 2.1 Legal standards for Ice cream, *Kulfi*, Chocolate Ice cream and Softy Ice cream (**FSSAI, 2023**).

Parameter	**Ice Cream or *Kulfi* or Chocolate ice cream or softy ice cream**	**Medium Fat Ice Cream or *Kulfi* or Chocolate ice cream or softy ice cream**	**Low Fat Ice Cream or *Kulfi* or Chocolate ice cream or softy ice cream**
TS, minimum, %, (m/m)	36.0	30.0	26.0
Weight, minimum, g/l	525.0	475.0	475.0
Milk Fat, %, (m/m)	10.0 (minimum)	More than 2.5 and less than 10.0	2.5 (maximum)
Milk Protein*, minimum, %, (m/m)	3.5	3.5	3.0

***** Protein content is 6.38 multiplied by the total nitrogen determined.

The process of making ice cream involves agitating and freezing a pasteurized mixture to introduce air and maintain consistency. The mixture consists of milk products, sugar, dextrose, liquid or dry corn syrup, water, and optional egg or egg

product additions, flavourings that are safe, and additional stabilizer or emulsifier—all of which are wholesome food ingredients. The category of frozen desserts mainly includes ice cream and similar items, such as ice cream, frozen custard, ice milk, sherbet, water ice, frozen confections. According to US government rules, ice cream is defined in the US. It must have a minimum of 10% milkfat and 20% total milk solids; if bulky flavours are included, the minimums for fat and solids are 8 and 16%, respectively. It needs to be at least 4.5 pounds per gallon, have no more than 0.5% stabilizer, and have 1.6 pounds of total food solids (TS) per gallon. Ice cream composition differs depending on the market and the area. The percentages of fat (12%), milk solids not fat (MSNF) (11%), sugar (15%), stabilizer and emulsifier (0.3%), and total solids (TS) (38.3%) make up an excellent average ice cream. The range of composition is as follows: TS, 36-43%; fat, 8-20%; MSNF, 8-15%; sugar, 13-20%; stabilizer-emulsifier, 0-0.7%. **(Arbuckle, 2013).**

Table 2.2 Types of Ice-creams and frozen dessert with their description **(Arbuckle, 2013).**

Ice Cream Type	Description
Plain Ice Cream	Less than 5% colour and flavouring ingredients. Examples: vanilla, coffee, maple, caramel.
Fruit	Flavoured with fruit. Examples: pineapple, strawberries, apricots.
Nut	Contains nutmeats like almonds, pistachios, or walnuts, with or without extra flavouring or colour.
French Ice Cream	Includes egg ingredients with at least 1.4% egg yolk solids content (1.12% for bulky flavoured goods).
Frozen Milk	Sweetened and flavoured frozen product with 2-7% fat and 12-15% moisture content.
Sherbet Made of Fruit	Made from milk products, sugar, stabilizer, and fruit juices, similar to ice but uses milk products.
Confection	Ice cream mixed with candy pieces like chocolate chips, buttercrunch, or peppermint sticks.
Bisque	Contains bits of baked goods like sponge cake, macaroons, ginger snaps, or grapenuts.

Ice Cream Type	Description
Puddings	Rich ice cream with assorted fruits, nut meats, raisins, and may include eggs, spices, or alcohol. Examples: plum puddings, Nesselrode.
Mousse	Whipped cream mixed with colouring, flavouring, and sugar, frozen without additional stirring. Sometimes includes condensed milk.
Variety	Plain vanilla mixed with syrup to create a marbled effect.
Neapolitan	Contains two or more unique flavours in the same box. Philadelphia (vanilla with extra colour) or New York (vanilla with extra fat and eggs). Also includes ice milk or soft serve.
Novelties	Distinctively shaped frozen dairy products and treats. Examples: ice cream sandwiches, pops or fudge, candy- or chocolate-coated ice cream bars, milk bars.
Frozen Yogurt	Cultured frozen food with 0.5% titratable acidity, at least 8.25% MSNF, and 3.25% milk fat. Must weigh at least 5 pounds per gallon.
Low Fat	Frozen yogurt with 0.5% to 2.0% milk fat.
Non-fat	Contains less than 0.5% fat. Current specifications not standardized.

2.2 Traditional dairy product *Kulfi*

The ingredients for *kulfi*, a frozen dairy product, are milk products, sugar, and flavour, either with or without colour or stabilizers. It is a native frozen dairy treat that resembles ice cream and is made by slowly boiling and stirring sweetened and flavoured milk until it evaporates. The caramelization of lactose and sugar during cooking gives *kulfi* its unique flavour. It is available in a number of tastes, including as pistachio, cardamom, saffron, cream, rose, and mango. In India, Bangladesh, and Sri Lanka, Aegle Marmelos, an herbal remedy used in Ayurvedic, Unani, and Siddha medical systems, has been utilized to treat diabetes. The study examines the commercial production of *kulfi* enhanced with wood apple pulp and evaluates the produced product's sensory attributes.

Kulfi, a popular frozen dessert originating from the Indian subcontinent, was traditionally made by condensing milk and adding various flavourings such as

cardamom, saffron, or nuts. They emphasized *kulfi*'s rich texture and unique slow-chilling process, which distinguished it from conventional ice creams. The authors underscored *kulfi*'s cultural significance and its appeal in both domestic and international markets due to its creamy consistency and distinctive flavour profile **(Singh *et al.*, 2019)**.

2.3 Nutrition benefits from Moringa

The Moringa tree, long revered in traditional medicine, is now being validated by modern scientific research for its exceptional nutritional benefits. Moringa leaves and pods are rich in essential vitamins, including A, C, and E, and minerals such as calcium, potassium, and iron (Table 2.2).

Table 2.3 The nutrient compositions of different parts of Moringa (*Moringa oleifera*) plant **(Fahey, 2005)**.

Nutrients	Fresh leaves	Dry leaves	Leaf powder	Seed	Pods
Calories (Cal)	92	**329**	205	-	26
Protein(g)	6.7	29.4	27.1	**35.97**	2.5
Fat (g)	1.7	5.20	2.3	**38.67**	0.1
Carbohydrates(g)	12.5	**41.23**	38.2	8.67	3.7
Fibre(g)	0.9	12.5	**19.2**	2.87	4.8
Vitamin B1(mg)	0.06	2.02	**2.64**	0.05	0.05
Vitamin B2(mg)	0.05	21.3	**20.5**	0.06	0.07
Vitamin B3(mg)	0.8	7.6	**8.2**	0.2	0.2
Vitamin C (mg)	**220**	15.8	17.3	4.5	120
Vitamin E (mg)	448	10.8	113	**751.67**	-
Calcium (mg)	440	**2185**	2003	45	30
Magnesium (mg)	42	448	368	**635**	24
Phosphorous(mg)	70	**252**	204	75	110
Potassium(mg)	259	1236	**1324**	-	259
Iron (mg)	0.85	25.6	**28.2**	-	5.3
Sulfur (mg)	-	-	**870**	0.05	137

The leaves are particularly noted for their high protein content, containing all nine essential amino acids necessary for human health. Moreover, Moringa is abundant in beta-carotene, which the body converts to vitamin A, essential for vision and immune function. It also provides significant amounts of antioxidants and anti-inflammatory compounds, which help protect cells from oxidative stress and reduce inflammation throughout the body. Additionally, Moringa contains a balanced mix of omega-3 and omega-6 fatty acids, which are crucial for cardiovascular and cognitive health (**Fahey *et al.*, 2005**).

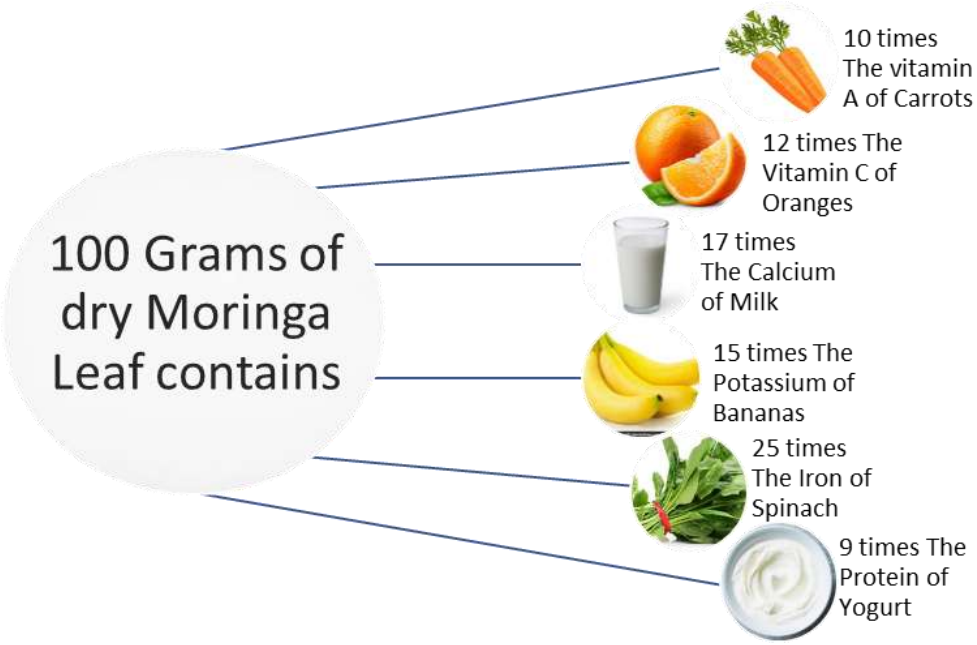


Figure 2.1 Nutritional benefits of *Moringa oleifera*.

Beyond these basic nutrients, Moringa leaves contain unique sugar-modified glucosinolates, which have shown potential in cancer prevention by inducing apoptosis in abnormal cells. These findings suggest that Moringa may help prevent the uncontrolled growth of cancer cells. Moreover, Moringa's anti-inflammatory properties have been validated through in vitro and in vivo studies, showing effectiveness in managing inflammation, hyperlipidemia, and hyperglycemia. The plant's phytochemicals, including flavanols and phenolic acids, contribute to its anti-inflammatory, antioxidant, and antibacterial activities (**Bennett *et al.*, 2021**).

Table 2.4 Health benefits of different parts of moringa (*Moringa oleifera*).

Health Benefit	Description	Reference
Anti-fibrotic/ Anti-ulcer	*Moringa oleifera* seed extract exhibits significant anti-fibrotic effects on liver fibrosis, reducing scarring and blocking the rise of crucial serum enzymes (AST and ALT), indicating hepatoprotective effects. Moringa leaf extract shows dose-dependent anti-ulcerogenic activity, decreasing acid-pepsin secretion and strengthening stomach lining defences.	(Verma *et al.,* 2018; Hamza, 2020)
Anti-inflammatory	*Moringa oleifera* contains phytochemicals like flavanols and phenolic acids, contributing to its anti-inflammatory, antioxidant, and antibacterial properties. These compounds help combat inflammation, alleviate pain, and fight infections.	(Bennett *et al.,* 2021; Fahey, 2005)
Antimicrobial	Moringa extracts from seeds, stem bark, leaves, and root bark exhibit antimicrobial activity, particularly against gram-positive bacteria like Staphylococcus aureus and Enterococcus faecalis. These findings suggest potential for treating bacterial infections.	(Arora *et al.,* 2019; Peixoto *et al.,* 2021)
Anti-hyperglycemic	*Moringa oleifera* shows promise in managing diabetes by lowering blood sugar levels and stimulating insulin release. Compounds like N-Benzyl thiocarbamates and benzyl derivatives within Moringa extracts are believed to enhance insulin secretion and reduce inflammation associated with diabetes.	(Ajit *et al.,* 2021; Francis *et al.,* 2020)
Antioxidant	*Moringa oleifera* is rich in polyphenols, which act as free radical scavengers, preventing oxidative damage to essential cellular components. Moringa extracts enhance the body's antioxidant defence system, promoting overall cellular health.	(Siddhuraju and Becker, 2019)
Anti-tumor	Compounds isolated from Moringa, such as niazimicin, exhibit anti-tumor promoting activity, delaying tumor progression and reducing papilloma incidence in mice. This suggests potential for cancer prevention and treatment.	(Guevara *et al.,* 2019; Fahey *et al.,* 2005; Murakami *et al.,* 2020)
Anti-cancer	*Moringa oleifera* shows potential in combating cancer by increasing levels of enzymes like cytochrome P450 and GST, which help metabolize and eliminate carcinogens. This highlights Moringa's chemo-preventive properties.	(Mehta *et al.,* 2018; Karadi *et al.,* 2020)
Anti-clastogenic	Studies indicate that *Moringa oleifera* does not exhibit clastogenic effects and may offer anti-clastogenic properties by neutralizing free radicals, thus protecting against chromosome damage. This supports the safe consumption of Moringa and its potential protective benefits.	(Promkum *et al.,* 2020)

Moringa also exhibits promising hepatoprotective effects. Studies indicate that *Moringa oleifera* seed extract can protect against liver fibrosis and cell injury, evidenced by reduced scarring and lower levels of liver enzymes indicative of damage. Additionally, Moringa leaf extract has shown potential in treating gastric ulcers by reducing acid-pepsin secretion and enhancing the stomach lining's defences (**Murakami *et al.*, 2020**).

