PROCESS STANDARDIZATION FOR DEVELOPMENT OF *SAMBHARAM*

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BY

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CERTIFICATE

This is to certify that the dissertation work entitled "PROCESS STANDARDIZATION OF *SAMBHARAM*" is a bonafide work done by Ms. SREELAKSHMI M.N (Reg No.VM21FPT020), student of ST. TERESA'S COLLEGE, ERNAKULAM, MAHATHMA GANDHI UNIVERSITY, in partial fulfillment of the degree of MASTER OF VOCATIONAL IN FOOD PROCESSING TECHNOLOGY. This dissertation work is carried out by her under my supervision and guidance.

SIGNATURE OF EXAMINERS | HEAD OF THE DEPARTMENT

1.

2.

DECLARATION

I, Sreelakshmi M.N, hereby declare that the Project Report entitled "**PROCESS STANDARDIZATION OF *SAMBHARAM*"** has been prepared by me and submitted to the St. Teresa's College, Ernakulam in partial fulfillment of requirements for the award of the Master of Food Processing Technology is a record of original work done by me under the supervision of **Dr. Dinker Singh**, Asst. Professor, department of dairy technology, Verghese Kurien Institute of Dairy and Food Technology, Mannuthy, Thrissur.

I also declare that this project work has not been submitted by me fully or partly for the award of any Degree, Diploma, Title or recognition before any authority.

Mannuthy SREELAKSHMI M.N

Date: VM21FPT020

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CONTENTS

LIST OF TABLES

ABSTRACT

Sambharam is a healthy refreshing drink. The *Sambharam* is prepared by mixing curd with water, shallot, ginger, chilly, salt, and curry leaves. This study was conducted to standardize the process of preparation of *Sambharam*. Response Surface Methodology (RSM) was used to optimize the different levels of ingredients such as water, shallot and ginger. The product was optimized on the basis of sensory analysis viz., colour and appearance, flavour, sourness, saltiness, consistency, spiciness and overall acceptability by judging panel consisting of five trained judges. An optimized product having 100 per cent water, 2 per cent shallot and 2 per cent ginger was found to be sensorily acceptable through Response Surface Analysis. Physicochemical analysis of optimized product was done and got values 93.26% for moisture, 6.73% for total solids, 1.44% for ash, 2.35% for fat, 2.08% for protein and 7.5 % LA for titratable acidity. Microbial qualities such as Standard Plate Count (SPC) was analysed and got 1.9×10^6 cfu/gm and no count were obtained for coliform test.

1. INTRODUCTION

India ranks first in global milk production. In India, the milk production was 198.4 Million Tonnes with per capita consumption of 406 gm/day recorded during the financial year 2019-20 (NDDB). Food is protected, its nutritional content is increased, and its sensory qualities are improved via fermentation, it is a practical and affordable process. Products made from fermented milk are renowned for their superior nutritional and health-related qualities, which include the ability to prevent gastrointestinal illnesses, lower blood cholesterol levels, and exhibit antimutagenic action. These are also advantageous for consumption by lactose intolerant people and those with atherosclerosis (Shiby and Mishra, 2013; Rasane *et al*., 2017). It can lessen atopy's signs and symptoms (Cross *et al*., 2001; Rasane *et al*., 2017). The known ethenic fermented foods in India include *Dahi* (curd), *Mishti Doi* (sweetened curd), Shrikhand, Lassi, and *Chhach* or *Mohi* (buttermilk), *Chhurpi*, *Somar*, *Philu*, and *Shyow* (Dewan and Tamang, 2007; Rasane *et al*., 2017).

Curd is known locally as "*Dahi*" (Dewan and Tamang, 2007; Rasane *et al*., 2017). Given that it is useful to humans nutritionally and therapeutically, it is regarded as a traditional fermented milk product and a functional food (Yadav *et al*., 2007; Rasane *et al*., 2017). According to the latest report by International Market Analysis Research and Consulting (IMARC, 2021) Group, the curd market in India size reached INR 1,184.3 Billion in 2021. As a result of the nutrients and probiotic bacteria it contains, daily consumption of fresh curd aids in the prevention of several ailments. Curd provides our digestive system with helpful and healthy bacteria because it is the richest source of probiotics. Streptococcus cremoris, Streptococcus lactis, Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus helveticus, and Lactococcus cremoris are the microorganisms responsible for curd formation (Madhu *et al*., 2013; Das *et al*., 2019)

Shallot *(Allium ascalonicum L.)* it is one of the most valuable spices for flavour food and for medicinal purposes, it is also a perennial crop (Swamy and Gowda, 2006). It is a member of the onion family and is renowned for having a flavour that is delicate, meaty, and onion-like. It is regarded as a significant plant in Asian medicinal practises and is frequently recommended as a potent treatment for a number of illnesses in Ayurvedic medicine (Sun *et al*., 2011).

Ginger *(Zingiber Officinale Roscoe)* is a popular and well-known spice and condiment, particularly in Asia. Ginger's rhizome is a fascinating source of bioactive compounds with various potential health benefits. It's interesting to note that in recent years, demand for ginger has increased in North America, not only for usage as a food ingredient but also for its protective properties against bacterial infections, osteoarthritis, heart disease, cancer, hypertension, obesity, and diabetes. Chinese, Ayurvedic, and Unani-Tibb remedies frequently use ginger (Ali, 2008; Memudu *et al*., 2012; Shahrajabian *et al*., 2019).

Green chillies *(Capsicum Frutescens)* are a widely utilized vegetable in the entire world due to their hot flavour. Capsaicin is the substance that gives green chilies their spiciness. Capsaicin, which gives hot and spicy foods their spiciness, also has a number of positive effects on the heart, stomach, and pain alleviation. (Chaiyasit *et al*., 2009).

Curry leaf *(Murraya koenigii)* is a significant leafy food. They are typically included in very little amounts to cuisine for their distinct aroma since they contain volatile oil and because they can aid in digestion. Additionally, many Indian Ayurvedic and Unani remedies contain the curry leaf (Singh *et al*., 2014). Salt (Sodium Chloride) is often known as "common salt," one of the most frequently used ingredients in daily life. It is also widely utilised in processing technologies and preservation. Different physiological processes require salt, which is also necessary for maintaining cellular homeostasis (Farquhar *et al*., 2015).

Sambharam is a traditional Indian dairy based beverage used mainly in southern states of India especially Kerala State. It is made from cultured milk i.e. curd/*dahi* (Vineetha *et al*., 2015). It is nutritionally balanced drink which keeps body hydrated and similar to buttermilk or *Chhach or Mattha*. Traditionally, Kerala style *sambharam* is prepared by blending curd with water, chilli, ginger, shallot and salt. But, there is no legal standards about *sambharam* and also the manufacturing procedures are changing region wise. Hence, this study is aims to standardize the process for manufacturing of *sambharam* through the optimization of its ingredients used in the preparation. By considering all the points, this research was framed with the following objectives:

1. To optimize *sambharam* based on sensory attributes.
2. To assess the physico-chemical attributes of optimized product
3. To assess the microbiological attributes of optimized product

Hypothesis:

We assumed that the ingredients used to make *Sambharam* have no adverse effect on human health.

2. REVIEW OF LITERATURE

Milk, was used by human at the beginning of seventh millennium BC (Evershed *et al*., 2008; Dudd, 1998) is a wholesome liquid nourishment produced by mammals' mammary glands (Zhang *et al*., 2021). To achieve a healthy, balanced diet, the US Department of Agriculture (USDA) specifically advises including milk and milk products. Milk can be consumed directly for its health advantages or transformed into other dairy products including cheese, butter, curd, clarified butter or ghee, dairy whitener, ice cream, cottage cheese or paneer, flavor-infused milk, or milk candies. Milk is a potential raw material that can be processed to produce milk products with useful qualities (Batta, 2016).

2.1. CURD

According to the Prevention of Food Adulteration (PFA) Act, *dahi* is "a semi-solid product" made from pasteurised or boiled milk that has been soured with the help of safe lactic acid or other bacterial cultures. *Dahi* could include more cane sugar. It must have a minimum of the same amount of solid-not-fat (SNF) and fat as the milk from which it was made (De, 1980; Kumar and Mishra, 2004; Rasane *et al*., 2019).

Table 1: The FSSR and BIS standards for buttermilk

Characteristics	FSSR (2011)	BIS
Acidity % lactic acid	-	0.6- 0.8
Total plate count	Not more than 1000000/g	-
Coliform Count	10 per g max	10 per g max
Escherichia coli	Absent in 1g	-
Salmonella	Absent in 25g	-
Shigella	Absent in 25g	-
Staphylococcus aureus	Not more than 100/g	-
Yeast and Mould	100 per g max	100 per g max
Anaerobic spore	Absent in 1g	-
Listeria monocytogenes	Absent in 1g	-

Phosphate test	-	Negative
Other requirements	It should have the same minimum percentage of fat and SNF as milk from which it is prepared. If no standards declared then standards prescribed for *dahi* from buffalo milk shall apply	*Dah*i shall conform to the requirements of milk fat and MSNF, as laid down in FSSR, 2011

(Source: Ministry of Food Processing Industries, Government of India)

Odyuo *et al*. (2019) suggest that curd contains a variety of healthful nutrients, including carbohydrates, protein, lipids, minerals, and vitamins. It has demonstrated a variety of defence mechanisms, including immune system control and protection. Additionally, it promotes hormone secretion and renal cleansing. It is used in the cosmetic industry because to its anti-inflammatory, antibacterial, and antioxidant properties.

Das *et al*. (2019) reported that curd is highly useful in the management, treatment, and prevention of several ailments, including AIDS, cancer, diabetes, insomnia, and liver disorders.

Kumar and Durgawati (2021) concluded that curd/dahi made from cooked milk offers benefits for Dhatu, digestion, stamina, and relishing in addition to calming Vata and Pitta. Peenasa, Nava Pratishyaya, Sheetajanya Vishamajwara, Aruchi, Arsha, Atisara, Mutrakricchra, Nashtartava, the fourth trimester of pregnancy, Nidranasha, Karshya, and Vataja Hridroga are among the illnesses that respond well to dadhi. Even though dadhi has many health advantages, it shouldn't be consumed on a daily basis because it affects digestion and can lead to herpes, blood disorders, skin conditions, anaemia, giddiness, jaundice, oedema, and diabetes. When utilised properly, dadhi can treat a number of illnesses as a processing agent, adjuvant, food supplement, and medication

Table 2: Composition and nutritional value of curd

Nutrients of curd	Values
Calories	61kcl
Carbohydrates	4.7 g
Proteins	5.1g
Fats	3.5 g
Minerals	
Calcium	121 mg
Iron	0.1 mg
Phosphorous	141 mg
Magnisium	12.0 mg
Selenium	2.2 mg
Zinc	0.6 Mg
Electrolytes	
Potassium	155 mg
Sodium	46.0 mg
Vitamins	
Vitamin A	99.0 IU
Vitamin C	0.5 mg
Vitamin K	0.2 µg
Vitamin B12	0.4 µg
Vitamin B2	0.2 mg
Vitamin D	0.1 mg

(Source: Milind et al., 2014)

2.1.1 Types of curd/*Dahi*

Kumar and Durgawati (2021) studied the qualities of Dahi (curd) and dietary concepts. According to rasa, origin, and concentration, they defined many forms of curd in this review from an Ayurvedic perspective.