2.4 Dairy products fortified with Moringa

Despite having numerous beneficial and nourishing qualities, milk and its derivative products are not typically thought of as being rich sources of specific bioactive compounds, such as antioxidants and polyphenols. As a result, consumers who are concerned about their health are calling for an increasing number of innovative dairy products that are made with extracts or medicines from herbs. Table 2.3 provides a compendium of recent articles that have reformulated dairy products utilizing various elements of the moringa plant. These investigations centre on cheese, curd, and yogurt. For yogurt, 0.5–2% dry moringa leaf concentration was used; however, in **Zhang *et al.* (2019)**'s study, the yogurt was supplemented with 0–0.2% moringa extract (ME; hot water extract, 100 °C, 30 min). Yogurt was supplemented with 0.1 and 0.2%**.** The preservation of sensory acceptability, increased antioxidant activity, and higher concentrations of certain amino acids (tyrosine, glutamine, and alanine acid) are associated with most of the positive characteristics mentioned in these studies. Additionally, moringa increases the level of Ca, P, K, and Fe while controlling the growth of harmful bacteria like *Salmonella spp., E. coli, S. aureus, L. monocytogenes*, and *L. rhamnosus* without adversely influencing their growth. But after five weeks of storage at 4 ºC, there was a decrease in *L. rhamnosus*, as well as a decrease in viscosity and whiteness and a decrease in cysteine, methionine, and histidine. A light green tint was also recorded in the majority of the cases. When moringa pod powder was added to curd, it had increased potassium, iron, fibre, and vitamin A and C levels than the control sample. Dry moringa leaves or an ethanolic extract of the leaves were used to make labneh cheese, soft white cheese, and cream cheese in amounts ranging from 1-3 percent. Even though they were less white than the control group, their nutritional value rose with acceptable organoleptic scores, as in earlier occurrences **(Trigo *et al.*, 2023)**.

Table 2.5 Dairy Products fortified with different parts of moringa **(Trigo *et al.*, 2023)**

Part of Moringa	Moringa Content	Dairy Product	Beneficial Features	Problematic Features
Dried leaf powder	2% milk replaced with 0.5-5% moringa	Yogurt	Sensory rating similar to control except appearance.	Sensory value decreased after 5 weeks at 4^0C.
	0.5, 1, 1.5, and 2% (w/w)	Yogurt	Higher flavour and taste content due to increased volatile fatty acids and soluble nitrogen.	--
	1, 2, or 3% (w/w)	Labneh cheese	Absence of yeast and increase in vitamins (A, B1, B2, E) and minerals Ca, Zn, Fe, Si during storage indicates quality.	--
	1, 2, or 3% (w/w)	Soft white cheese	Rich in glutamic acid, proline, and leucine, enhancing appearance, texture, and flavour.	--
Aqueous moringa leaf extract	0.05, 0.1, and 0.2% (w/v)	Yogurt	Enhances sensory experience, increases Lactobacillus acidophilus proliferation, and improves antioxidant properties.	--
Seed flour	0.1% (M1) and 0.2% (M2) (w/w)	Buffalo yogurt	High phenolic content and significant antioxidant and antibacterial activity, particularly against Salmonella spp and monocytogenes in M2 yogurt.	--
Pod powder	1.5% (w/w)	Curd	Higher levels of iron, fibre, potassium, and vitamins A and C compared to control sample.	--
Ethanol extract of dried leaves	2, 3, and 4g/100 g skimmed UF-retentate	Cream cheese	Useful as a preservative and nutritional supplement.	--

Chapter III

MATERIALS AND METHODS

This research work on topic **"FORMULATION OF INSTANT *KULFI* PREMIX INCORPORATED WITH MORINGA (*Moringa oleifera*) LEAF POWDER"** was performed at Department of Dairy Science and Food Technology, Banaras Hindu University, Varanasi, Uttar Pradesh, India in collaboration with Shyam Dairy Products, Prayagraj, Uttar Pradesh, India. This research explored a formulation for instant *kulfi* premix that incorporated moringa leaf powder using a dry blending method. Experimental and control premixes were created, followed by *kulfi* samples prepared from each according to a standardized recipe. To gain a deeper understanding of their properties, all dry ingredients were characterized, with particular focus placed on the moringa leaf powder (MLP).

3.1 Technical Programme

Phase I- The process of formulation of Instant *Kulfi* premix incorporated with Moringa Leaf Powder (MLP) and its optimization on the basis of sensory parameter and melting profile was done.

Sensory properties	**:**	9-point hedonic scale (Appearance, body and texture, flavour, melting properties and overall acceptability), melting rate

Phase II- Physico-chemical analysis, textural profile, microbial analysis, were studied.

Physico-chemical properties	**:**	Proximate composition of *kulfi* premix i.e., moisture, fat, protein, ash, fiber, carbohydrate, pH, antioxidant properties (DPPH scavenging activity, total phenolics and total flavonoids).
Mineral analysis	**:**	Calcium and Iron.
Texture profile analysis	**:**	Hardness, adhesiveness, cohesiveness, springiness and gumminess.
Microbial analysis	**:**	Total plate count, Yeast and Mould count and Coliform count.

Phase III- Characterization of optimized *kulfi* premix was done by colour hunter, FT-IR and HRMS analysis.

3.2 Materials

Different types of materials were used for the manufacturing of Instant *Kulfi* Premix incorporated with Moringa Leaf Powder as follows:

Dairy whitener (contains 20% Sugar) was obtained from Shyam Dairy Products, Prayagraj, Uttar Pradesh, India. The source of the moringa leaf was Sam Higginbottom University of Agriculture, Technology, and Sciences agricultural land. Cardamom was bought from local store. Carrageenan powder (stabilizer) was procured online with the brand KREAMAZE®. Sulphuric acid, 2,2-diphenyl-1picrylhydrazyl, deionized water, distilled water, 0.313 N NaOH, potassium thiocyanate, sodium nitrite, aluminium chloride and Folin-Ciocalteu reagent were procured from Bioquest Research Solution, Prayagraj, UP, INDIA.

3.2.1 List of instruments used

Different types of instruments are used in the manufacturing of Instant *Kulfi* Premix incorporated with MLP as mentioned in Table 3.1.

Table 3.1 List of all instruments used for the dissertation.

Name of instrument	**Company**
Electronic weighing balance	Metllertoledo, Switzerland
Digital pH meter	Lab-stream instruments, Gujarat
Kjeldahl apparatus	KELPULS – ELITE EX (VA)
Solar dryer	Rudra Solar Energy, India
Spectrophotometer (operating at 517)	Shimadzu, Japan
Autoclave	Tommy SX – 500, UK
Spray drier	LABH PROJECTS PVT. LTD. India
Incubator	Remi, India

Name of instrument	**Company**
Laminar Air Flow	Lab tech LCB 1201v, Daihan Pvt. Ltd, India
Grinder	Morphy Richards, England
HRMS Machine	Thermo Fisher Scientific, US
FT-IR machine	Alpha II, Bruker, Germany
Centrifuge	Sigma, 3-30k, Germany
Colourimeter	Minolta CR-400, Indonesia

3.3 Method

Instant *Kulfi* Premix incorporated with Moringa Leaf Powder was prepared by using different treatments and procedures mentioned below:

3.3.1 Preparation of Moringa leaf powder

Moringa leaves were procured from the crop research farm of SHUATS. It was ensured that the leaves were not contaminated and was of good quality.

Primary processing begins with the meticulous selection of raw moringa leaves. Trained workers inspect each batch, carefully considering factors like colour, variety, and maturity. Vibrant green leaves, typically from the most prized *Moringa oleifera* variety, are chosen at their peak, ensuring optimal nutrient content. These chosen leaves are then gently washed in a pot filled with clean water. The water is swirled and refreshed multiple times to remove any lingering dirt, debris, or unwanted microbes that might compromise the final product. After this thorough cleansing, the leaves are carefully sieved out of the water, ensuring all moisture is removed for the next step. Finally, the pristine leaves are spread out on a clean sheet, ready to be transformed. Simultaneously, a solar dryer harnesses the natural power of the sun, offering a sustainable and economical way to dry the leaves. Through this meticulous primary processing, the foundation is laid for high-quality moringa products.

Solar dryer

The solar dryer is a revolutionary method of dehydrating moringa leaves, utilizing the sun's energy to create a gentle, eco-friendly process. This innovative method differs from traditional dryers that rely on fossil fuels. The process involves a chamber with air as the engine, which extracts moisture from the leaves through convection. The washed leaves are spread on a mesh tray for optimal airflow, and the sun's energy continuously drives the drying process. The process is monitored by periodically weighing the leaves before sunset, allowing for adjustments in drying time and trays positioning. This ensures uniform drying throughout the chamber, ensuring the final product retains its vibrant green colour, rich nutrient content, and characteristic flavour profile. Additionally, the solar drying process is gentler than high-heat alternatives, preserving heat-sensitive nutrients and volatile compounds, resulting in a more potent and flavourful final product.

Figure 3.1 Image of solar drying of Moringa Leaf

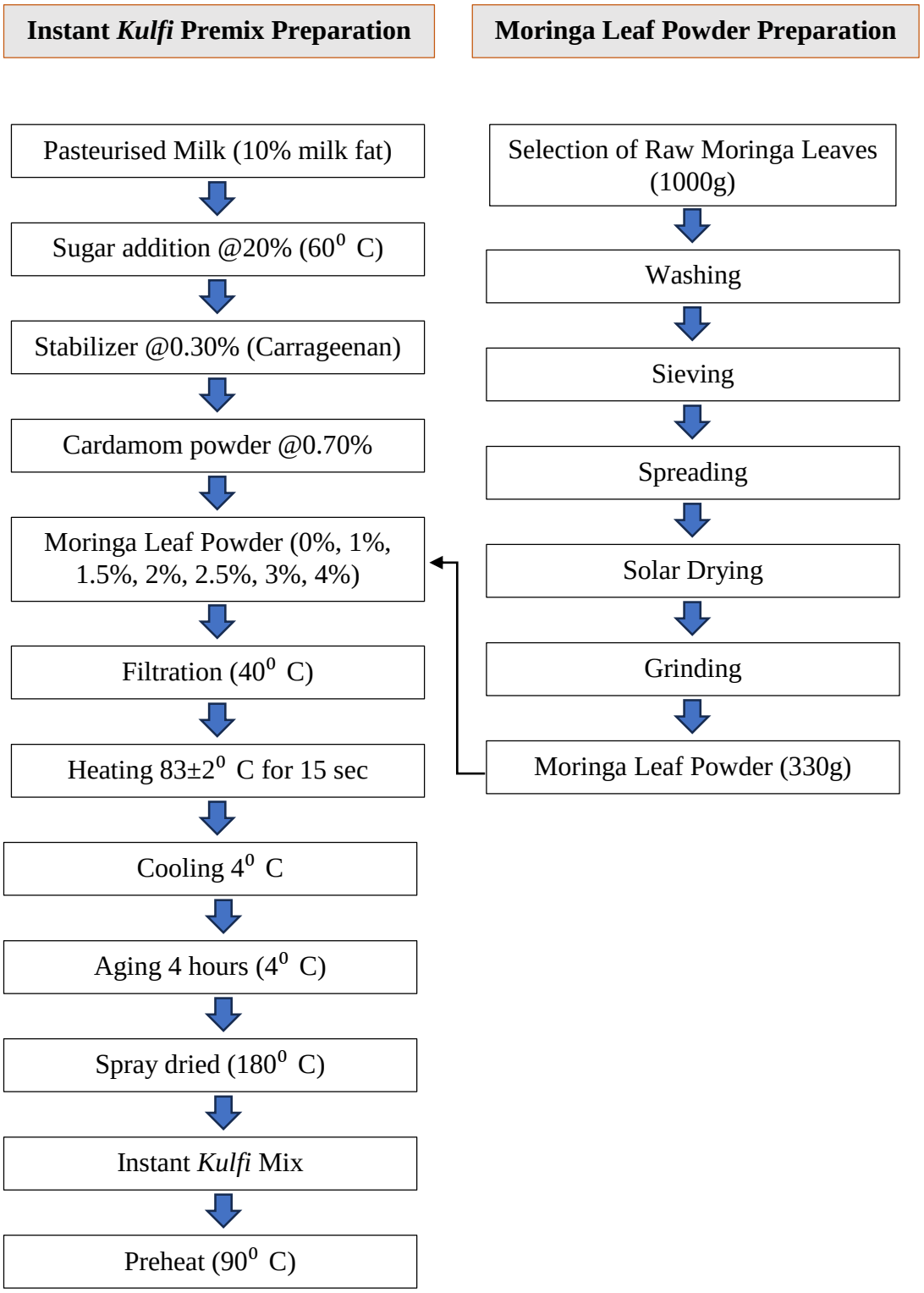


Fig 3.2 Process flow diagram showing the preparation of Instant *Kulfi* premix Incorporated with Moringa leaf powder

3.3.2 Preparation of instant *Kulfi* premix incorporated with MLP

Milk (contains 10% fat) was procured from Shyam Dairy Products, Prayagraj. Cardamom procured from local market was grinded into fine particles. Stabilizer and emulsifier blend (contains mono and diglycerides of fatty acid, carrageenan and sodium alginate) was purchased online with the brand KREAMAZE®. Moringa Leaf Powder was added at 0g (for control sample), 1g, 1.5g 2g, 2.5g, 3g and 4g to make 100g Instant *Kulfi* Premix (w/w).

The manufacturing process for Instant *Kulfi* Premix incorporated with Moringa Leaf Powder involves two main stages: the preparation of Moringa Leaf Powder and the formulation of the Instant *Kulfi* Premix. Firstly, raw moringa leaves are selected and thoroughly washed to remove any impurities. The washed leaves are then sieved, spread out for even drying, and subjected to solar drying until they reach the desired dryness. After drying, the leaves are ground into a fine powder, resulting in moringa leaf powder.

For the Instant *Kulfi* Premix, pasteurized milk constituting 10% fat. Sugar is added at a concentration of 20%, and the mixture is heated to 60°C to ensure complete dissolution of the sugar. A stabilizer is incorporated at 0.30% to maintain the texture and consistency of the premix, followed by the addition of 0.70% cardamom powder to enhance flavour. The prepared moringa leaf powder is then added in varying ratios, depending on the desired nutritional and flavour profile. The mixture is filtered at 40°C to remove any undissolved particles or impurities and subsequently heated to 83±2°C for 15 seconds to ensure pasteurization and stability.

The heated mixture is rapidly cooled to 4°C to stop any further cooking and maintain its quality, and then aged for 4 hours at 4°C to allow the flavours to meld and the stabilizer to work effectively. Before drying, the mixture is preheated to 90°C and then spray-dried at 180°C to convert it into a dry powder form. The final product is the Instant *Kulfi* Mix, which is enhanced with the nutritional benefits of moringa leaf powder while retaining the traditional taste and texture of *kulfi*.

3.3.3 Preparation of *Kulfi* mix for analysis

The *kulfi* mix was prepared by combining *kulfi* premix powder with lukewarm distilled or RO water in the ratio of 1:2. The water temperature used was between 35-40 degrees Celsius. This specific temperature range facilitated the dissolution of the premix components during preparation.

3.3.4 Treatment details of MLP incorporated instant *Kulfi* premix

For the preparation of incorporated Instant *Kulfi* Premix, several proportions of Moringa Leaf powder (MLP) were used to study the antioxidant properties of *Kulfi*. Different concentrations of MLP and Dairy Whitener are used as per Table 3.2.

3.3.5 Sensory analysis

A 9-point hedonic test was conducted to evaluate the sensory attributes of the *kulfi* samples. Permission to conduct consumer acceptance was granted by the Ethics Committee. A respondent's consent form was first signed by the panellist to confirm they met the inclusion criteria for the study and had no allergies to the ingredients in *kulfi* samples. A 9-point hedonic scale mentioned in appendix 1 was used for evaluation. Five attributes were evaluated for each sample, namely, colour and appearance, melting properties, texture, taste, and overall acceptability. The panellists consisted of twenty semi trained panelist from DSFT, IAS, BHU. The analysis was conducted in an environmentally controlled room (25 ± 2 ºC) under white light in DSFT, IAS, BHU. The samples were randomly presented in a transparent cup marked with a 3-digit code. A cup of water was also given for rinsing the mouth between tasting (**Kaur *et al.*, 2021**).

3.3.6 Melting test of *Kulfi*

A day before the melting characteristics were determined, samples were placed in a freezer set at -20°C and left there for the entire night in order to analyse the melting behaviour of ice cream. The time it took for 100 g of ice cream to melt at room temperature (25 °C) was measured after the samples were taken out of their plastic containers, which were roughly 6 cm tall and 3 cm in diameter (**Akhter *et al.*, 2022**).

Fig 3.3 Image of instant *kulfi* premix obtained after nozzle type spray drying.

3.3.7 Physico-chemical analysis of MLP incorporated instant *Kulfi* premix

Various physicochemical properties were tested by using different methods and protocols. The brief of the methodologies and their respective methods are listed below:

3.3.7.1 pH Determination

pH determination of *kulfi* mix samples was done using a benchtop pH meter (VS Industries, Gujarat, India). The pH meter was calibrated with buffer solution (pH 7) prior to commencement of analysis. The samples were first mixed thoroughly before recording any measurements. The electrode was submerged to the immersion level in each sample container three times to obtain readings of the samples. This corresponded to three replicates and average values were then calculated.

Table 3.2 Different combination of treatment samples prepared with MLP incorporated Instant *Kulfi* Premix prepared.

Treatment Sample (with different % of MLP)	**Dairy Whitener (g/100g)**	**MLP (g/100g)**
T0 (control sample)	100.0	0
T1 (1%)	99.0	1.0
T2 (1.5%)	98.5	1.5
T3 (2%)	98.0	2.0
T4 (2.5%)	97.5	2.5
T5 (3%)	97.0	3.0
T6 (4%)	96.0	4.0

3.3.7.2 Determination of protein content

Briefly, 2 g of the sample was introduced into a Kjeldahl digestion flask together with 10g of copper sulphate and sodium sulphate in the ratio of 5:1. After adding 25 millilitres of concentrated sulfuric acid to the digestion flask, the process was continued in the fume cupboard at 1500 until frothing stopped. A clear and light blue colouration was observed. The digest was cooled and diluted up to the mark with distilled water in 100 mL volumetric flask. 10 mL of the diluted mixture was poured into the distillation apparatus and 18 mL of 40% of sodium hydroxide was added. 25 mL of 2% boric acid was added into the receiving conical flask and two drops of bromocresol green and methyl red mixed indicator were added. The distillation was continued until the boric acid solution turned from pink to yellowish green. After the distillation, the solution in the conical flask was titrated against 0.1N hydrochloric acid until the endpoint was reached. A blank was taken using the same procedure using only distilled water. The protein was calculated as:

*% Crude protein = % Nitrogen * 6.38*

*% Nitrogen = ((ml standard acid – ml blank) * N of acid * 1.4007)/ sample in gram*

IS: 1479 (Part II) – 1961

3.3.7.3 Determination of fat content

Fat content in *kulfi* mix was determined by Gerber method, where 10 ml 85% H_2SO_4 was poured in 20% Gerber butyrometer. 5g *kulfi* mix sample was poured in the butyrometer followed by 1 ml amyl alcohol. Then butyrometer was closed by stopper with the help of key. Butyrometer was then kept in Gerber centrifuge for 5 minutes. After 5 minutes butyrometer was check for fat content.

3.3.7.4 Determination of moisture content

Moisture content in *kulfi* mix was calculated by formulation method, *kulfi* mix was prepared by mixing *Kulfi* premix with Distilled or RO water in the ratio of 1:2.

3.3.7.5 Determination of total solids and solid not fat content

The total solids were obtained from moisture content analysis as described in section 3.3.5.4. The weight of the residue obtained from moisture content was determined and expressed as a percentage of total solid by the relation:

% Total Solids= (100 - Moisture%)

Total solids-non-fat (SNF) was determined by taking the difference between % total solids and % fat content.

Solids-non-fat= %Total Solids - %Fat content.

3.3.7.6 Determination of carbohydrate content

Carbohydrate content of *kulfi* mix samples were calculated by the difference method,

% Carbohydrate = 100 – (% Moisture + % Fat + % Protein + % Ash).

3.3.7.7 Determination of ash content

10g of *kulfi* premix sample was taken in a silica dish which was ignited and then cooled in desiccator. Sample was evaporated to dryness and ignite in a muffle furnace

at temperature 500^0 C for 4 hours. Then sample was cooled in a desiccator and weighed.

% Ash (by weight) = 100 w/W

Where, w = weight in g of the final ash, and

W = weight in g of the sample taken initially for ash content determination.

IS: 1479 (Part II) – 1961

3.3.7.8 Determination of energy value

Energy value in terms of total calorie content was measured as per **Arbuckle (2013).**

*Total calorie content = (%Carbohydrate * 3.87) + (%Fat * 8.79) + (%Protein * 4.27).*

3.3.7.9 Determination of Crude fibre

Two grams of feed were accurately weighed and then digested in a boiling solution of 200 ml of 0.255 N H2SO4 for 30 minutes. After filtration, the residue was meticulously washed to ensure it was completely free of acid.

The acid-washed residue was then subjected to further digestion using 200 ml of 0.313 N NaOH solution. This digestion mimicked the previous one, boiling for 30 minutes.

The residue was then filtered and thoroughly washed to remove any traces of alkali. To ensure complete dryness, the residue was squeezed and transferred to a clean silica crucible. Crucially, throughout both digestion stages, the condensing flask was maintained at a cool temperature. This prevented evaporation of the acid or alkali solution and ensured no residue was lost. The crucible containing the residue was then placed in a preheated oven at 110°C overnight to completely remove any moisture. After drying, it was cooled in a desiccator before being weighed along with the residue. Subsequently, the crucible was heated with a Bunsen burner to ash the residue. This

heating continued until a whitish ash remained. Finally, the crucible was allowed to cool to room temperature and weighed again.

$$\% \text{ Crude fibre} = ((a - b) * 100)/c$$

Where, a = weight in g of the crucible with dry residue, b = weight in g of the ash, c = weight in g of the sample.

(A0AC, 962.09, 16TH edition)

3.3.7.10 Analysis of antioxidant activity

The antioxidant activity of the *kulfi* premix was assessed based on their scavenging abilities towards the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) following the method described by Bozin *et al.* (2006). Samples were prepared in a concentration range of 0.5 to 15.5 µg / mL. One millilitre of 90 µM DPPH solution was then added to each sample, followed by 95% MeOH to achieve a final volume of 4 mL. This ensured consistent reaction conditions throughout the experiment. The absorbance of the resulting solutions along with a blank (without *kulfi* premix) was measured after incubation for 1 hour at room temperature. Butylated hydroxytoluene (BHT) was included as a positive control.

The percentage inhibition of DPPH by the samples (%I) was calculated using the following equation:

$$I(\%) = 100 \times (A\,blank - A\,sample / A\,blank)$$

Where, A blank represents the absorbance of the control reaction mixture excluding the test compounds (*kulfi* premix), and A sample denotes the absorbance of the test compounds. The concentration of *kulfi* premix required to scavenge 50% of the DPPH radicals (IC50) was determined by plotting the inhibition percentage against the sample concentration. This value serves as a quantitative measure of the antioxidant potency of the *kulfi* premix.

Hussain *et al.*, (2008)

Fig 3.4 *Kulfi* made from Instant premix for sensory analysis.

3.3.7.11 Analysis of bioactive compounds

Total phenolics and total flavonoid contents of *kulfi* premix were analysed by their respective methods.

3.3.7.11a. Determination of total phenolic content

The total phenolic content of the samples was determined using the Folin-Ciocalteu assay, as described by **Chaovanalikit and Wrolstad (2004**). An extract solution of *kulfi* premix was prepared at a concentration of 0.05 grams per 5 millilitres.