Types of Dadhi according to Rasa:

- **Manda Avyakta Rasa** (tasteless): Kinchit Ghana (slightly concentrated), Srishtavidmuta (evacuate faeces and urine), Tridoshakara (aggravates all humors), and Vidahi (causing burning).
- **Svadu Madhura Rasa** (sweet taste): Samyaka Ghana (properly concentrated), Abhishyandi (congestive), Vrishya (aphrodisiac), Medakara (increases fat), Kaphakara (increases mucus), Vatashamaka (alleviate Vata), Madhura Vipaka (sweet after assimilation), and Raktapittaprasadana (purify blood and bile).
- **Svadvamla Madhura** (sweet): Amla Rasa (sour taste) and Kashaya Anurasa (astringent in anurasa), Sandra (concentrated), its properties are explained in Samanya (common) properties of Dadhi.
- **Amla Amla Rasa** (sour taste): overcomes Madhura Rasa (sweet taste), Deepana (enhances digestive fire), aggravates Pitta-Rakta-Kapha.
- **Atyamla Dantaharsha** (sensitive for teeth): Romaharsha (causes piloerection), Kanthadahakara (causes burning throat), Deepana (enhances digestive fire) and aggravates Rakta-Vata-Pitta.

Types of *Dadhi* According to Origin:

- **Gavya Dadhi** (curd prepared with cow milk): Snigdha (unctuous), Madhura Vipaka, Deepana, Balavardhana (enhances strength), Vatashamaka (alleviate Vata), Pavitra (auspicious), and Ruchiprada (creates taste). It is the best among all other types of *Dadhi*.
- **Aja Dadhi** (curd prepared with goat milk): Alleviates Kaphapitta, Laghu (light for digestion), Deepana, Vata and Kshaya Roga Nashaka (alleviates Vata and emaciation). It is beneficial in Arsha (hemorrhoids) and Shvasha Roga [dyspnoea]).
- **Mahisha Dadhi** (curd prepared with buffalo milk): Atisnigdha (very unctuous), Madhura Vipaka, Vrishya, alleviate Vatapitta and aggravates Kapha.

- **Aushtrika Dadhi** (Curd prepared with camel milk): Katu Vipaka (pungent after assimilation), Ishatkshara, Guru (heavy to digest), Bhedya (purgative), Vata Roga (joint disorders), Arsha, Kushtha, Krimi (infestation), and Udara Roga Nashaka (alleviates abdominal disorders).
- **Avika Dadhi** (curd prepared with sheep milk): Doshala (aggravates all Dosha) and aggravates Kaphavata, Madhura Rasa, and Vipaka, Atyabhishyandi and Arsha.
- **Vadava Dadhi** (curd prepared with mare milk): Deepana, Achakshushya (harmful for eyes), Vatavardhaka, Ruksha (unctuous), Ushna (hot potency), Kashaya, alleviates Kaphaand Mutra Roga.
- **Narya Dadhi** (curd prepared with human milk): Snigdha, Madhura Vipaka, Balya, Santarpana (nourishing), Guru, Chakshushya (beneficial for eyes), and alleviates Dosha.
- **Nagya Dadhi** (curd prepared with elephant milk): Laghupaki (easy to digest), alleviates Kapha, Ushnavirya (hot potency), Agninashaka (reduce digestive fire), Kashayanurasa, and Malavardhaka (stool forming).

Types of Dadhi According to Concentration:

- **Ghola**: Churned Dadhi with skin and without adding water is Ghola. It has property like Rasala (mango pulp) when mixed with Sharkara (sugar) and it is alleviates Vatapitta.
- **Mathita**: Churned Dadhi devoid of skin and without adding water is Mathita. It is aggravates Kaphapitta and Ahladakara (pleasant) .
- **Takra**: Churned Dadhi after adding one fourth of water is Takra. It is Kashya, Amla and Madhura Rasa, Madhura Vipaka, Laghu, Ushna Virya, Deepana, Vrishya, Preenana, Vatashamaka. It is Pathya for GrahaniRoga due to its Laghu and Sangrahi properties. It does not aggravate Pitta due to MadhuraVipaka. It is Kaphashamaka due to its Kashaya, Ushna, Vikashi (clarity) and Ruksh (unctuous) properties. The people who consume Takra do not suffer from diseases. It is as beneficial for human as Amrita (nectar) for Devata.
- **Udashvita**: Churned Dadhi after adding half of water is Udashvita. It is Kaphavardhaka, Balya and Amanashaka.
- **Chacchika**: Churned Dadhi after adding plenty of water and removing whole fat is Chacchika. It is sheeta (cold potency), Laghu, alleviates Pitta and Kapha, Shrama

(fatigue) and Trisha (thirst) and aggravates Vata. It is Deepana when consumed with Lanvana (salt).

- Takra after proper extraction of Ghrita (Half of Ghrita) is Pathya (wholesome) and Laghu
- Takra after complete extraction of Ghrita is Guru, Vrishya and alleviates Kapha
- Takra without extraction of Ghrita is Sandra, Guru, Pushti and Kaphakaraka (digestive fire), Kashayanurasa, and Malavardhaka (stool forming).

2.2. YOGURT

According to the Code of Federal Regulations of the FDA (FDA, 1996), yogurt is defined as the "food produced by culturing one or more of the optional dairy ingredients (cream, milk, partially skimmed milk, and skim milk) with a characterizing bacteria culture that contains the lactic acid-producing bacteria, Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophiles". The FAO/WHO Codex Alimentarius Commission defines yogurt as "a coagulated milk product obtained by lactic acid fermentation through the action of Lb. delbrueckii subsp. bulgaricus and St. thermophiles from milk (pasteurized or concentrated milk) with or without additions (milk powder, skim milk powder, etc.). The finished product's microbes must be healthy and plentiful" (Mareschi and Cueff, 1989; Rasic, 1987; Trachoo, 2002)

Hadjimbei, 2022 yoghurt and probiotic fermented milk consumption have been shown to have favourable health effects in a variety of pathological disorders. Along with gut health promotion and immune system regulation, they include osteoporosis, cardiovascular conditions, and diabetes. Yoghurt and fermented milks may be a great approach to supply useful components, providing alternative methods for disease prevention, towards the promotion of good health, as their positive effects are becoming more and more established.

Table 3: A basic nutrient report for yogurt

Nutrients	100g	1 Cup (245g)
Water (g)	87.90	215.35
Energy (Kcal)	61	149
Protein (g)	3.47	8.50
Total lipid (g)	3.25	7.96
Carbohydrate (g)	4.66	11.42
Minerals		
Calcium (mg)	121	296
Magnesium (mg)	12	29
Phosphorus (mg)	95	233
Potassium (mg)	155	380
Zinc (mg)	0.59	1.45
Vitamins		
Riboflavin (mg)	0.142	0.348
Vitamin B12 (µg)	0.37	0.91
Vitamin A (IU)	99	243
Vitamin D (IU)	2	5

(Source: USDA, National Nutrient Database for Standard Reference, 2016; Hadjimbei *et al*., 2022)

As per FSSAI specifications, Yogurt (including Flavoured Yogurt) and Flavoured *Dahi* shall conform to the following compositional specifications

Table 4: FSSAI compositional specifications of Yogurt and Flavoured *Dahi*

Parameters	Yogurt and Flavoured *Dahi*	Partly Skimmed Yogurt and Flavoured Partly Skimmed *Dahi*	Skimmed Yogurt and Flavoured Skimmed *Dahi*
Milk Fat, %, (m/m)	Not less than 3.0 and not more than 15	More than 0.5 and less than 3.0	0.5 (maximum)
Milk solids-not-fat, minimum, %, (m/m)	8.5	8.5	8.5
Milk protein*, minimum, %, (as lactic acid)	2.9	2.9	2.9
Titratable acidity, minimum, %, (as lactic acid)	0.6	0.6	0.6

* Protein content is 6.38 multiplied by the total nitrogen determined (Source: FSSAI, 2021)

2.3. GINGER

Nutakor *et al*. (2020) reported that ginger is a medicinal plant having a variety of biological and pharmacological properties, such as antioxidant, anti-inflammatory, antibacterial, and analgesic activity, and that it may be used to treat conditions like colds, headaches, toothaches, etc. Extensive scientific investigations that demonstrate that it also has anticancer, antidiabetic, and anti-hypercholesteremic effects support its usage as a medicinal plant. The phytoconstituents of ginger, such as shogaols, zingerones, gingerols, and gingerenones, are what give it its therapeutic benefits. Additionally, it has a lot of nutritional elements like proteins, carbs, vitamins, and minerals

Gunathilake and Rupasinghe (2015) demonstrated via their research that ginger has significant promise because of its anti-inflammatory, lipid-lowering, anti-platelet aggregation, hypoglycemic, and hypotensive qualities.

Mao *et al.* (2019) concluded that ginger contains a variety of bioactive compounds. Ginger has the potential to be a component of functional foods or nutraceuticals, and it may be used to treat or prevent a number of illnesses, including cancer, cardiovascular disease, diabetes mellitus, obesity, neurodegenerative diseases, nausea, emesis, and respiratory disorders.

Al-Awadi (2017) was found tat ginger has been reported to be beneficial for a variety of acute and chronic ailments, including menstrual cramps, asthma, congestive diseases, congestive heart failure, and as an aphrodisiac.