A 0.5 millilitre aliquot of the extract solution was combined with 0.5 millilitres of Folin-Ciocalteu reagent and 7.5 millilitres of deionized water. This mixture was incubated at room temperature for 10 minutes. After incubation, 1.5 millilitres of a 20% (w/v) sodium carbonate solution were added to the mixture. The mixture was then heated in a water bath at 40°C for 20 minutes, followed by cooling in an ice bath. The final absorbance of the reaction solution was measured at a wavelength of 755 nm using a spectrophotometer. The results were expressed in milligrams per gram of dry matter based on gallic acid equivalents (GAE). This indicates the amount of gallic acid with antioxidant potential equivalent to the total phenolics present in one gram of the sample on a dry weight basis.

3.3.7.11 b. Determination of total flavonoid content

The total flavonoid content of the *kulfi* premix samples was determined using the method described by **Dewanto *et al.* (2002)**. An aqueous extract of the *kulfi* premix was prepared to achieve a concentration of 0.01 grams of dry matter per millilitre (g/mL). One millilitre of this extract was then transferred to a 10-millilitre volumetric flask. Distilled water (5 mL) was added to the extract in the flask. Subsequently, 0.3 mL of a 5% sodium nitrite (NaNO2) solution was introduced, followed by a 5-minute incubation period. Then, 0.6 mL of a 10% aluminium chloride (AlCl3) solution was added, and the mixture was incubated for another 5 minutes. Finally, 2 mL of 1 M sodium hydroxide (NaOH) solution was added, and the volume was adjusted to 10 mL with distilled water.

3.3.7.12 Mineral analysis

The mineral content such as Ca and Fe was estimated by AAS (atomic absorption spectroscopy): Model-Thermo Fisher Scientist-IN at λ 422.7 and 248.3, respectively by wet digestion method **(Chis *et al.*, 2020)**.

3.3.8 Textural profile analysis

Ice cream's texture profile analysis (TPA) was conducted using the Stable Micro System TAXT2 plus Texture Analyzer. TPA, or the "two-bite" test, consists of the

compression cycles for the first and second. The force vs. time data obtained during the product's first and second compression by the instrument probe is displayed in the first and second compression cycles.

The following characteristics were noted: cohesion, hardness, fractur-ability, springiness, and gumminess. With minor adjustments, texture analysis was performed using the methodology of **Akalin *et al.* (2008)**. Using a Texture Analyser (TAXT, SDS) fitted with a P/36R stainless steel cylindrical probe, TPA was carried out at room temperature. Before examination, ice cream samples that had been kept at -18 °C for five days were tempered for 24 hours at -10 °C. The following parameters were set for the analysis: penetration distance = 15 mm, force = 5.0 g, probe speed = 3.3 mm s-1 during penetration, and probe speed = 3.0 mm s-1 before and after penetration **(Veer *et al.*, 2020)**. The peak compression force (kg) during the sample's penetration was used to calculate the hardness. The test is set up so that the load and displacement at predefined places on the TPA curve are used to calculate the TPA parameters, hardness, adhesiveness, cohesiveness, and gumminess at the time of the test. The highest load, stated in kilograms, that was applied to the samples during the initial compression was known as hardness (F1). The area under the curve for the second compression (A2) divided by the area under the curve for the first compression (A1) was the measure of cohesiveness (A2/A1). The region under the negative peak, adhesiveness (A3), was measured in kilograms per second. Hardness and Cohesiveness produced Gumminess (F1x A2/A1).

3.3.9 Microbial analysis

10 g sample was diluted in 90 ml warm sterile peptone water (45^0 C). Then serial dilution was done up to 10^{-6} aseptically. Test tubes were capped securely and mixed thoroughly using vortex to ensure homogenous mixing. Test sample (1ml) was added into sterile petri plates from dilution 10^{-5} and 10^{-6}. For total plate count, Tryptic Soy Agar (15 ml) was poured, then petri plates were left for few minutes until agar solidify, then kept in incubator at 35^0 C for 48 hours **(AOAC 2002.07).**

For Coliform count, test samples from dilutions 10^{-4} and 10^{-5} were transferred using micro pipette (1000 µL) then, Voilet red bile lactose agar (15 ml) was poured into petri plates and after solidification petri plates were kept in incubator at 44^0 C for 48 hours **(ISO 8523).** For Yeast and Mold analysis, 15 ml Rose Bengal agar was poured after transferring 1 ml test sample with help of micro pipette. Then after solidifying petri plates were kept at 25^0 C for 5 days **(ISO 7954).** All after incubation colonies were counted using colony counter.

3.3.10 Colour analysis

To conduct a colour analysis of *kulfi*, the *kulfi* samples were first prepared according to the desired formulation and stored at 4°C to prevent colour degradation. A colourimeter, Minolta CR-400, was used, and the instrument was calibrated with a standard white calibration tile according to the manufacturer's instructions. A uniform layer of the *kulfi* sample was placed in a petri dish, ensuring a smooth, even surface without air bubbles or irregularities. The colour of the sample was measured, with multiple readings taken from different parts to ensure accuracy, and the L* (lightness), a* (red-green), and b* (yellow-blue) values were recorded. The average L*, a*, and b* values for each sample were calculated and compared to standard references or other samples to evaluate colour consistency and differences. The readings, calibration details, and sample conditions were thoroughly documented. This standardized approach to colour analysis was essential for maintaining the quality and consumer acceptance of *kulfi* **(Sharma and Shukla, 2021).**

3.3.11 FT-IR analysis

An investigation into the chemical composition of *kulfi* premix sample was conducted using Fourier-Transform Infrared (FTIR) spectroscopy. A small portion of the sample was first ground into a fine powder using a mortar and pestle. This increased the surface area of the sample, allowing for better interaction with the infrared radiation during analysis. The powdered sample was then mixed with a specific carrier compound, often potassium bromide (KBr), to form a homogenous mixture. This mixture was pressed into a thin pellet suitable for FTIR analysis. The prepared pellet

was placed within the FTIR spectrometer. The instrument then passed infrared radiation through the sample. Functional groups within the *kulfi* premix molecules absorbed specific wavelengths of this radiation. The FTIR detected these absorption patterns and converted them into a spectrum, essentially a fingerprint of the chemical bonds present in the sample. By analysing the peak positions and intensities within the spectrum, researchers could identify the various organic molecules present in the cooked spaghetti, such as carbohydrates, proteins, and lipids. This information provided valuable insights into the overall composition of the sample **(Du *et al.*, *2019*)**.

3.3.12 HRMS analysis

Solvent Composition for small molecule, Solvent A: 100% Water + 0.1% Formic Acid, Solvent B: 80% Acetonitrile + 0.1% Formic Acid and Solvent C: 100% Methanol + 0.1% Formic Acid Column. Hypersil GOLD™ C18 Selectivity HPLC Column, Particle size 1.9 µm, Diameter 2.1 mm, Length 100 mm was used. Analysis: All the analyses were performed by the default parameters of "Compound discoverer 3.3.3.200" using online databases. Food Research Unknown ID w Database Searches and Molecular Networks Untargeted food research ID workflow without statistics: Detect and identify unknown compounds. - Performs retention time alignment, unknown compound detection, and compound grouping across all samples. Predicts elemental compositions for all compounds, and hides chemical background (using Blank samples). Identifies compounds using mzCloud (ddMS2 and/or DIA), ChemSpider (exact mass or formula) and local database searches against Mass Lists (exact mass with or without RT). Performs spectral similarity search against mzCloud for compounds with ddMS2. Applies mzLogic to rank order structure candidates from ChemSpider and mass list matches. And applies spectral distance scoring to ChemSpider and mass list matches. Generate molecular networks based on spectral similarity matching and transformations **(Singh *et al.*, 2023)**.

3.4 Statistical analysis

Statistical analysis was done and the results were expressed as means of three values. One-way analysis of variance (ANOVA) was used to compare means at the

significant level of p-value <0.05. All analysis were performed by using SPSS software version 29.0.2.0 for statistical analysis and Origin Lab student version for analysis of FT-IR graph.

RESULTS AND DISCUSSION

In the present study entitled **"FORMULATION OF INSTANT *KULFI* PREMIX INCORPORATED WITH MORINGA (*Moringa oleifera*) LEAF POWDER"** was performed at Department of Dairy Science and Food Technology, Banaras Hindu University, Varanasi, Uttar Pradesh, India in collaboration with Shyam Dairy Products, Prayagraj, Uttar Pradesh, India.

4.1 Sensory analysis

The process of formulation for the production of Instant *Kulfi* premix incorporated with Moringa Leaf Powder was optimized on the following criteria, Sensory attribute and Melting test of *kulfi*.

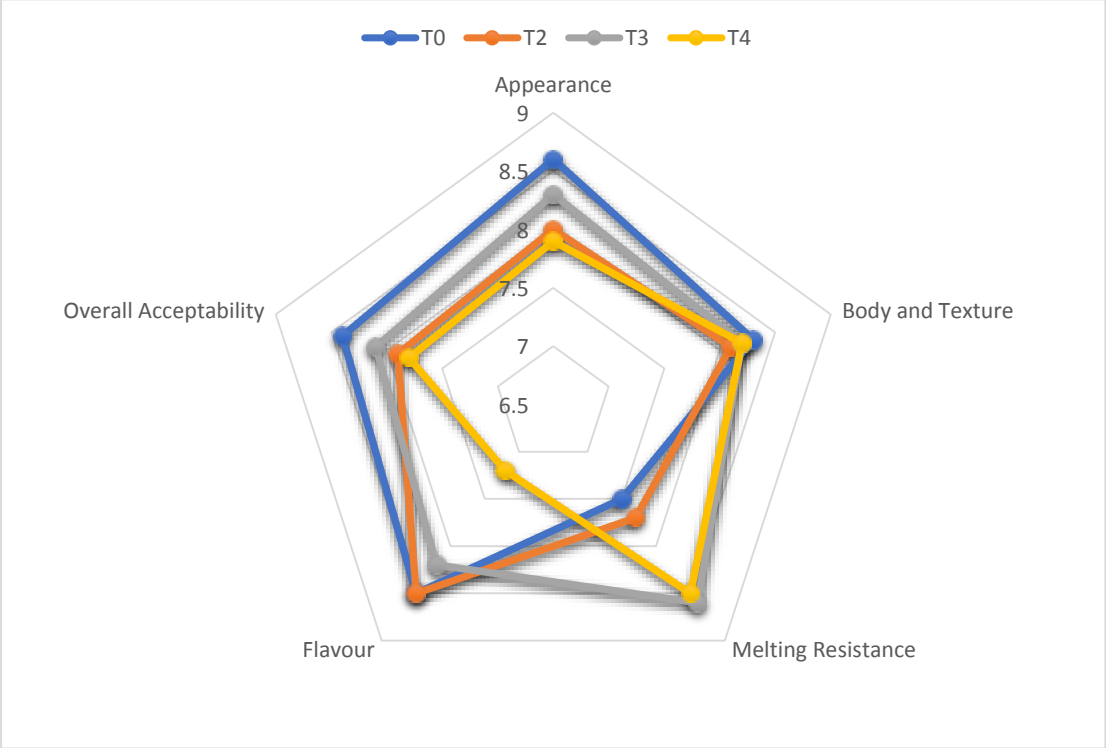


Fig 4.1 Sensory analysis of *Kulfi* incorporated with MLP in different composition.

Sensory analysis revealed that T3 excelled across all evaluated categories. It possessed the most favourable flavour, appearance, melting resistance, body and texture, and achieved the highest overall acceptability rating. While T0, T1, T2, and T4 exhibited decent flavour, both T1 and T2 displayed poor melting resistance, and T4's flavour fell short of optimal. Based on these sensory and melting characteristics, T3 was optimized to be the having the best composition among all six samples. Sensory analysis encompassed a broad range of factors for all samples, including flavour, appearance, and overall acceptability.

Table 4.1 Represents the sensory evaluation

Sensory Attributes	T0	T2	T3	T4
Appearance	8.55± 0.39^{a}	8.16±0.25^{b}	**8.33±0.35^{bc}**	7.72±0.26^{c}
Body and Texture	8.50± 0.43^{a}	8.22±0.26^{ab}	**8.38± 0.33^{b}**	7.88±0.69^{b}
Melting Resistance	7.61± 0.41^{a}	7.72± 0.26^{a}	**8.22± 0.36^{b}**	8.50±0.35^{b}
Flavour	8.83± 0.25^{a}	8.33± 0.35^{b}	**8.50±0.43^{bc}**	7.72±0.44^{c}
Overall Acceptability	8.66± 0.35^{a}	7.77± 0.44^{b}	**8.00± 0.35^{b}**	7.22±0.26^{c}

Data is represented as mean ± S.D. where (n=20).

Values represented with small alphabets as superscript (^{abc}) are significantly different at (Duncan test, $p < 0.05$).

4.1.1 Melting rate

The study investigated the influence of moringa leaf powder (MLP) on the melting rate of kulfi, a traditional Indian frozen dessert. The experiment comprised seven samples: a control group (C) with 0% MLP and six treatment groups (T1-T6) with increasing MLP concentrations of 1%, 1.5%, 2%, 2.5%, 3%, and 4%, respectively.

Initial Increase in Melting Time when we increase MLP concentration in *kulfi* was observed when we move from sample T0 (48), T1 toT3(51.5) and after that it starts decreasing. So, we can see that as MLP concentration increases, the melting rate progressively slowed, reaching a peak value at T3. This trend can be attributed to the

increased dry matter content from MLP, which enhances structural integrity and slows the melt. The water-binding capacity of the dietary fibre in MLP likely absorbed free water, reducing mobility and slowing the melt.

Table 4.2 Represents the melting time for control and formulated samples.

Sample	MLP Concentration (%)	Melting Time (minutes)
T0	0	48
T1	1	49
T2	1.5	50
T3	2	51.5
T4	2.5	51
T5	3	50.7
T6	4	50

Decrease in Melting Time (T4-T6) mainly due to beyond T3, the melting time slightly decreased for T4 to T6, with T6 (4% MLP) melting in 50 minutes. Higher MLP concentrations may have influenced ice crystal formation, affecting ice crystal size and distribution. This alteration in ice crystal morphology could lead to a faster melt compared to the peak observed at T3. This decreasing result might be due to reducing sugar levels in kulfi, as observed in a study that found lower melting rates for kulfi with guava pulp (**As et al., 2021**) and another study noted similar results with the addition of mango pulp (**Nalkar et al., 2018**). Despite this, higher fiber content can still slow down the melting rate, improving the final product's quality (**Xavier and Ramana, 2021**). Similarly, **Hartel et al. (2004)** emphasized that the slow melting rate in ice cream samples was associated with reduced heat transfer rates. Samples with freeze-dried betalain extracts showed a lower melting rate due to the stabilizing effect of these extracts, which maintained their shape and decreased the melting rate by absorbing liquid from kulfi samples and rehydrating rather than causing them to melt. This slow melting rate can be advantageous for using relatively lower freezing temperatures during storage, thus minimizing power consumption. These findings confirm that functional kulfi samples can achieve a lower melting rate through processes like microencapsulation, potentially replacing high fat in frozen dairy products and opening new opportunities for enhancing the quality of dairy frozen food products.

4.2 Physical and chemical analysis of MLP incorporated *Kulfi* premix

The incorporation of Moringa (*Moringa oleifera*) leaf powder into the instant *kulfi* premix has resulted in significant alterations in its nutritional profile, as evidenced by the proximate analysis. The total solids content significantly increased from 96.17% (T0) in the control to 97.30% in T4 and 97.07% in optimized sample, likely due to the addition of the Moringa leaf powder as TSS increases with the addition of MLP. Similar trends have been reported in studies where Moringa is added to yogurt, enhancing their total solid content due to the presence of bioactive compounds and fibers in Moringa (**Adetuyi & Ibrahim, 2014**).

The fat content showed a progressive significant increase from 9.8% in the control to 10.07% in T4 and in T3 which was best sample had 10.01% fat, which is consistent with study showed that Moringa leaf powder elevated the fat content in mango puddings due to its inherent fat content (**Chinma *et al*., 2014**). This increment in fat content is beneficial as it may improve the creaminess and mouthfeel of the *kulfi*.

The solids-not-fat (SNF) content also significantly increased from 86.3% in the control to 87.05 in T3 and 87.2% in T4, attributable to the high levels of proteins, vitamins, and minerals in Moringa leaf powder, which complement the solids-not-fat fraction in dairy products (**Oluduro, 2012**).

A significant enhancement was observed in the protein content, rising from 24.56% in the control to 24.95% in T3 and 25.07% in T4. Moringa leaves are rich in protein, and similar increase in protein content was studied by **Tesfay *et al*., (2016)** by incorporating MLP in yogurt.

The carbohydrate content also significantly increased from 56.03% in the control to 57.12 in optimized sample and 57.30% in T4, due to the presence of complex polysaccharides and dietary fibers in Moringa leaf powder, which is supported by research on the nutritional enhancement of foods through Moringa integration in herbal tea (**Afolabi *et al*., 2018**).

The moisture content significantly decreased from 4.75% in the control to 3.26% in T3 and 2.82% in T4. This reduction is expected as Moringa leaf powder has a dehydrating effect on the mixture, absorbing water due to its fibrous nature, and is beneficial for the shelf-life of the premix (**Alfa *et al.*, 2014**).

The ash content, indicative of mineral content, significantly increased from 5.006% in the control to 5.40% in T3 and 5.507% in T4, as Moringa leaf powder, being mineral-rich, significantly enhances the ash content in the functional yogurt (**Gopalakrishnan *et al*., 2016**).

The crude fiber content increased from 0.543% in the control to 1.06 in T3 and 1.198% in T4. The high fiber content in Moringa leaves is responsible for this increase, improving the dietary fiber profile of the *kulfi* and contributing to better digestive health (**Fahey, 2005**).

Table 4.3 Result of proximate analysis of different sample incorporated with MLP

Parameters	Treatments				
	T0	**T1**	**T2**	**T3**	**T4**
Total Solids %	96.17± 0.021^{a}	96.38±0.029^{ab}	96.73±0.015^{ab}	**97.07±0.023^{b}**	97.30±0.033^{c}
Fat %	9.78± 0.006^{a}	9.89± 0.005^{b}	9.96± 0.005^{c}	**10.01±0.008^{d}**	10.07±0.003^{e}
SNF %	86.31± 0.007^{a}	86.39± 0.008^{b}	86.79± 0.026^{c}	**87.05±0.013^{d}**	87.16±0.006^{e}
Protein %	24.56± 0.022^{a}	24.74± 0.022^{b}	24.83± 0.005^{c}	**24.95±0.006^{d}**	25.07±0.018^{e}
Carbohydrates %	56.03± 0.025^{a}	56.56± 0.003^{b}	56.84± 0.008^{c}	**57.12±0.004^{d}**	57.30±0.007^{e}
Moisture %	4.75± 0.040^{a}	3.85± 0.039^{b}	3.51± 0.008^{c}	**3.26± 0.036^{d}**	2.82± 0.017^{e}
Ash %	5.00± 0.005^{a}	5.21± 0.010^{b}	5.31± 0.006^{c}	**5.40± 0.008^{d}**	5.50± 0.005^{e}
Crude Fibre %	0.54± 0.001^{a}	0.79± 0.002^{b}	0.94± 0.001^{c}	**1.06± 0.001^{d}**	1.19± 0.005^{e}

Data is represented as mean ± S.D. where (n=3).

Values represented with small alphabets as superscript are significantly (^{abcde}) different at (Duncan test, $p < 0.05$).

4.2.1 Antioxidant activity, Total Phenolics and Total Flavonoid content of MLP incorporated *Kulfi* premix

The antioxidant activity of the instant *kulfi* mix formulations, measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, exhibited a significant ($p < 0.05$)

increase with increasing moringa leaf powder (MLP) concentration. The control sample (0% MLP) had the lowest antioxidant activity (5.30%), while the formulation with the highest MLP concentration (T4, 2.5% MLP) showed the highest antioxidant activity (18.48%), whereas the optimized sample showed 15.79% antioxidant activity. Similar increase in antioxidant activity due to incorporation of moringa leaves in tea were observed in the study by **Sreelatha & Inbathiran, (2018)**.

The total phenolic content of the instant *kulfi* mix formulations exhibited a significant ($p < 0.05$) increase with increasing moringa leaf powder (MLP) concentration. The control sample (0% MLP) had the lowest total phenolic content (54.10 mg GAE/g), while the formulation with the highest MLP concentration (T4, 2.5% MLP) showed the highest total phenolic content (84.36 mg GAE/g) whereas the optimized sample showed 76.77 mg GAE/g phenolic content. Incorporating moringa leaves in tea exhibited increase in phenolic compounds, which are well-documented for their antioxidant and anti-inflammatory properties **(Sreelatha & Inbathiran, 2018)**.

The total flavonoid content of the instant *kulfi* mix formulations exhibited a significant ($p < 0.05$) increase with increasing moringa leaf powder (MLP) concentration. The control sample (0% MLP) did not contain any detectable flavonoids (0 mg/g), while the formulation with the highest MLP concentration (T4, 2.5% MLP) showed the highest total flavonoid content (134.41 mg/g) whereas the optimized sample showed 109.6mg/g flavonoid content. Significant increase in flavanol glycosides, quercetin, kaempferol, and myricetin which are flavonoids were observed by **Anwar *et al.*, 2017** due to incorporation of moringa leaf in gluten free cookies.

4.2.2 Mineral analysis

The calcium content of the instant *kulfi* mix formulations exhibited a gradual increase with increasing moringa leaf powder (MLP) concentration. There was a statistically significant difference ($p < 0.05$) between the control group (0% MLP) and the MLP-incorporated formulations. The control sample had the lowest calcium content (856 mg/100g), while the formulation with the highest MLP concentration (T4, 2.5% MLP) showed the highest calcium content (922 mg/100g) whereas the optimized

sample showed 905 mg/100g calcium. Moringa leaves boast a remarkable calcium content **(Foidl *et al.*, 2001),** when incorporated in yogurt.

The iron content of the instant *kulfi* mix formulations exhibited a significant ($p < 0.05$) increase with increasing moringa leaf powder (MLP) concentration. The control sample (0% MLP) had the lowest iron content (3.92 mg/100g), while the formulation with the highest MLP concentration (T4, 2.5% MLP) showed the highest iron content (5.73 mg/100g) whereas the optimized sample showed 5.37 mg/100g iron. Moringa leaves are a rich source of iron, often exceeding the iron content of commonly consumed vegetables like spinach. When moringa leaf powder incorporated in yogurt there was significant increase in the iron content **(Moyo *et al.*, 2014).**

Table 4.4 Antioxidant activity, bioactive compound and mineral content of MLP incorporated *Kulfi*.

Parameters	Treatments				
	T0	**T1**	**T2**	**T3**	**T4**
DPPH scavenging %	5.30±0.02^{a}	10.58±0.06^{b}	13.18±0.05^{bc}	**15.79±0.04^{c}**	18.48±0.11^{d}
Total Phenolics (mg GAE/g)	54.10±0.19^{a}	69.18±0.03^{b}	76.77±0.18^{c}	**84.36±0.04^{d}**	91.76±0.09^{e}
Total Flavonoids (mg/g)	--	54.80±0.11^{a}	79.81±0.02^{ab}	**109.60±0.16^{b}**	134.41±0.17^{c}
Iron (mg/100g)	3.92±0.05^{a}	4.64±0.01^{b}	5.09±0.03^{c}	**5.37±0.09^{d}**	5.73±0.15^{e}
Calcium (mg/100g)	856±1.53^{a}	877±2.75^{b}	889±2.58^{c}	**905±2.01^{d}**	922±1.97^{e}

Data is represented as mean ± S.D. where (n=3).

Values represented with small alphabets as superscript (^{abcde}) are significantly different at (Duncan test, *$p < 0.05$).*

4.3 Textural profile analysis of *kulfi*

The incorporation of Moringa (*Moringa oleifera*) leaf powder significantly altered the textural properties of the instant kulfi premix. Hardness increased from 2931 g in the control to a peak of 3931 g in T1 and then decreased slightly in T2, T3, and T4, likely due to the fibrous nature of Moringa which initially made the kulfi firmer but later disrupted the matrix structure **(Oluduro, 2012; Akinmoladun *et al.*, 2019)**.

Adhesiveness became more negative, indicating increased stickiness, which was attributed to the moisture retention and water-binding capacity of Moringa fibers **(Afolabi *et al.*, 2018)**. Cohesiveness initially rose from 0.78 in the control to 0.88 in T1, suggesting enhanced binding properties from Moringa's high protein content, but then decreased to 0.55 in T4 due to excessive fiber content disrupting the gel network **(Tesfay *et al.*, 2016; Gopalakrishnan *et al.*, 2016)**. Springiness increased significantly from 1.45 mm in the control to 9.58 mm in T4, reflecting improved elasticity from the fibrous Moringa **(Chinma *et al.*, 2014)**. Gumminess increased from 403 g in the control to a peak of 953 g in T1 and then decreased, remaining higher than the control across all treatments. This indicated that Moringa's high fiber content made the kulfi denser and more cohesive **(Adetuyi & Ibrahim, 2014)**. Overall, Moringa leaf powder enhanced the firmness, stickiness, cohesiveness, elasticity, and density of the kulfi premix, aligning with findings in similar studies.