Table 5: Nutritional composition of ginger

Nutrients	Values
Moisture	75.20±0.53%
Carbohydrate	2.01±0.23%
Crude ash	0.81±0.01%
Crude fat	11.71±0.19 %
Crude fibre	1.39±0.50%
Crude protein	8.91±0.04 %
Sodium	7.32±0.02mg
Zinc	4.99±0.04 mg
Calcium	182.67±0.04 mg
Iron	9.68±0.02 mg

(Source: Nutakor *et al*., 2020; Onimawo *et al*., 2019)

2.4. SHALLOT

Due to their distinctive aroma and advantageous health properties, alliums are widely produced around the world and are eaten either as vegetables or as a condiment. The phytochemicals found in all Alliums, including domesticated varieties like garlic, leeks, common onions, and shallots as well as wild varieties like Allium ursinum L., are what give them their health advantages. These phytochemicals include polysaccharides, saponins, phenolic compounds, and organosulfur compounds. The high phenolic content of shallots is well-known, particularly the flavanol quercetin, which is conjugated with saccharides (Wiczkowski *et al*., 2008; Major *et al*., 2022)

Table 6: Nutritional compositions of Shallot (per 100 g)

Parameters	**Shallots**
Water	79.8 g
Energy	301 KJ
Calories	72Kcl
Protein	2.5 g
Fat	0.1 g
Carbohydrates	16.8 g
Fiber	3.2
Vitamin B6	20% of the Daily Value (DV)
Manganese	13% of the DV
Copper	10% of the DV
Folate	9% of the DV
Vitamin C	9% of the DV
Potassium	7% of the DV
Iron	7% of the DV
Pantothenic acid	6% of the DV
Magnesium	5% of the DV
Thiamin	5% of the DV

DV: Daily Value (Source: USDA, 2019)

Motlagh *et al*. (2011) and Sun *et al*. (2019) revealed that shallot may be a candidate for the prevention and treatment of numerous inflammatory and cancerous disorders.

Sun *et al.* (2019) reported that shallots' top health benefits include their high antioxidant content, improvement of heart health, cancer prevention, diabetes, anti-inflammatory, and antibacterial properties, potential for battling obesity, and potential for treating or preventing allergies.

Moldovan *et al.* (2022) provided key results about the shallot's antibacterial properties and its various uses in cardiovascular disorders.

Table 7: Health benefits of shallot

S/N	**Benefits**
1	Reduction of cancer risk
2	Improve heart health
3	Aid detoxification
4	Help control diabetes
5	Improve brain health
6	Help fight obesity
7	Help treat allergies
8	Boost bone health
9	Might maintain vision health
10	Boost immunity
11	Improve skin health
12	Enhance abdominal health
13	Keep Hair healthy

(Source: Sun *et al*., 2019)

2.5. CHILLY

Pawar *et al*. (2011) given findings on chilli extract, its primary ingredient, capsaicin, is used to treat heart arrhythmia, lower cholesterol levels, and diabetic neuropathy. Additionally, peppers are utilised to treat cancer.

Mehta (2017) highlighted on the benefits of chillies for one's health and diet, stating that they are low in calories, low in salt, high in vitamins A and C, and a good source of folic acid, potassium, and vitamin E. They have a lengthy history of use as a conventional treatment for conditions like vertigo and anorexia. They are used in the treatment of burns, postherpetic neuralgia (shingles), blood clots, cluster headaches, asthma, arthritis, and rheumatoid arthritis.

Pundir *et al*. (2016) suggest that chilli pepper is a crucial component of Indian cuisine. Numerous vitamins, including vitamins A, C, B2, B6, and E, are present in the little chilli pepper fruit. Only the capsicum plant produces the hotness-causing alkaloid component. Capsaicinoids are a key component of pharmaceuticals that are useful in neurology. Chilli peppers provide medicinal benefits that include anti-inflammatory, anti-cancer, anti-inflammatory, antioxidant, anti-hemorrhoidal, anti-obesity, gastroprotective, antipyretic, analgesic, and relief from arthritis, migraine, diabetes, and rhinitis-sinusitis. Numerous health advantages are provided by including a tiny bit of chilli in our regular diet.

Salehi *et al*. (2018) mentioned about the multifunctional qualities of capsicum and capsaicin have been discussed and seem to hold promise for the treatment/prevention of the metabolic syndrome. This includes potential for preventing diabetes, lowering cholesterol and triglycerides, and having positive cardiovascular effects.

Milind *et al*. (2012) indicated that green chilies encourage perspiration, which cleanses the skin and improves complexion. The potato family includes the naturally hot, spicy, and flavorful green chilli, or Capsicum frutescens. Capsaicin, the substance that gives it its intensely fiery flavour, is primarily found in the seeds or the white pith to which the seeds are connected.

Table 8: Composition and nutritional value of chilly (per 100 gm)

Parameters	Chillies green
Moisture	85.700 gm
Protein	2.900 gm

Fat	0.600 gm
Minerals	1.000 gm
Fibre	3.000 gm
Carbohydrates	6.800 gm
Energy	29.000K.gm
Calcium	30.000 mg
Phosphorus	80.000 mg
Iron	4.400 mg
Carotene	175.000 µg
Thiamine	0.190 mg
Riboflavin	0.300 mg
Niacin	0.900 mg
Vitamin C	111.000 mg
Sodium	-
Potassium	-
Phytin Phosphorus	7.000 mg
Magnesium	272.000 mg
Copper	1.400 mg
Manganese	1.380 mg
Molybdenum	0.070 mg
Zinc	1.780 mg

(Source: National Institute of Nutrition. Hyderabad, 2012)

2.6. CURRY LEAVES

Chaudhury and Kumar (2021) has been hypothesised that the plant exhibits a wide range of pharmacological properties, including phagocytic activity, anti-diabetic, antimicrobial, anti-diarrheal, anti-oxidative, anti-ulcer, cytotoxic, and anti-cancer activity.

Jain *et al*. (2017) concluded that Murraya koenigii is a Rutaceae plant with green leaves and leafy medicinal leaves. The plant's multiple pharmacological effects have been observed, including phagocytic effects as well as antiulcer and antidiarrheal properties. Murraya koenigii contains terpenoids and alkaloids that are found in essential oils.

Bhandari (2012) reported that Murraya koenigii leaf crude organic extracts have shown anti-aging properties after being tested for a variety of pharmacological activities. The therapeutic potential of additional plant parts, including seeds, leaves, and seed oil, which also have important medical benefits, has to be further researched.

Igara *et al.* (2016) proven that the curry leaf, or Murraya Koenigii, has a significant number of phytochemicals and phytonutrients when employed as a spice or flavouring agent in cuisine. The proximate analysis revealed significant levels of fat, protein, carbohydrate, and fibre. The plant leaves' content of vitamins and minerals indicated that they could be consumed to supplement these insufficient nutrients. Curry leaf's anti-cancer and cardio-protective properties, which come from its abundance of anti-oxidants such flavonoids, phenols, and vitamins E and C, promote its usage as a medicinal plant.As a result, more phytochemical, pharmacological, and clinical research on curry leaves is warranted in order to create a natural medicine that can provide lead compounds with therapeutic value.

Table 9: Composition and nutritional value

Nutrients	Value of fresh curry leaves (100g)	Value of Dehydrated curry Leaves (100g)
Protein	6 g	12 g
Fat	1 g	5.4 g
Carbohydrate	18.7 g	64.31 g
Calcium	830 mg	2040 mg

Iron	0.93 mg	12 mg
B-carotene	75600 g	52920 g

(Source: Singh *et al*., 2015)

2.7. SALT

In 2018, total world production of salt was 300 million tons, the top six producers being China (68 million), the United States (42 million), India (29 million), Germany (13 million), Canada (13 million) and Australia (12 million) (Nagendra *et al*., 2020)

Table 10: Types of Salt

Name of the salt	**Description**
Himalayan pink salt	It contains almost 84 natural minerals and elements found in the human body. Its mineral content gives it a bolder flavor than many other salts. Hence it is used as a cooking and finishing salt. It retains temperature for hours. It is harvested from Himalayan Mountains of India and Pakistan
Kala Namak (Black Salt)	Himalayan salt that's been packed in a jar with charcoal, herbs, seeds and bark. Then fired in a furnace for about 24 hours before it's cooled, stored and aged. It has a faint, sulfurous aroma of eggs. It's often used in vegan and vegetarian dishes to give the taste of egg, as well as used in ayurvedic practice.
Table salt	It is the most common salt used. It is harvested from salt deposits found underground. Impurities and trace minerals removed in the process. It's also treated with an anti-caking agent to keep from clumping.
Kitchen salt	A coarse salt that is used in cooking but not at the table
Sea Salt	Generic term for salt derived from evaporation or reduction of sea water. Mineral content varies with locally and drying process.
Pickling salt	A non-iodized salt used for pickling and brining

(Source: Nagendra *et al*., 2020)

2.7.1. <u>Salt recommendations</u>:

Nagendra *et al*. (2020) reported that the salt is a chemical compound called as "sodium chloride" since it contains both sodium and chlorine. Food preservation and flavouring are major uses for it. In actuality, salt is the diet's principal supply of sodium, which is necessary for the body to operate normally. The amount of salt that the body needs is substantially lower than what is typically consumed. According to data provided by the National Nutrition Monitoring Bureau (NNMB), salt consumption varies between 5 and 30 grams in different Indian states

- For adults: WHO recommends that adults consume less than 5 g (just under a teaspoon) of salt per day.
- For children: WHO recommends that the recommended maximum intake of salt for adults be adjusted downward for children aged two to 15 years based on their energy requirements relative to those of adults. This recommendation for children does not address the period of exclusive breastfeeding (0-6 months) or the period of complementary feeding with continued breastfeeding (6-24 months).
- All salt that is consumed should be iodized or “fortified” with iodine, which is essential for healthy brain development in the fetus and young child and optimizing people’s mental function in general (WHO, 2016)
- 1g of sodium Chloride (Nacl) contains contain 39% Sodium.

2.8. BUTTERMILK

According to Food Safety and Standards (Food Products and Food Additives) Regulations, 2011 (FSSR), Buttermilk is refers to a product made by pasteurizing or sterilizing what remains after butter is manufactured from milk, cream or a product made by powdering the above said product.