Table 4.5 Textural profile analysis of *Kulfi*.

	Control	**T1**	**T2**	**T3**	**T4**
Hardness (g)	2931	3931	3801	**2989**	3124
Adhesiveness (mJ)	-17.3	-37.3	-26.6	**-10.4**	-27.5
Cohesiveness	0.78	0.88	0.65	**0.66**	0.55
Springiness (mm)	1.45	2.45	5.93	**1.48**	9.58
Gumminess (g)	403	953	661	**435**	686

4.4 Microbial analysis

Standard plate count for sample T1, T2, T3 and T4 were 1.50, 1.38, 1.33 and 1.30 cfu x 10^{-3} respectively on day 0 and 1.60, 1.53, 1.45 and 1.36 cfu x 10^{-3} respectively on 60^{th} day, therefore the microbial growth was controlled in all the samples till 60 days and was best in T4 sample. Yeast and Mold count were nil for all the samples throughout the period of 60 days of microbial testing, similarly coliform was also nil throughout the period.

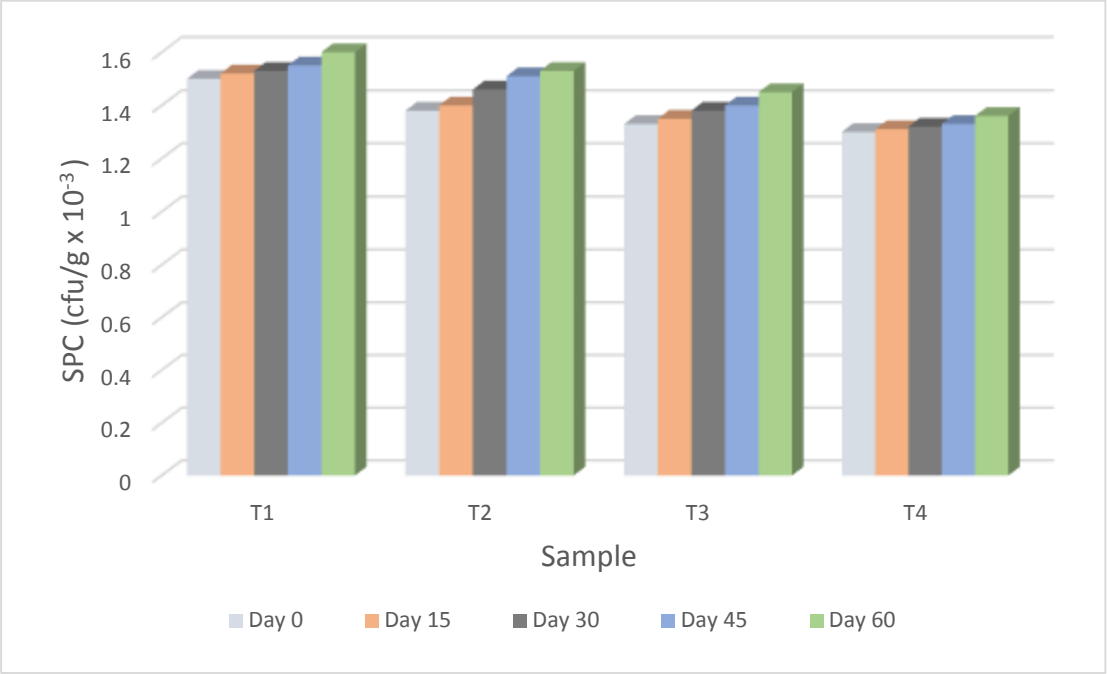


Fig 4.2 Graphical representation of microbial analysis of *kulfi* premix.

4.5 Colour analysis

The table below presents the colour analysis data for the control and T3 *kulfi* samples. The control sample exhibited higher lightness (L*) at 65.10, with a* and b* values of 0.95 and 8.09, respectively. In contrast, the T3 sample showed lower lightness (L*) at 46.24, with a* and b* values of 3.69 and 5.20, respectively, indicating a darker and more red-yellow hue compared to the control.

Table 4.6 Colour analysis of control and T3 sample.

Sample	L* (Lightness)	a* (Red-Green)	b* (Yellow-Blue)
Control	65.1	0.95	8.09
T3 Sample	46.24	3.69	5.2

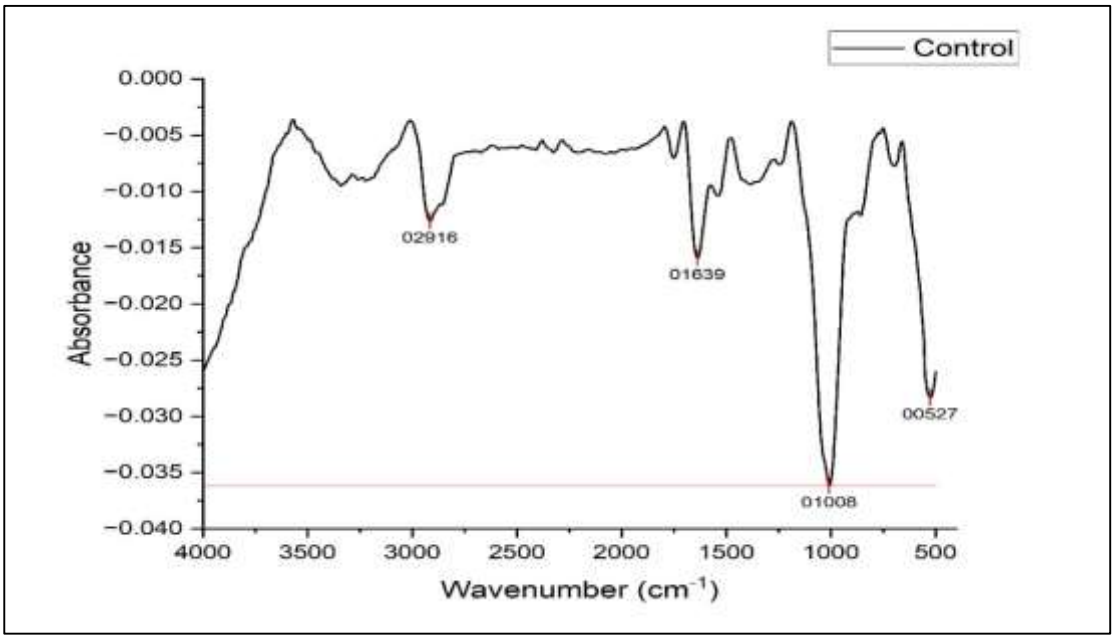
4.6 FT-IR analysis

The FTIR analysis revealed significant differences between the control sample and the *kulfi* premix incorporated with moringa leaf powder (T3 sample). The T3

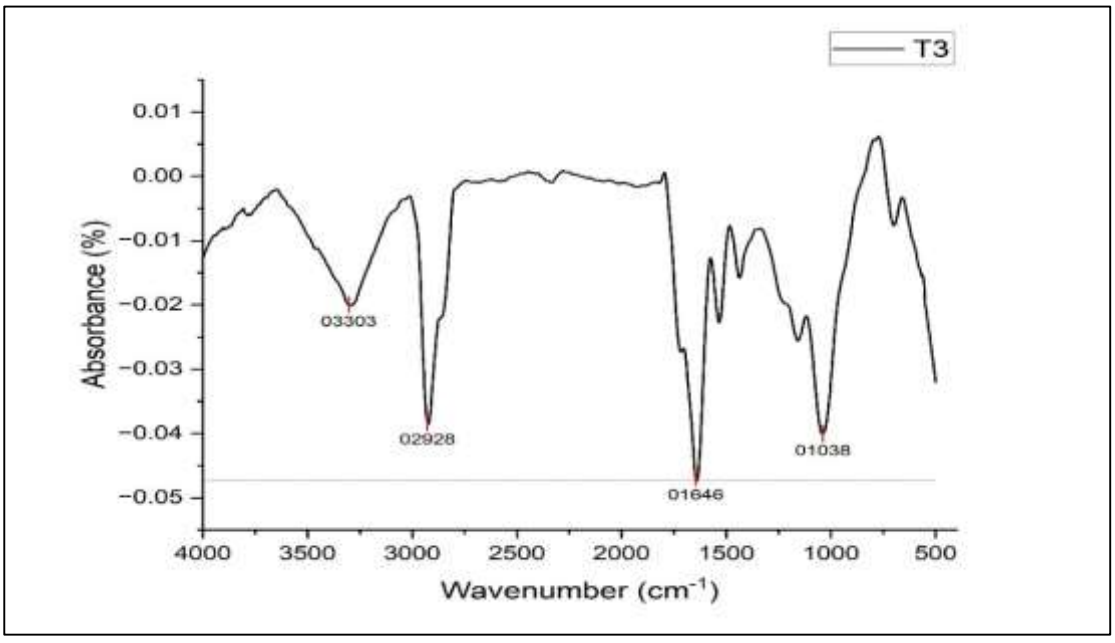
sample showed an O-H stretching peak at 3303 cm^{-1}, indicating the presence of additional hydroxyl groups from moringa. The C-H stretching peak shifted from 2916 cm^{-1} in the control sample to 2928 cm^{-1} in the T3 sample, suggesting interactions between the *kulfi* base and moringa components. A slight shift in the amide I (C=O stretching) peak from 1639 cm^{-1} to 1646 cm^{-1} indicates changes in protein structures, likely due to the protein-rich moringa leaf powder. The C-O stretching peak shifted from 1008 cm^{-1} to 1038 cm^{-1}, consistent with the presence of additional carboxyl or alcohol groups from moringa. The absence of the C-H bending peak at 527 cm^{-1} in the T3 sample suggests potential suppression or overlap due to moringa components. These findings were compared with the study by **Du *et al.*, (*2019*)** where, MLP was incorporated in yogurt and obtained peaks which were common have been mentioned.

Table 4.7 FTIR analysis table for control and T3 sample representing class and groups according to peak obtained.

Peak	**Control (cm^{-1})**	**T3 (cm^{-1})**	**Reference Peaks for Milk Products (cm^{-1})**	**Reference Peaks for Moringa Products (cm^{-1})**	**Class**
O-H Stretching	-	3303	3300-3400	3200-3400	Hydroxyl Group
C-H Stretching	2916	2928	2850-2950	2850-2950	Alkyl Group
Amide I (C=O Stretching)	1639	1646	1650-1660	1600-1650	Carbonyl Group
C-O Stretching	1008	1038	1000-1100	1000-1100	Carboxyl/Alcohol Group
C-H Bending	527	-	Not prominent	Not prominent	Alkyl Group



a



b

Fig 4.3 Graph obtained after FT-IR analysis of *Kulfi* premix (a) Control and (b) optimized sample T3.

4.7 HRMS analysis

High-Resolution Mass Spectrometry was utilized to identify chemical compounds within the *kulfi* premix. The analysis revealed the presence of 949 different metabolites. The mass spectrum generated by the Orbitrap instrument provided insights into the relative abundance based on retention time and mass/charge ratio (m/z). Within the mass spectrum, 21 prominent peaks were detected **(Figure 4.4)**, representing 53 compounds including 7 amino acids, neochlorogenic acid among phenolic acids, 8 flavonoids, 3 organic acids which helps in treatment of metabolic disorder, synthesis of pharmaceuticals and are energy producing **(Zhang *et al*., 2019; Lee *et al*., 2017; Tian *et al*., 2020)**. 6 fatty acids were identified and amines which have antibacterial, antimicrobial, antifungal, antioxidant, anticancer and antitumor functional benefits. **(Table 4.8)**. Identification was conducted using calculated molecular weight, m/z, and retention time **(Table 4.8)**. The analysis identified 4 essential amino acids along with 3 non-essential amino acids in the *kulfi* premix. Among the phenolic acids, neochlorogenic acid was found to be abundantly present. Among vitamins nicotinic acid and pantothenic acids were found and among flavonoid quercetin and kaempferol were found in abundant amount having anti-inflammatory and anti-microbial functional benefits respectively.