Buttermilk is the watery phase that is discharged when cream is churned to make butter. It includes all the water-soluble elements found in cream, including minerals, milk protein, and lactose (Sodini *et al*., 2006). Around 3.2 million tonnes of buttermilk are produced annually as a byproduct of making butter, which accounts for 6.7% to 7.0% of all milk produced worldwide (Kumar et al., 2015; Mascarello *et al*., 2019). It is nourishing and offers all the components required for a healthy, balanced diet, including proteins, carbs, minimum fats, vitamins, and vital enzymes (Yeragi and Maske, 2016)

Table 11: Different types of buttermilk and their properties

Sweet Cream Buttermilk	Sour Cream Buttermilk	Cultured Buttermilk	Commercial Buttermilk
Result of the churning process of cream separated from milk.	End product of churning process of ripened cream.	Produced by culturing of skim milk	Commercial buttermilk is most widely available in grocery stores.
Produced from fresh, or sweet, milk, which is converted to cream that convert into butter and buttermilk and has a taste similar to regular skim milk.	Made from raw, unpasteurized sour milk. The milk is allowed to sour naturally prior to churning.	Produced by addition of bacterial culture, which provide a rich, fuller and tangier flavor.	Milk is not used to make butter, instead manufactures add bacterial cultures to skim or low-fat cow's milk and let it mature
Exact taste of the sweet cream milk depends on the flavor of the original milk. For example, goat's milk has a naturally more pungent taste than cow's milk.	Sour cream buttermilk has a tart taste, similar to yogurt or sour cream. As with sweet cream milk, the source of the milk provides slight variations in taste.	The resulting buttermilk has a similar tangy flavor.	Over time, the milk thickens and develops its characteristic sour taste. Commercial manufacturers also make powdered buttermilk. They use the same process as with wet milk, then remove the liquid

(Source: Kumar *et al*., 2015)

2.8.1. <u>Classification of Buttermilk according to Ayurveda</u>: (Jyoti and Kavita, 2016; Chaudhari et al., 2018).

Acharya Charak and *Acharya Vaghabhata* has classified buttermilk into three types based on fat content namely *Ruksha Mandatama* (obtained by churning of curd and completely removing of fat), *Ardhodruta sneha* (prepared by churning of curd from which half of fat is removed), *Anudhrut*a *sneha takra* (prepared by churning of curd from which no fat is removed: Jyoti and Kavita, 2016). Another way of classification by *Bhavamisra* is based on the method of preparation as *Ghola* (churning of curd without adding water and without removing butter), *Madita* (obtained after preparation of butter using churning curd without water), *Takra* (by adding ¼th quantity of water to Madita), *Udashvita* (by adding half the quantity of water to *Madita*) and *Chachika* (Obtained by adding large quantity of water to *Madita*)

Table 12: Chemical composition of Buttermilk

Components	**Sweet Cream Buttermilk**	**Cultured Buttermilk**	**Whey based Cultured Buttermilk**
Total Solids (%)	7-10	8-10	6.97
Fat (%)	0.3-1	0-2.5	1.34
Lactose (%)	3.5-4.9	4.8-5.8	3.28
Phospholipids (%)	0.075-0.25	ND	-
Minerals (%)	0.6-0.75	0.6-0.9	0.75
Acidity (% LA)	0.13	0.5-0.7	0.67

(Source: Libudzisz and Stepaniak, 2002; Aneja *et al*., 2002; Sonali *et al*., 2017; Chaudhari *et al*., 2018)

2.8.2. <u>Health Benefits of Buttermilk</u> (Yeragi and Maske, 2016)

1. <u>Buttermilk contain all essential Macronutrients</u>: Buttermilk is a complete food. It has proteins, carbohydrates, minimal lipids, vitamins and essential enzymes and makes for a complete meal anywhere, anytime. As over 90 percent of buttermilk is water, its consumption helps to maintain the water balance of the body. It is absorbed slowly from the intestines as its contents are mostly combined with proteins. Fermented buttermilk

is sour to taste, but biologically is very nutritive for the human body and tissues (Laudman, 2013)

2. Has a cooling effect on the digestive tract: Buttermilk has a tendency to wash down spicy food and soothes the lining of the stomach when consumed after a piquant meal. The additives of ginger, cumin powder and other condiments also relieve the stomach of any irritation that may have been caused due to the food. Buttermilk is instrumental in reducing body heat. It is well-liked by women, both pre- and post-menopausal, as it reduces body heat and alleviates many symptoms these women suffer from. Even men who have a high metabolic rate and body temperature can avail of the advantages of buttermilk to reduce body heat (Laudman, 2013; Gajanana and Supriya)

3. Encourages digestion and treats stomach ailments: Buttermilk has a fair amount of acid in it which works against bacteria and helps clear stomach and aids in digestion. All the condiments added to buttermilk make very good digestive agents. Ginger, pepper and cumin all make excellent digestive properties. As they are carminative substances, they expel gas from the stomach. Consumed together they have a cooling and digestive effect on the gastrointestinal tract. A preparation of buttermilk drunk regularly also helps gastrointestinal conditions. Some of the digestive ailments cured by buttermilk include: Irregular bowel movements, Irritable bowel syndrome, Cancer of the colon, Stomach infections (Laudman, 2013)

4. Effective against dehydration: Made from yogurt with added salt and spices, buttermilk is an effective therapy to prevent dehydration. It is full of electrolytes and is one of the best drinks to fight against the heat and loss of water from the body. In summer, it is truly a drink to relish (Laudman, 2013; Gajanana and Supriya)
5. Provides calcium without the fat: Buttermilk provides calcium and nutritional supplements without the added calories. Getting the required calcium in your meal plan helps slow bone loss as one grows older. It provides sustenance for new bone development and keeps off osteoporosis (Laudman, 2013; Gajanana and Supriya)
6. Rich in vitamins: Buttermilk contains vitamins such as B complex vitamins and Vitamin D. This makes buttermilk a good choice to overcome weakness and anemia caused by vitamin insufficiency. Vitamin D present in buttermilk strengthens the immune system, making it less susceptible to infections. One serving of this drink gives you over 21 percent of the suggested daily intake (Isenberg, 2010; Laudman, 2013; Gajanana and Supriya)

7. Assists in lowering cholesterol: A natural remedy for lowering and controlling blood cholesterol is buttermilk. Its constituents are very effective in keeping cholesterol under check (Laudman, 2013; Gajanana and Supriya)
8. Strengthens body's skeletal frame: It is a rich source of calcium, an essential building block for the bones and skeletal system of the body. It also helps the teeth become strong. The calcium in this drink gets absorbed by the bone tissue and helps in maintaining bone density. It also provides nourishment to the tissues of the heart and other organs, including nerves and muscles (Hunziker, 1923)
9. Raises immune levels by warding of illnesses: This drink is rich in lactic acid bacteria. This bacterium boosts the immune system and helps the body fight off detrimental pathogens present in everyday foods. Many benefits of buttermilk are related to keeping illnesses at bay by acting on bacteria. It also helps keep blood pressure in check. Special proteins in buttermilk regulate the blood pressure and the high levels of elements such as calcium, potassium and magnesium help in lowering blood pressure. As a probiotic, it is active against vaginal infections and infections of the urinary tract. Candida infections are a common problem in diabetic women and consumption of buttermilk regularly has shown a decrease in such incidences (Hunziker, 1923)
10. Natural therapy against ulcers: Several case studies have been documented to prove that drinking buttermilk is a natural therapy against ulcers. As buttermilk helps neutralize acids in the stomach by coating the stomach lining, it prevents heartburn and keeps the acids from moving up into the oesophagus. This drink is great for people suffering from GERD. Overall, because of its cooling effect, ulcers too are prevented from erupting (Hunziker, 1923; Mishra, 2003)
11. Can be used to treat haemorrhoids: Buttermilk can be used for the relief from haemorrhoids by adding a cup of buttermilk to a mixture of rice and banana. Consume it twice a day to get some respite from haemorrhoids. It is one of the most effective health benefits of buttermilk (Hunziker, 1923; Mishra, 2003)
12. Aids in building body mass: Buttermilk also increases protein intake. Every cell in the body comprises of proteins. All tissues depend on proteins to repair damage and maintain themselves. As this drink is amply rich in proteins, which is essential for building muscles, buttermilk is the drink of choice for many body builders. It provides vitamins for good body health and is nutritious without adding unnecessary calories. Protein is the key for robust bones, tough muscles and healthy skin. This makes buttermilk indispensable in any diet regimen (Mishra, 2003)

2.8.3. Therapeutic Uses of Buttermilk (Takra) (Anubha and Mohanlal, 2016)

1. ***Jwar*** (Fever): *Sushrut* has stated that if there is *Arochak* (uninterested in food) in Jwar due to *Kaph, Takra* should be used mixed with *Vyosh* (Sushrut, 2013).
   Vagbhatt has advised use of *Anamlatakra* (not sour in taste) as *Pathya* (as food material) in Jwar (fever). Food cooked with *Anamla* (not sour in taste) Takra is *Ruchikarak* (increases appetite) (Vagbhatt, 2007).
2. ***Atisar*** (Diarrhoea): *Carak* has advised use of *Takra* in *Atisar* (diarrhoea) as *Anupan* (vehicle) of *Laghuann* (light to digest) Further *Carak* has advised *Takra* as *Anupan* (vehicle) for different *Yogas* (formulations) for *Kaphaj Atisar*. *Carak* has also advised use of *Takra* with *Chitrak* (Plumbago zylenica) churn for treatment of *Atisar* (Agnivesh, 2012). *Vagbhatt* has also advised use of *Takra* in the form of *Tarpanyavagu* (gruel with nourishing property) in *Atisar* (Vagbhatt and Chikitsa, 2007).
3. ***Chardi*** (Vomiting): *Carak* has advised use of *Takra* as *Aahardravya* (food article) in management of *Kaphaj Chardi* (Agnivesh 2012).
4. **Respiratory Disorders**: *Carak* has advised use of *Takra* as *Anupan* (vehicle of drugs) in the management of *Kaphaj Kaas* (cough due to *Sleshm dosh*) (Agnivesh, 2012) *Vagbhatt* has mentioned a special use of *Takra* in *Swas- Kaas* (~ asthma and cough). According to *Vagbhatt* apply thick paste of *Pippali* (*Piper longum*), *Pippalimula* (*Piper longum*), *Pathya* (*Terminalia chebula*), Vidang (*Embelia ribes*) and *Chitrak* (*Plumbago zylenica*) to the inner side of earthen pot of ghee (butter). When the paste gets dried fill the earthen pot with Takra and leave for a month. This *Takra* is said to be *Agnideepak* (improves digestion) and *Swas- kaasnashak* (~ asthma and cough reliever) (Vagbhatt, 2007).
5. ***Aanah***: *Vagbhatt* has stated that use of *Takra* in *Aanah* for *Vat-kaph* patients is equivalent to elixir (Vagbhatt, 2007)
6. ***Udar Rog*** (~ abdominal distention): *Carak* has advised use of *Takra* in *Udarrog* as follows: *Udarrog |Takra* mixed with *Carak* has also indicated use of *Takra* in *Udar Rog* an *Anupan* (vehicle) of *Narayan Churn* (Agnivesh, 2012).
7. ***Shawathu*** (oedema): *Carak* has clearly indicated that if a patient of *Shawathu* is suffering from *Aamatisar* then *Takra* mixed with *Vyosh*, *Sauvarchal*, and *Makshik* (honey) should be used for the treatment. *Takra* is a content of *Chitrakghrit*, used for the treatment of *Shawathu* (Agnivesh, 2012). *Vagbhatt* has advised that for treatment of *Shawathusauvarchal*, *Trikatu*, and honey mixed with *Takra* should be used. Further *Vagbhatt* has advised use of *Takra* as *Anupan* (vehicle) of *Haritaki* (*Terminalia chebula*)

used with *Gud* (jaggery) or of *Aadrak* (*Zingiber officinale*) with *Gud* (jaggery) (Vagbhatt, 2007).

8. ***Gulm***: *Carak* has advised use of *Takra* mixed with *Yavani* (*Trachyspermum ammi*) churn and *Vidlavan* as *Pathya* (useful) in *Gulm*. This *Takra* is *Agnideepak* (improves digestion) and *Vaat- Kaph- Mutraanulomak* (promotes excretion of flatus and urine). *Vagbhatt* has advised use of *Takra* as *Anupan* of various *Ghrit* and churn such as *Triyushanadyaghrit* etc. Further *Vagbhatt* has advised *Takra* as *Pathya* for patients of *Kaphaj Gulm* (Agnivesh, 2012; Vagbhatt, 2007)
9. ***Arsh*** (piles): *Carak* has used Takra in the management of *Arsh* on a broad level. *Carak* has advised use of *Haritaki* (*Terminalia chebula*) and *Triphala* (three myrobalans) churn with *Takra*. *Carak* has advised special formulation of *Takra*, namely *Takrarisht* in the management of *Arsh*. *Carak* has clearly said that there is no better medicine than Takra for the management of *Vaat- Kaphaj Arsh*. *Carak* has further advised that, all those food materials that causes *Vaatanuloman* (promotes excretionof flatus) and are *Agnideepak* (improves digestion) should be used for *Arsh* patients and *Takra* possesses both the qualities. *Vagbhatt* has advised use of *Takra* as *Anupan* of fruits of *Peelu* (*Salvadora persica*). Further *Vagbhatt* has advised use of *Takra* mixed with *Hapusha* (*Juniperus communis*), *Hingu* (*Ferula narthex*) and *Chitrak* (*Plumbago zylenica*) (Agnivesh, 2012; Vagbhatt, 2007).
10. ***Grahni** Dosh* (sprue): *Carak* has described the properties of *Takra* in the *Grahnidoshchikitsaadhyay*. *Carak* has stated that *Takra* is best for management of *Grahni dosh* due to *Deepan* (stomachic), *Grahi* and *Laghu* (light to digest) properties of *Takra*. Further, *Carak* has stated that all types of *Takra* should be used extensively for the management of *Grahnidosh*. *Takra* is component of various formulations mentioned for management of *Grahnidosh* such as *Takrarisht*, *Panchmoolyadhya ghrit* and Churn. *Sushrut* has also stated that in management of *Grahni*, Churn of *Pachniya*, *Sanghrahi* and *Deepniyagana* should be used with *Anupan* of *Takra* or only *Takra* is enough to treat the diseases (Agnivesh, 2012; Sushrut, 2013)
11. ***Anupan*** (vehicle) of various formulations: Apart from these direct therapeutic indications, *Takra* has also been indicated as *Anupan* (vehicle) for various formulations in *Ayurved*. *Anupan* is any liquid with which medicine is taken orally or which is drink after taking medicine. *Anupan* (vehicle) helps in quick assimilation and distribution of drug in body. Various formulations whose *Anupan* is *Takra* are *Trivrittadikalk*, *Laghugangadhar churn*, *Narayan churn*, *Navayasloha* and *Mandurvatak* (Sharma, 2006).

2.8.4. <u>Utilization of buttermilk</u> (Chaudhari *et al*., 2018)

Sweet cream buttermilk (SCBM) is used to prepare different type of cheeses like cheddar cheese, processed cheese (Joshi and Thakar, 1996; Gokhale *et al*., 1999; Shodjaodini *et al*., 2000) and is also used to substitute skim milk powder in the preparation of yoghurt (Trachoo and Mistry, 1998). SCBM used in traditional Indian dairy products like channa, paneer, shrikhand, basundi, rasogolla etc. (Sharma *et al*., 1998; Karthikeyan *et al*., 1999 and 2000; Patel and Upadhyay, 2004; Kumar, 2006). Many researchers had prepared beverages using buttermilk added with fruit juice or fruit pulp (Shukla *et al*., 2004; Kankhare *et al*., 2005). Prajapati *et al*. (2014) developed technology for manufacturing of burfi from sweet cream buttermilk standardized to 6% fat and by addition of 34% sugar to buttermilk khoa. Jana and Kadiya (2015) standardized the manufacturing of kulfi replacing 20% of milk solids with sweet cream buttermilk solids.

2.8.5. SAMBHARAM

Sambharam, is Kerala-style buttermilk flavoured with curry leaves, green chillies, shallot and ginger.

Table 13: Nutritional Composition of *Sambharam*

Nutrients	Values
Calories	82 kcal
Carbohydrates	7 gm
Protein	4 gm
Fat	4 gm
Saturated Fat:	2 gm
Cholesterol	16 mg
Sodium	685 mg
Potassium	193 mg
Sugar	6 gm
Vitamin A	265 IU
Vitamin C	77 mg
Calcium	172 mg
Iron	0.1 mg

(Source: Anjali Rao, 2022)

3. MATERIALS AND METHODS

This chapter describes the study material and methodologies employed during the present investigation. The study was carried out at the Department of Dairy Technology, Varghese Kurian Institute of Dairy and Food Technology, Kerala Veterinary and Animal Sciences University, Mannuthy.

3.1. RAW MATERIALS AND EQUIPMENTS

3.1.1. Procurement of raw materials:

1. Milk: Pasteurised cow milk procured from the university dairy plant Mnnuthy.
2. Culture: Culture is procured from Microbiology lab of department of microbiology in the VKIDFT, Mannuthy.
3. Shallot (*Allium ascalonicum L*): Good quality shallot was procured from the local market, Mannuthy.
4. Ginger (*Zingiber officinale Roscoe*): Good quality ginger was procured from the local market, Mannuthy.
5. Green chilly (*Capsicum frutescens*): Fresh, good quality chilly was procured from the local market, Mannuthy.
6. Curry leaf (Murraya koenigii): Fresh curry leaves was procured from the local market, Mannuthy.
7. Sait: commercially available powdered salt obtained from the market was used in the preparation of the product.

3.1.2. Equipments and manufacturer:

1. Micro Kjeldhal unit: Kelplus supra-LX VA
2. Hot air oven: LABTOP
3. Muffle Furnace: HINDUSTAN
4. Precision Balance: SAFRON
5. Incubator

3.2. TECHNOLOGICAL METHODOLOGY

3.2.1. Preparation of *Sambharam*

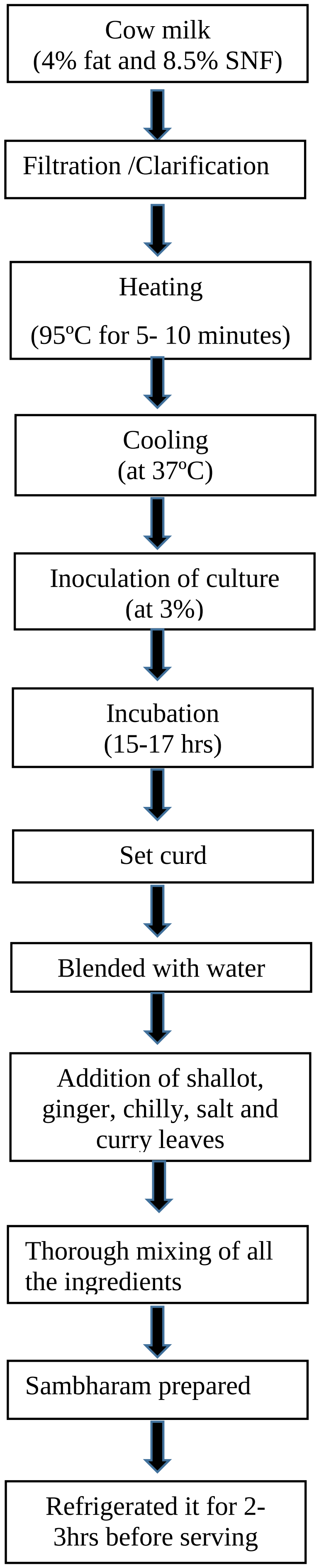
The sambharam was prepared according to the procedure done by Vineetha et al., 2015 with slight modifications. Curd was prepared by incorporating bacterial culture into the heated and cooled milk. The ingredients such as ginger (Zingiber officinle Roscoe), chilly (Capsicum frutescens) and shallot (Allium ascalonicum L.) after washing were crushed for making spicy mix using grinder. This spicy mix and fresh, chopped curry leaves (Murraya koenigii) were added into the curd water mix. Salt was added as a final step. Hence the *sambharam* is ready.

Fig 1: Milk

Fig 2: Shallot, chilly, ginger and curry leaves

Fig 3: Sambharam

Fig 4: Flow chart of *sambharam* preparation:



3.3. PHYSICO-CHEMICAL ANALYSIS

The chemical methods employed are described below:

3.3.1. MOISTURE

Fig 5: Hot Air Oven

Moisture content was determined according to AOAC 2000.

Apparatus

1. Flat bottom dish
2. Hot air oven

Procedure

1. Dry an empty dish in the oven for 105°C for 3 hours and cool it in the desiccator then take the empty weight of the dish.
2. Weigh about 3g sample into the dish and spread the sample uniformly.
3. Place the dish with sample into the hot air oven for about 3 hours at 105°C.
4. Take the dish, cool it in the desiccator and take the weight.
5. Repeat the process till similar readings are obtained.

Calculation

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

Where,

W1 = Weight (g) of sample before drying.

W2 = Weight (g) of sample after drying.

3.3.2. TOTAL SOLIDS

The total solid content includes both the suspended and dissolved salts. The total solid content also used to determine a sludge dry weight expressed as a percentage. The total solid content is expressed as a ratio of weights obtained before and after drying process.

Calculation

Total solids% = 100 – moisture %

3.3.3. FAT

Fig 5: Mojonnier flask

Fat percentage in prepared Sambharam was estimated according to ISI 1983 by Mojonnier method.