DL-Malic Acid is involved in energy production (**Zhang *et al*., 2019**). Orotic Acid is used in the treatment of metabolic disorders (**Lee *et al.*, 2017**). D-(-)-Quinic Acid is utilized in the synthesis of pharmaceuticals (**Tian *et al*., 2020**). Nicotinic Acid (Vitamin B3) plays a crucial role in metabolism and energy production (**Grundy *et al.*, 2016**). Pantothenic Acid (Vitamin B5) is essential for the metabolism of carbohydrates, proteins, and fats (**Yang *et al*., 2014**). Rutin exhibits anti-inflammatory properties (**Smith *et al*., 2020**). Isorhamnetin is known for its anticancer effects (**Johnson *et al.*, 2017**). Luteolin has neuroprotective properties (**Brown *et al.*, 2018**). Kaempferol acts as an anticoagulant and antimicrobial agent (**Yang *et al.*, 2017**). Neochlorogenic Acid exhibits antioxidant, anti-inflammatory, and hepatoprotective properties (**Yan *et al.*, 2021**). L-Phenylalanine is a precursor for neurotransmitter synthesis (**Fernstrom, 2016**). L-Isoleucine is important for muscle metabolism (**Harper *et al.*, 2017**). L-

Tyrosine is essential for neurotransmitter synthesis (**Young, 2018**). DL-Tryptophan is involved in serotonin synthesis (**Fernstrom *et al.,* 2016**). L-Glutamic Acid acts as a neurotransmitter in the brain (**Meldrum, 2015**). L-(+)-Arginine supports wound healing (**Boger, 2017**). L-Histidine is involved in histamine synthesis and immune response (**Young *et al.,* 2016**). Ethyl Palmitoleate is used for skin moisturizing (**Puglia *et al.,* 2008**). Arachidonic Acid plays a role in cell signaling and inflammation regulation (**De *et al.,* 2017**). Docosahexaenoic Acid supports brain health and cognitive function (**Swanson *et al.,* 2022**). Ethyl Levulinate acts as a flavoring agent and fragrance (**Xu *et al.,* 2013**). Ethyl Myristate is used for skin conditioning and as an emollient (**Miller *et al.,* 2005**). Palmitoleic Acid is known for its skin moisturizing and anti-inflammatory properties (**Ichihashi *et al.,* 2008**). Stearic Acid acts as a skin conditioning agent and emollient (**Thiele *et al.,* 2014**). Oleic Acid functions as a skin penetration enhancer and emollient (**Puglia *et al.,* 2017**). Linoleic Acid supports skin barrier repair and has anti-inflammatory effects (**Denda *et al.,* 2002**). Myristic Acid acts as a cleansing agent and emulsifier (**Goffin *et al.,* 2014**). Leupyrrin D possesses antimicrobial and antitumor properties, making it useful in treating infections and potentially in cancer therapy (**Helfet *et al.,* 2017**). Alterporriol P is an effective antimicrobial agent, potentially useful in combating bacterial infections (**Hu *et al.,* 2014**). Aspergillicin E exhibits antimicrobial properties, which could be leveraged to treat infections (**Shen *et al.,* 2017**). Noursamycin D is known for its antibiotic properties, useful in treating bacterial infections (**Wang *et al.,* 2013**). Agrocin 84 functions as a biological control agent against plant pathogens, aiding in agricultural pest management (**Kerr *et al.,* 2008**). Grassystatin E combines antimicrobial and anti-inflammatory benefits, useful in treating infections and inflammation (**Zabriskie *et al.,* 2015**). Sulformycin F is an antibiotic that can be used to combat bacterial infections (**Luzzatto *et al.,* 2019**). Samholide A exhibits antimicrobial properties, making it useful against various infections (**Kim *et al.,* 2015**). Moriniafungin B is known for its antifungal properties, useful in treating fungal infections (**Zeng *et al.,* 2015**). Aeruginosamide 639 has antitumor and antimicrobial properties, potentially useful in cancer therapy and infection control (**Okino *et al.,* 2013**).

Table 4.8 Different Compounds obtained from HRMS Analysis of Instant *Kulfi* Premix

Group	Compound	Mol. wt.	m/z	RT (min)	Area (Max.)	Functional Benefit	Ref.
Organic acids	DL-Malic acid	134.02	133.01	0.87	139765045	Energy producing	**Zhang *et al.*, 2019**
	Orotic acid	156.01	155.01	0.87	40032776	Treatment of metabolic disorders.	**Lee *et al.*, 2017**
	D- (-)-Quinic acid	192.06	191.05	1.00	42318833	Synthesis of pharmaceuticals.	**Tian *et al.*, 2020**
Vitamins	Nicotinic Acid	123.03	124.03	0.92	13845788	Metabolism and energy production,	**Grundy *et al.*, 2016**
	Pantothenic acid	219.11	218.10	0.93	27171713	Metabolism of carbohydrates, proteins, and fats.	**Yang *et al.*, 2014**
Flavonoids	Rutin	610.15	609.14	11.8	791716223	Anti-inflammatory,	**Smith *et al.*, 2020**
	Isorhamnetin	316.05	317.06	13.4	9537780	Anticancer	**Johnson *et al.*, 2019**
	Luteolin	286.04	285.04	15.5	15472233	Neuroprotective	**Brown *et al.*, 2018**
	Apigenin 7-rhamnosyl- (1->2) -galacturonide	592.14	591.13	13.5	28781265	Anti-anxiety	**Garcia *et al.*, 2021**
	Quercetin-3β-D-glucoside	464.09	463.08	0.84	67943342	Anticancer	**Chen *et al.*, 2019**
	Isorhamnetin 3-glucuronide-7-sulfate	572.04	573.05	13.3	8173320	Hepatoprotective	**Lee *et al.*, 2020**
	Isoetin 7-glucoside-2'- (4''-acetylxyloside)	638.15	637.14	21.3	3576082	Anticancer	**Wang *et al.*, 2019**
	Kaempferol	286.04	287.05	0.90	75319627	Anticoagulant, antimicrobial,	**Yang *et al.*, 2017**
Phenolics	Neochlorogenic acid	354.09	353.08	0.82	603522096	Antioxidant, anti-inflammatory, hepatoprotective properties.	**Yan *et al.*, 2021**
Amino Acids	L-Phenylalanine	165.07	166.08	1.31	243406334	Precursor for neurotransmitters,	**Fernstrom, 2016**
	L-Isoleucine	131.09	132.10	1.10	26562234	Muscle metabolism,	**Harper *et al.*, 2017**
	L-Tyrosine	181.07	182.08	0.94	8843784	Neurotransmitter synthesis	**Young 2018**
	DL-Tryptophan	204.08	188.07	1.20	133650053	Serotonin synthesis,	**Fernstrom *et al.*, 2009**
	L-Glutamic acid	147.05	148.06	0.86	30271195	Neurotransmitter	**Meldrum, 2015**
	L- (+)-Arginine	174.11	175.11	0.72	8762225	Wound healing	**Boger, 2017**
	L-Histidine	155.06	156.07	0.72	1857814	Histamine synthesis, immune response, nerve function	**Young *et al.*, 2016**
Ester	Ethyl palmitoleate	282.25	283.26	24.8	19497355	Skin moisturizing,	**Puglia *et al.*, 2008**
	Arachidonic acid ethyl ester	332.52	187.09	13.2	85438805	Cell signalling	**De *et al.*, 2017**

	Docosahexaenoic acid methyl ester	328.59	201.11	15.2	11943859	Brain health, cognitive function	**Swanson *et al.*, 2022**
	Ethyl levulinate	144.07	145.08	24.7	14113928	Flavouring agent, fragrance	**Xu *et al.*, 2015**
	Ethyl myristate	256.43	184.09	9.08	16047588	Skin conditioning, emollient	**Miller *et al.*, 2003**
Fatty acid	Palmitoleic acid	254.22	237.22	23.3	53650446	Skin moisturizing, anti-inflammatory	**Ichihashi *et al.*, 2008**
	Stearic acid	284.27	283.26	26.8	24056528	Skin conditioning, emollient	**Thiele *et al.*, 2008**
	Oleic acid	282.25	281.24	25.5	272327252	Skin penetration enhancer, emollient	**Puglia *et al.*, 2012**
	Arachidonic acid	304.24	303.23	24.5	11365569	Inflammation regulation, cell signaling	**De *et al.*, 2017**
	Linoleic acid	280.24	279.23	24.7	83316830	Skin barrier repair, anti-inflammatory	**Denda *et al.*, 2009**
	Myristic acid	228.20	227.20	23.9	21914050	Cleansing agent, emulsifier	**Goffin *et al.*, 2014**
Amida	Leupyrrin D	722.37	721.36	22.5	8974745	Antimicrobial, antitumor	**Hertweck *et al.*, 2017**
	Alterporriol P	602.14	601.13	1.04	22753390	Antimicrobial	**Hu *et al.*, 2014**
	Aspergillicin E	754.42	755.42	13.5	20561760	Antimicrobial	**Shen *et al.*, 2017**
	Noursamycin D	775.37	776.38	22.5	6242495	Antibiotic	**Wang *et al.*, 2021**
	Agrocin 84	702.16	701.15	21.9	18388859	Biological control agent against plant pathogenic bacteria	**Kerr *et al.*, 2008**
	Grassystatin E	944.56	945.56	18.8	2179075	Antimicrobial, anti-inflammatory	**Zabriskie *et al.*, 2015**
	Sulfurmycin F	841.35	421.68	23.7	4849412	Antibiotic	**Luzzatto *et al.*, 2019**
	Samholide A	1857.08	620.03	12.1	11226857	Antimicrobial	**Kim *et al.*, 2015**
	Moriniafungin B	662.32	663.33	23.0	3219039	Antifungal	**Zeng *et al.*, 2018**
	Aeruginosamide 639	639.31	640.31	16.1	6936801	Antitumor, antimicrobial	**Okino *et al.*, 2013**
	Squalestatin W2	652.27	653.28	13.2	14207231	Inhibits cholesterol biosynthesis	**Harned *et al.*, 2014**
Amina	Tryptoquivaline W	522.21	521.20	11.7	61685529	Antifungal, antitumor	**Kumar *et al.*, 2017**
	Hostanox O3	794.47	793.46	23.1	1389666	Antioxidant	**Matsui *et al.*, 2015**
	Herbicidin H	537.17	536.16	1.05	14059468	Antibacterial, Antifungal	**Oku *et al.*, 2021**
	Lonomycin C	814.50	815.51	23.0	8982176	Antibacterial	**Chakraborty *et al.*, 2017**
	Daldinin	523.29	524.29	23.8	6044859	Cytotoxic activity against cancer cell	**Jiang *et al.*, 2021**
	Cylindrocyclophane E	610.42	609.41	21.4	3422474	Anticancer activity	**Mo *et al.*, 2015**
	Aldecalmycin	594.37	595.38	23.0	12600210	Antibiotic properties	**Chuaboon *et al.*, 2012**
	Dehydroechinulin	459.28	460.29	22.7	13338028	Antioxidant, cytoprotective	**Song *et al.*, 2020**
	Arbumycin	513.30	512.29	22.8	11484144	Antitumor	**Kurina *et al.*, 2014**
	Buanmycin	593.15	594.16	21.3	6997890	Antimicrobial	**Yoon *et al.*, 2015**