Apparatus

1. Mojonnier flask with lid.
2. Beakers

Reagents

1. Ammonia solution: approximately 25%.
2. Ethyl alcohol.
3. Diethyl ether.
4. Light petroleum ether.

Procedure

1. Weigh 1g of sample into a beaker.
2. Add 8mL of hot distilled water and 3 mL of ammonia solution and shake well.

3. Add 10 mL ethyl alcohol and mix well and transfer the contents into the Mojonnier fat extraction flask.
4. Add 25 mL diethyl ether and shake well.
5. Add 25 mL light petroleum and shake well.
6. Allow the apparatus to stand for a while until the upper layer has become clear and is distinctly separated from the aqueous layer.
7. Decant the solution and repeat the steps for 4 times by using 15 mL diethyl ether and light petroleum instead of 25 ml.
8. Evaporate the solvent.
9. Dry in a pre-dried dish in hot air oven at 100°C for 1 hour.
10. Cool in desiccator and measure the value.

Calculation

$$\text{Fat (\%)} = \frac{[(\text{weight of dish + fat}) - (\text{weight of dish})]}{\text{Weight of sample}}$$

3.3.4. **PROTEIN**

Fig 6: Micro Kjeldhal Apparatus

The total protein content in herbal paneer spread was determined by Kjeldhal method AOAC 2000.

Apparatus

1. Kjeldhal apparatus.
2. Beakers.
3. Standard flask.

Reagents

1. Kjeldhal catalyst: Mix 9 part of potassium sulphate (k_2SO_3) with 1 part of copper sulphate ($CuSO_4$)
2. Sulphuric acid (H_2SO_4).
3. 40% NaOH
4. 0.2 N HCl
5. 4% Boric acid
6. Indicator solution: Mix 100 mL of 0.1% methyl red (in 95% ethanol) with 200 mL of 0.2% bromocresol green (in 95% ethanol).

Procedure

1. Place sample (0.5 - 1.0 g) in digestion flask.
2. Add 5g Kjeldhal catalyst and 200 mL of con. H_2SO_4. Prepare a tube containing the above chemical except sample as blank.
3. Place the flasks in an inclined position and heat gently unit frothing ceases. Boil it until solution clears.
4. Cool the solution and add 60 mL of distilled water carefully.
5. Then immediately connect the flask to digestion bulb on condenser and condenser tip immersed in standard acid and 5 - 7 drops of mix indicator in receiver. Then rotate the flask to mix the content thoroughly and heat the content until all ammonia NH_3 is distilled.
6. Remove the receiver, wash tip of condenser and titrate excess standard acid distilled with standard NaOH solution.

Calculation

$$\text{Protein (\%)} = \frac{(A-B) \times N \times 14.007 \times \text{volume made up of digest} \times 100 \times 6.3}{\text{Aliquot of digest taken} \times W \times 10}$$

Where,

A = volume (mL) of 0.2 N HCl used sample titration.

B = volume (mL) of 0.2 N HCl used blank titration.

N = Normality of HCl.

W = weight (g) of sample.

14.007 = atomic weight of Nitrogen

1.3.6. ASH

Fig 7: Muffle Furnace

Principle

Heating the Sambharam at higher temperature decomposes organic matter and soluble inorganic salts are left behind in the form of ash.

Apparatus

1. Silica crucible.
2. Desiccator.
3. Muffle furnace.
4. Bunsen burner.

Procedure

1. Weigh 3g of sample in a silica crucible.
2. Heat it in Bunsen burner until the smoke coming out is getting stopped.
3. Transfer the dish into a muffle furnace and gradually increase the temperature of furnace until 550°C.
4. Heating is continued for about 4 - 5 hours until the residue in dish is free from black carbon particles.
5. Cool it in the desiccator and weigh accurately.
6. Repeat heating for 1 hour, cool in desiccator and weigh again.

Calculation

$$\text{Ash (\%)} = \frac{\text{Weight of ash} \times 100}{\text{Weight of sample taken}}$$

3.3.7. TITRATABLE ACIDITY

Apparatus

1. Burette
2. Beaker

Reagents

1. Phenolphthalein indicator
2. 0.1 N NaOH

Procedure

1. Weigh about 10g of well mixed sample into a 100 mL beaker.
2. Add 10 mL of hot distilled water and mix well.
3. Add few drops of phenolphthalein indicator.
4. Titrate against 0.1N NaOH with continuous stirring till a faint pink colour persisting for 30 seconds.

Acidity was expressed as lactic acid per 100 grams of product.

Acidity as percent lactic acid = (V/W) × 0.9

Where,

V= Volume of 0.1 N NaOH required for titration.

W= Weight of the sample (grams).

3.4. MICROBIOLOGY TESTS

3.4.1. SANDARD PLATE COUNT

Standard plate count of Sambharam spread was estimated by pour plate technique. From the selected tenfold dilution of sample, one millilitre of the inoculum was transferred in to duplicate Petri dishes of uniform size. To each inoculated plate about 20-25ml sterile molten nutrient agar maintained at 45ºc was poured and mixed with the inoculum, by gentle rotatory movement i.e., clockwise and anticlockwise, forward and backward. The inoculated plates were left at room temperature and allowed to solidify, and incubated at 37ºc for 48hours. At the end of incubation, lates showing between 30 and 300 colonies were selected and count were taken with the help of a colony counter. The number of colonies forming units (cfu) per ml of sample was calculated by multiplying the mean colony count in duplicate plates with the dilution factor.

3.4.2. COLIFORM COUNT

Coliform count of Sambharam was estimated by pour plate technique. From the selected tenfold dilution of sample, one millilitre of the inoculum was transferred in to duplicate Petri dishes of uniform size. To each inoculated plate about 20-25ml sterile molten VRBA agar (Violet Red Bile Agar) maintained at 45ºC was poured and mixed with the inoculum, by gentle rotatory movement i.e., clockwise and anticlockwise, forward and backward. The inoculated plates were left at room temperature and allowed to solidify, and incubated at 37ºC for 48hours. At the end of incubation, lates showing between 30 and 300 colonies were selected and count were taken with the help of a colony counter. The number of colonies forming units (cfu) per ml of sample was calculated by multiplying the mean colony count in duplicate plates with the dilution factor.

3.5. EXPERIMENTAL DESIGN

Response surface methodology (RSM) of Design-Expert Software (version 13) was used for the optimization of sambharam, The ranges for experimental parameters were selected based on preliminary tials.The process variables considered for optimization were levels of water, shallot and ginger. The response to variation in process parameters was measured in terms of colour and appearance, flavour, consistency, sourness, saltiness, spiciness and overall acceptability. Experimental design was applied after selection of ranges. Twenty experiments were performed with three independent variables. The data collected from the preliminary analysis were statistically analyzed. The data obtained from sensory analysis were compared using t test.

4. RESULT

The present study was carried out to standardize the process and optimize the ingredients for the preparation of *Sambharam*. Optimization levels of ingredients such as shallot, ginger and water was undertaken in the first part of the study. It is based on the sensory attributes by using RSM software. In the second phase, sensory, physico-chemical and microbiological attributes of optimized product was analysed.

4.1. SELECTION OF MAXIMUM AND MINIMUM LEVELS OF SHALLOT, GINGER AND WATER IN THE PREPARATION OF *SAMBHARAM*

In the preliminary trials, the ingredients such as shallot, ginger and water for the preparation of *Sambharam* were analyzed at different levels. The product was optimized on the basis of sensory analysis viz., colour and appearance, flavour, sourness, saltiness, consistency, spiciness and overall acceptability by judging panel consisting of five trained judges. The statistical analysis for sensory scores was done using RSM software

4.1.1. Selection of levels of Water in *Sambharam*

The preliminary trials were conducted by preparing *Sambharam* with addition of 2 different levels of water ie,100 per cent and 200 per cent is based on different local videos available in the internet which is dealing with the preparation of *Sambharam*. The levels of water were fixed as 100 per cent (as lower limit) and 200 per cent (as upper limit). The resultant products were used for sensory attributes by a panel of expert judges. The sensory scores were subjected to statistical analysis.

4.1.2. Selection of levels of Shallot in *Sambharam*

The preliminary trials were conducted by preparing *Sambharam* with addition of different levels of shallot (2%, 2.5%, 3.5% and 4%) is based on different local videos available in the internet which is dealing with the preparation of *Sambharam.* The levels of shallot were fixed as 2 per cent (as lower limit) and 4 per cent (as upper limit). The resultant products were used for sensory attributes by a panel of expert judges. The sensory scores were subjected to statistical analysis.

4.1.3. Selection of levels of Ginger in *Sambharam*

The preliminary trials were conducted by preparing *Sambharam* with addition of different levels of ginger (2%, 2.5%, 3.7% and 4%) is based on different local videos available in the internet which is dealing with the preparation of Sambharam. The levels of ginger were fixed as 2 per cent (as lower limit) and 4 per cent (as upper limit). The resultant products were used for sensory attributes by a panel of expert judges. The sensory scores were subjected to statistical analysis.

4.2. OPTIMIZATION OF LEVELS OF SHALLOT, GINGER AND WATER BY RESPONSE SURFACE METHODOLOGY

Sambharam was prepared by incorporating selected levels of shallot, ginger and water. These levels were selected on the basis of preliminary trials. The design matrix representing different combinations of the three factors are presented in the table 14

4.2.1. Effect of different levels of water, shallot and ginger on sensory attributes of *Sambharam*

4.2.1.1 Diagnostic check of the Quadratic model

The sensory responses viz., colour and appearance, flavour, consistency, sourness, saltiness, spiciness and overall acceptability as a result of proposed experimental design (Table 15) obtained as a result of proposed experimental designs which were subjected to regression analysis in order to assess the effect of levels of water, shallot and ginger on the sensory attributes of *Sambharam*. A second order polynomial regression model for the dependent variables was established to fit the experimental data for each response. Regression models developed from the experimental data were found to be significant with the observed p-values. The partial regression coefficients of linear, quadratic and interaction terms for each model and their R^2 values are shown in the Table 16

Table 14: Response surface methodology design for three factors

Run order	Factor 1 A: Water (%)	Factor 2 B: Shallot (%)	Factor 3 C: Ginger (%)
1	65.9104	3	3
2	150	3	4.68179
3	150	3	3
4	150	3	3
5	200	4	4
6	150	3	3
7	150	3	1.31821
8	150	3	3
9	200	2	2
10	100	4	4
11	234.09	3	3
12	150	4.68179	3
13	200	4	2
14	150	1.31821	3
15	100	2	4
16	100	2	2
17	150	3	3
18	200	2	4
19	100	4	2
20	150	3	3

Table 15: **Sensory characteristics of *Sambharam* with different levels of shallot, ginger and water**

Run No.	Response 1	Response 2	Response 3	Response 4	Response 5	Response 6	Response 7
	Colour & Appearance	**Flavour**	**Consistency**	**Sourness**	**Saltiness**	**Spiciness**	**Overall Acceptability**
1	7.14	7.8	7.6	7.21	7.4	7.1	7.7
2	7.4	6.74	6.8	6.8	7.5	7.2	6.8
3	7.4	6.9	7.5	7.5	7.1	7.1	7.2
4	7.35	7.2	7.4	7.4	7.2	7.1	6.9
5	7	6.4	6.3	6.6	7.1	6.7	6.4
6	7.42	6.8	7.4	7.6	7.1	7	7.2
7	7.51	7.3	7.5	7.2	7.42	7.2	7.4
8	7.51	7	7.2	7.4	7.2	7.2	7
9	7.2	7	7.4	7	6.9	7.32	7.4
10	7.5	7.1	7	6.9	7	7.2	7.35
11	6.41	6.5	6.6	6.6	6.9	6.8	6.5
12	7.57	6.8	6.8	6.8	6.6	6.5	6.9
13	6.9	6.7	7	7	7	6.8	6.5
14	7.7	7.2	7.6	7.1	7	7	7.4
15	7.4	7.4	7.6	7.17	7.5	7.3	7.3
16	7.6	7.6	7.58	7.3	7.5	7.2	7.6
17	7.4	7	7.6	7.6	7.2	7	7
18	7	6.5	6.74	6.5	7	6.74	6.8
19	7.4	7.5	7.4	6.9	7	6.7	7.5
20	7.45	6.9	7.4	7.5	7.1	7.1	7.3

Table 16. **Estimated parameters model for sensory attributes and responses of Sambharam with different levels of shallot, ginger and water**

Partial coefficient	**Sensory characteristics**						
	Colour and appearaence	**Flavour**	**Consistency**	**Sourness**	**saltiness**	**spiciness**	**Overall acceptab-ility**
Intercept	7.42	6.97	7.24	7.50	7.15	7.08	7.10
A-Water	-0.2217**	-0.3798**	-0.2798**	-0.1608**	-0.1348**	-0.0985**	-0.3418**
B-Shallot	-0.0453*	-0.1078*	-0.2171**	-0.0787**	-0.1078**	-0.1465**	-0.1604**
C-ginger	-0.0282ns	-0.1715**	-0.2136**	-0.1247**	+0.0245ns	-0.0059ns	-0.1581**
AB	-0.0250ns	+0.0000ns	-0.0075ns	+0.0962**	+0.1500**	+0.0050ns	-0.1562**
AC	+0.0000ns	-0.0250ns	-0.1225**	-0.0963**	+0.0250ns	-0.1600**	-0.0312ns
BC	+0.0750**	-0.0000ns	-0.0575ns	+0.0288ns	+0.0000ns	+0.1100**	+0.0813ns
A^2	-0.2350**	+0.0570ns	-0.1133**	-0.2098**	-0.0023ns	-0.0397*	-0.0024ns
B^2	+0.0690**	+0.0040ns	-0.0779*	-0.1939**	-0.1260**	-0.1104**	+0.0153ns
C^2	+0.0054ns	+0.0110ns	-0.0956**	-0.1762**	+0.1073**	+0.0487*	-0.0024ns
Lack of fit	0.9196ns	0.9048ns	0.9749ns	0.9002ns	0.9347ns	0.9430ns	0.9523ns
Model F value	101.62**	23.99**	27.55**	52.65**	62.61**	31.46**	19.77**
R^2	0.9892	0.9557	0.9612	0.9793	0.9826	0.9659	0.9468
Press	0.0495	0.3651	0.3496	0.1003	0.0476	0.0875	0.3730
Adeq. Press	43.1120	18.2589	18.8336	21.1047	29.9832	18.7316	15.6493

*- Significant at five per cent level ($p<0.05$), **- Significant at one per cent level ($p<0.01$),
ns- Non significant ($p\geq0.05$)

4.2.1.2. Effect on Colour and Appearance

The sensory scores for colour and appearance and partial regression coefficients are shown in Table 15. Figures (8a, 8b &8c) illustrates the 3D response surface graphs obtained for colour and appearance. The model showed a significant F-value of 101.62 whereas lack of fit was found to be non-significant. A coefficient of determination (R^2) of 98 per cent was obtained which indicated 98per cent variations in the response shown by the variables in the model. Adequate precision ratio of 43.11was obtained which indicates an adequate signal.

The following response surface equation was generated to forecast the variation in colour and appearance with various amounts of factors:

Colour and Appearance= $7.42 - 0.2217*A - 0.0453*B - 0.0282*C - 0.0250*AB + 0.0000*AC + 0.0750*BC - 0.2350*A^2 + 0.0690*B^2 + 0.0054*C^2$

4.2.1.3. Effect on Flavour

The sensory scores for flavour and partial regression coefficients are shown in Table 15. Figures (9a, 9b &9c) illustrates the 3D response surface graphs obtained for flavour. The model showed a significant F-value of 23.99 whereas lack of fit was found to be non-significant. A coefficient of determination (R^2) of 95 per cent was obtained which indicated 95 per cent variations in the response shown by the variables in the model. Adequate precision ratio of 18.25 was obtained which indicates an adequate signal.

The following response surface equation was generated to forecast the variation in colour and appearance with various amounts of factors:

Flavour= $6.97 - 0.3798*A - 0.1078*B - 0.1715*C + 0.0000*AB - 0.0250*AC - 0.0000*BC + 0.0570*A^2 + 0.0040*B^2 + 0.0110*C^2$

4.2.1.4 .Effect on consistency

The sensory scores for Consistency and partial regression coefficients are shown in Table 15. Figures (10a, 10b &10c) illustrates the 3D response surface graphs obtained for flavour. The model showed a significant F-value of 27.55 whereas lack of fit was found to be non-significant. A coefficient of determination (R^2) of 96 per cent was obtained which indicated 96per cent variations in the response shown by the variables in the model. Adequate precision ratio of 18.83 was obtained which indicates an adequate signal.

The following response surface equation was generated to forecast the variation in consistency with various amounts of factors:

Consistency= 7.24 -0.2798*A -0.2171*B -0.2136*C -0.0075*AB -0.1225*AC -0.0575*B -0.1133*A^2 -0.0779*B ^{2}-0.0956*C^2

4.2.1.5. Effect on sourness

The sensory scores for sourness and partial regression coefficients are shown in Table 15. Figures (11a, 11b &11c) illustrates the 3D response surface graphs obtained for flavour. The model showed a significant F-value of 52.65 whereas lack of fit was found to be non-significant. A coefficient of determination (R^2) of 97 per cent was obtained which indicated 97 per cent variations in the response shown by the variables in the model. Adequate precision ratio of 21.10 was obtained which indicates an adequate signal.

The following response surface equation was generated to forecast the variation in sourness with various amounts of factors:

Sourness= 7.50 -0.1608*A -0.0787*B -0.1247*C +0.0962*AB -0.0963*AC +0.0288*BC -0.2098*A^2 -0.1939*B^2 -0.1762*C^2

4.2.1.6. Effect on Saltiness

The sensory scores for sourness and partial regression coefficients are shown in Table 15. Figures (12a, 12b &12c) illustrates the 3D response surface graphs obtained for flavour. The model showed a significant F-value of 62.61 whereas lack of fit was found to be non-significant. A coefficient of determination (R^2) of 98per cent was obtained which indicated 98 per cent variations in the response shown by the variables in the model. Adequate precision ratio of 29.98 was obtained which indicates an adequate signal.

The following response surface equation was generated to forecast the variation in saltiness with various amounts of factors:

Saltiness= 7.15 -0.1348*A -0.1078*B +0.0245*C +0.1500*AB +0.0250*AC +0.0000*BC -0.0023*A^2 -0.1260*B^2 +0.1073*C^2

4.2.1.7. Effect on Spiciness

The sensory scores for sourness and partial regression coefficients are shown in Table 15. Figures (13a, 13b &13c) illustrates the 3D response surface graphs obtained for flavour. The model showed a significant F-value of 31.46 whereas lack of fit was found to be non-significant. A coefficient of determination (R^2) of 96 per cent was obtained which indicated 96per cent variations in the response shown by the variables in the model. Adequate precision ratio of 18.73 was obtained which indicates an adequate signal.

The following response surface equation was generated to forecast the variation in spiciness with various amounts of factors:

Spiciness=7.08-0.0985*A-0.1465*B-0.0059*C+0.0050*AB-0.1600*AC+0.1100*BC-0.0397*A^2-0.1104*B^2+0.0487*C^2

4.2.1.8. Effect on Overall acceptability

The sensory scores for overall acceptability and partial regression coefficients are shown in Table 15. Figures (14a, 14b &14c) illustrates the 3D response surface graphs obtained for flavour. The model showed a significant F- value of 19.77 whereas lack of fit was found to be non-significant. A coefficient of determination (R^2) of 94 per cent was obtained which indicated 94per cent variations in the response shown by the variables in the model. Adequate precision ratio of 15.64 was obtained which indicates an adequate signal.