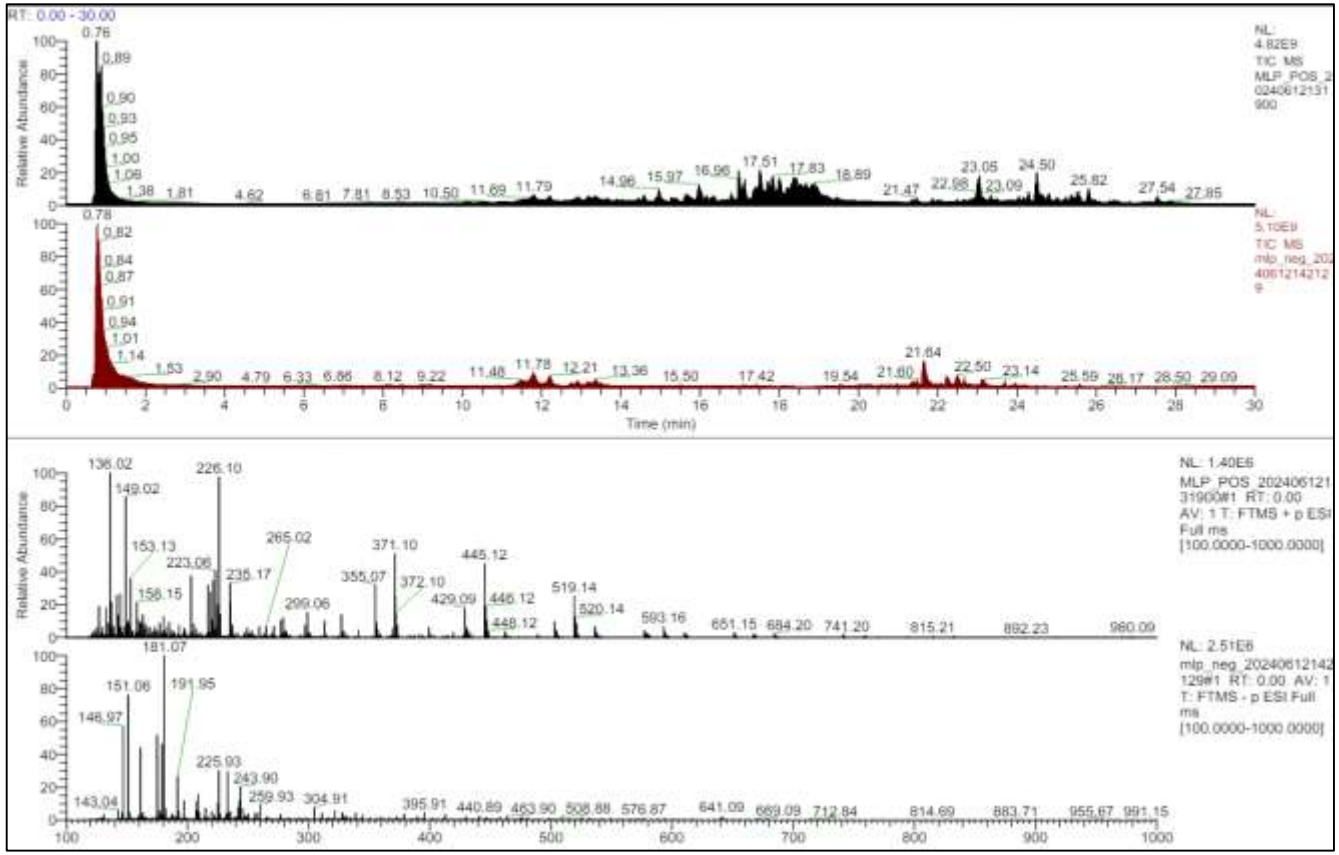


Fig 4.4 Mass spectrum of HRMS analysis of Instant *Kulfi* Premix.

Chapter v

SUMMARY AND CONCLUSION

SUMMARY

During the years 2022-2024, a comprehensive research project titled "**FORMULATION OF INSTANT *KULFI* PREMIX INCORPORATED WITH MORINGA (*Moringa oleifera*) LEAF POWDER**" was performed at Department of Dairy Science and Food Technology, Banaras Hindu University, Varanasi, Uttar Pradesh, India in collaboration with Shyam Dairy Products, Prayagraj, Uttar Pradesh, India.

The production process of Instant *Kulfi* premix incorporated with Moringa Leaf Powder was optimized based on sensory attributes and melting tests. Sensory analysis determined that the T3 sample excelled in flavor, appearance, melting resistance, body and texture, and overall acceptability. The melting rate analysis indicated that increasing Moringa Leaf Powder (MLP) concentration initially slowed the melting rate, peaking at T3, due to increased dry matter and water-binding capacity. However, beyond T3, the melting rate slightly decreased, possibly due to changes in ice crystal formation.

The total solids content significantly increased from 96.170% in the control (T0) to 97.30% in T4, fat content showed a progressive significant increase from 9.8% in the control to 10.07% in T4. Solids-not-fat (SNF) content also significantly increased from 86.3% in the control to 87.2% in T4, owing to the high levels of proteins, vitamins, and minerals in MLP. A notable enhancement was observed in the protein content, rising from 24.56% in the control to 25.07% in T4. The carbohydrate content significantly increased from 56.03% in the control to 57.30% in T4 due to the presence of complex polysaccharides and dietary fibers in MLP. The ash content, indicative of mineral content, significantly increased from 5.01% in the control to 5.50% in T4.

Whereas, the moisture content significantly decreased from 4.75% in the control to 2.82% in T4.

The antioxidant activity of the *kulfi* mix was measured using the DPPH assay. The results indicated a significant increase in antioxidant activity with higher MLP concentrations. The control sample showed an antioxidant activity of 5.30%, while the T3 sample exhibited a much higher activity of 15.79%. This increase is attributed to the high levels of phenolics and flavonoids in MLP, which are known for their strong antioxidant properties. These compounds enhance the free radical scavenging capacity of the *kulfi* mix, potentially offering greater health benefits to consumers.

The inclusion of MLP also enriched the *kulfi* mix with essential minerals. Moringa leaves are a good source of calcium, potassium, iron, and zinc, among other minerals. The study demonstrated significant increase in Fe and Ca by 1.4 mg/100g and 49 mg/100g respectively, which is beneficial for improving the nutritional value of the dessert. This mineral enrichment is crucial for addressing nutritional deficiencies and promoting overall health.

The textural profile analysis of the control and T3 samples of instant kulfi premix revealed several differences. The hardness of the control sample was measured at 2931 g, while the T3 sample exhibited a slightly reduced hardness of 2989 g. Adhesiveness showed a significant difference, with the control sample having an adhesiveness of -17.3 mJ, compared to -10.4 mJ in the T3 sample, indicating that the T3 sample was less sticky. Cohesiveness decreased from 0.78 in the control to 0.66 in the T3 sample, suggesting a reduction in binding properties. Springiness increased marginally from 1.45 mm in the control sample to 1.48 mm in the T3 sample, indicating a slight improvement in elasticity. Gumminess rose from 403 g in the control to 435 g in the T3 sample, showing an increase in the sample's density and cohesiveness.

Microbial analysis in which SPC (1.36 cfu x 10^{-3}), coliform count (nil), and yeast and mould count (nil) tests were performed, which revealed that *kulfi* premix samples were safe for consumption for 60 days.

FTIR analysis revealed significant molecular changes in the *kulfi* premix with Moringa Leaf Powder (T3). Key shifts included O-H stretching at 3303 cm^{-1}, C-H stretching at 2928 cm^{-1}, amide I (C=O stretching) at 1646 cm^{-1}, and C-O stretching at 1038 cm^{-1}. The absence of the C-H bending peak at 527 cm^{-1} indicates MLP's influence, enhancing texture, stability, and nutritional properties of the *kulfi*.

High-Resolution Mass Spectrometry (HRMS) identified 949 metabolites in the *kulfi* premix, including 21 prominent peaks representing 53 compounds such as amino acids, phenolic acids, flavonoids, organic acids, fatty acids, and amines. Key components include neochlorogenic acid, nicotinic acid, pantothenic acid, quercetin, and kaempferol, offering various health benefits like antioxidant, anti-inflammatory, antimicrobial, anticancer, and antitumor properties. The T3 sample, optimized as the best composition, exhibited improved sensory, physicochemical, and functional qualities, highlighting the extensive nutritional and health benefits of incorporating Moringa Leaf Powder into the Instant *Kulfi* premix.

The study highlighted the potential functional benefits of incorporating MLP into instant *kulfi* mix. The increased dietary fiber content from MLP can aid in digestion and promote gut health. Additionally, the enhanced antioxidant properties may help in reducing oxidative stress and inflammation, contributing to overall well-being. These functional benefits make the MLP-enriched *kulfi* mix a healthier alternative to traditional *kulfi*.

CONCLUSION

The study highlights the potential of incorporating Moringa Leaf Powder (MLP) into instant *kulfi* premix as a means to enhance both nutritional and functional properties of the dessert. The sample T3 with 2% MLP was optimized as best on the basis of sensory attributes and melting resistance, which shows the benefits of MLP addition. The physico-chemical analysis revealed significant increase in total solids, fat, SNF, protein, carbohydrate, ash content in per cent by 1.2, 0.3, 0.9, 0.45, 0.9, 0.4 respectively, significant decrease in moisture content by 1.25% and antioxidant properties showed significant increase in % DPPH scavenging and total phenolic content (mg GAE/g) by

10.3 and 30 respectively whereas, total flavonoid content in T3 (2%) sample was 109.5 mg/g which was not found in control sample. AAS was used for determination of calcium and iron percentage in the *kulfi* premix incorporated with 2% MLP where there was significant increase in both calcium and iron content by 49 mg/100g and 1.4 mg/100g. Textural profile analysis demonstrated that MLP enhanced the firmness, stickiness, cohesiveness, elasticity, and density of the kulfi premix. Microbial analysis (SPC, coliform count and yeast and mould count) for 60 days revealed that the *kulfi* premix was safe for consumption. FTIR analysis of T3 (2%) sample showed peaks at 3303 cm^{-1} (O-H stretching), 2928 cm^{-1} (C-H stretching), 1646 cm^{-1} (amide I: C=O stretching), and 1038 cm^{-1} (C-O stretching). HR-MS analyses further demonstrated molecular changes and the presence of 53 beneficial compounds such as 7 amino acids (4 essential and 3 non-essential), neochlorogenic acid a phenolic acid, 8 flavonoids, 3 organic acids, 6 fatty acids (2 saturated and 4 unsaturated), 5 esters, 21 amines and nicotinic and pantothenic acid among vitamins, contributing to enhanced health benefits. These findings indicate that MLP-enriched *kulfi* mix offers a healthier alternative to traditional kulfi, with improved texture, stability, and nutritional value and cab be serve as functional dessert food. The functional benefits including better digestion, gut health, and reduced oxidative stress, make this innovation a promising option for developing nutritious frozen desserts.

The future scope includes upgrading formulation, production optimization, retention of MLP's nutritional properties, comprehensive nutritional and clinical studies, storage and shelf-life trials, consumer acceptance and market potential evaluations. At the same time, increasing MLP utilization for value addition and making sure there is supportable production compliant with the requirements will enhance product appeal and marketability.

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APPENDICES

APPENDIX-I: SENSORY EVALUATION CARD

DEPARTMENT OF DAIRY SCIENCE AND FOOD TECHNOLOGY IAS, BHU, VARANASI

PRODUCT ***Kulfi*** **with the Incorporation of Moringa leaf powder**

Date

Time

Name of the panellist

INSTRUCTION: Given below is the sample of "***Kulfi*** **with the Incorporation of Moringa Leaf powder".** You are requested to judge the sample on the 9 points hedonic scale for the parameters listed below:

Sample	Colour & appearance	Flavour	Melting property	Body and texture	Overall acceptability
T1					
T2					
T3					
T4					
T5					
T6					

Hedonic Scale- Score
Like extremely- 9
Like very much- 8
Like moderately- 7
Like slightly- 6
Neither like or nor dislike- 5
Dislike slightly- 4
Dislike moderately- 3
Dislike very much- 2
Dislike extremely- 1

Remarks:
Signature: _____

APPENDIX-II: ANOVA

ANOVA- Proximate						
		Sum of Squares	df	Mean Square	F	Sig.
SOLIDS	Between Groups	56.482	6	9.414	7975.482	.001
	Within Groups	.017	14	.001		
	Total	56.499	20			
FAT	Between Groups	31.353	6	5.226	1115.631	<.001
	Within Groups	.066	14	.005		
	Total	31.419	20			
SNF	Between Groups	784.699	6	130.783	34560.414	<.001
	Within Groups	.053	14	.004		
	Total	784.752	20			
PROTEIN	Between Groups	68.124	6	11.354	3766.920	<.001
	Within Groups	.042	14	.003		
	Total	68.166	20			
CARBOHYDRATES	Between Groups	4.558	6	.760	462.185	<.001
	Within Groups	.023	14	.002		
	Total	4.581	20			
MOISTURE	Between Groups	8.430	6	1.405	353.028	<.001
	Within Groups	.056	14	.004		
	Total	8.485	20			

ANOVA-Sensory						
		Sum of Squares	df	Mean Square	F	Sig.
Apprearence	Between Groups	3.361	3	1.120	10.938	<.001
	Within Groups	3.278	76	.102		
	Total	6.639	79			
Texture	Between Groups	1.917	3	.639	2.992	.045
	Within Groups	6.833	76	.214		
	Total	8.750	79			
Melting	Between Groups	4.743	3	1.581	12.648	<.001
	Within Groups	4.000	76	.125		
	Total	8.743	79			
Flavour	Between Groups	5.854	3	1.951	13.707	<.001
	Within Groups	4.556	76	.142		
	Total	10.410	79			
OA	Between Groups	9.639	3	3.213	25.009	<.001
	Within Groups	4.111	76	.128		
	Total	13.750	79			

FORMULATION OF INSTANT KULFI PREMIX INCORPORATED WITH MORINGA (Moringa oleifera) LEAF POWDER by Shashank R Kiran (ID: 22412MDT014)

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