The following response surface equation was generated to forecast the variation in spiciness with various amounts of factors:

Overall acceptability= 7.10 -0.3418*A -0.1604*B -0.1581*C -0.1562*AB -0.0312*AC +0.0813*BC -0.0024*A^2 +0.0153*B^2 -0.0024*C^2

Fig.8: Response surface plots for colour and appearance score of sambharam added with water, shallot and ginger

Fig.8a

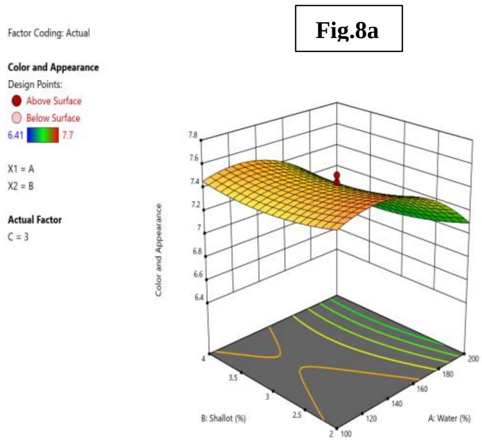


Fig.8b

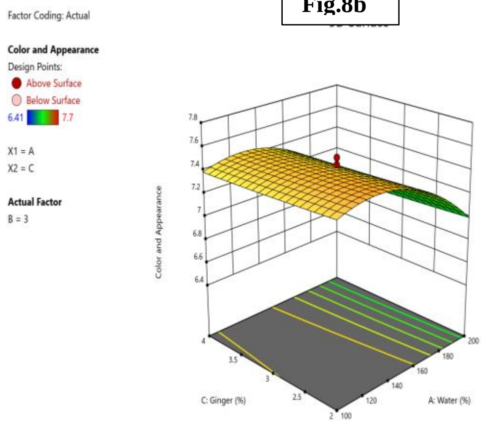


Fig.8c

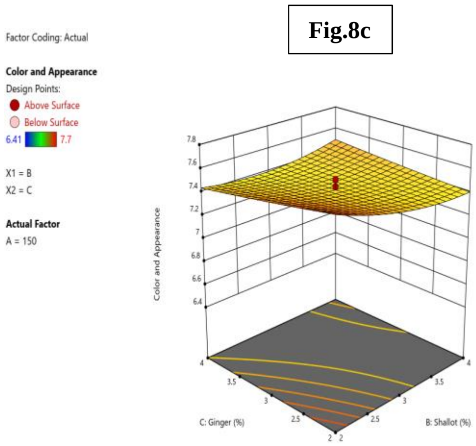


Fig.9: Response surface plots for flavour score of sambharam added with water, shallot and ginger

Fig.9a

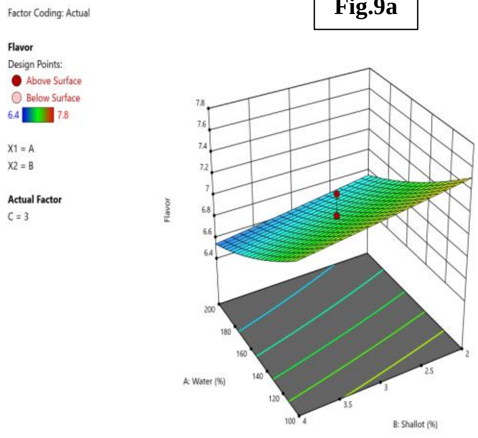


Fig.9b

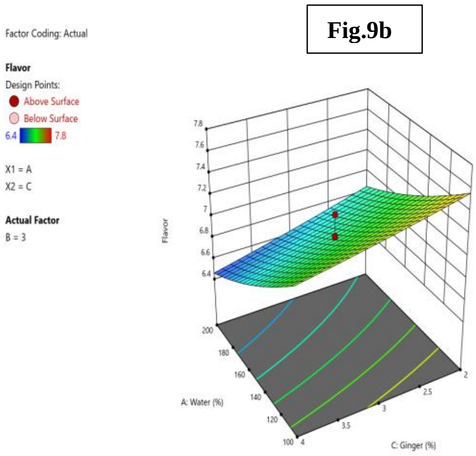


Fig.9c

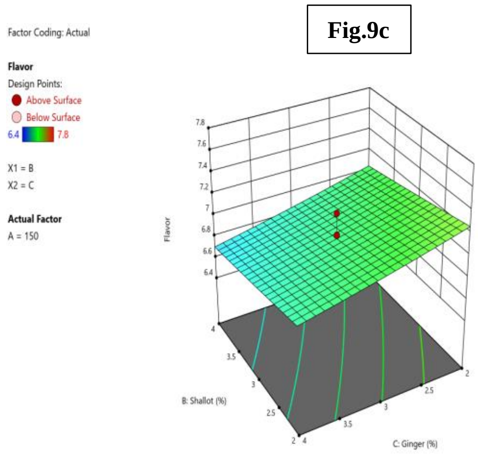


Fig.10: Response surface plots for consistency score of sambharam added with water, shallot and ginger

Fig.10a

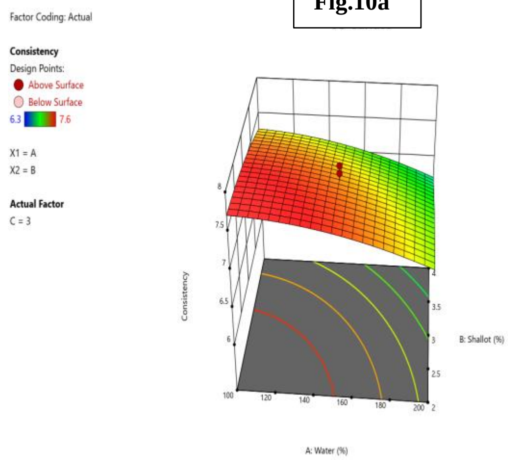


Fig.10b

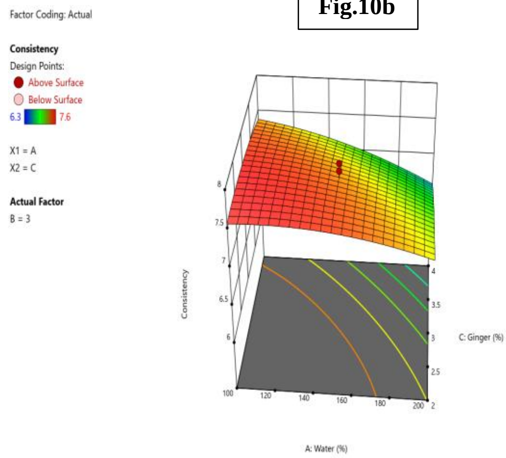


Fig.10c

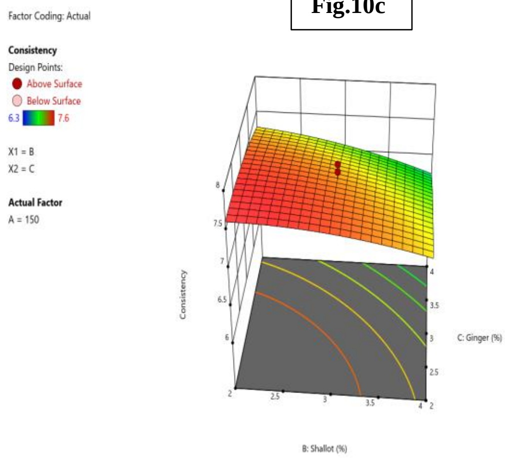


Fig. 11: Response surface plots for sourness score of sambharam added with water, shallot and ginger

Fig.11a

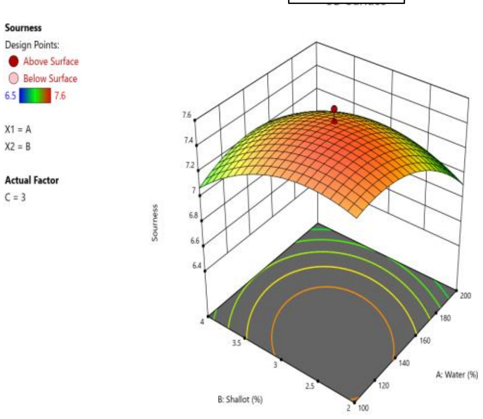


Fig.11b

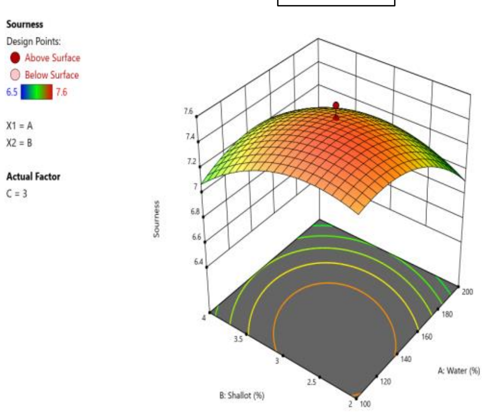


Fig.11c

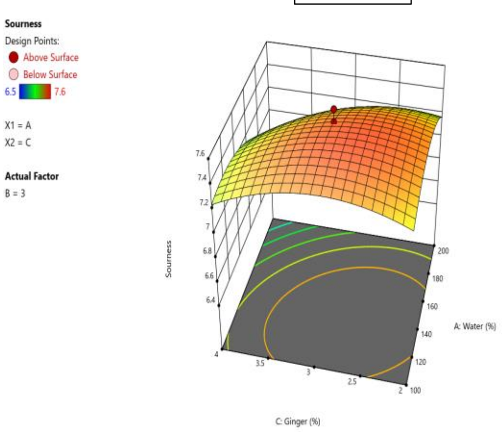


Fig.12: Response surface plots for saltiness score of sambharam added with water, shallot and ginger

Fig.12a

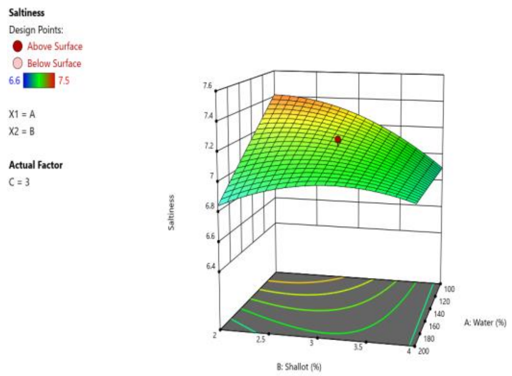


Fig.12b

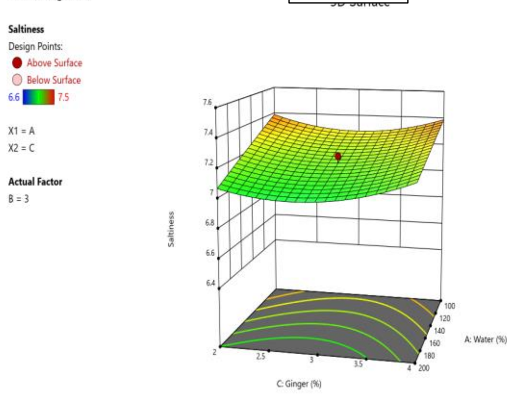


Fig.12c

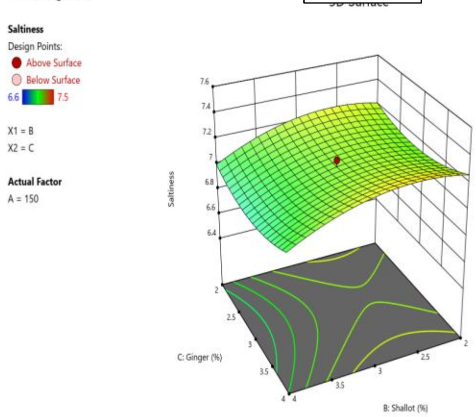


Fig.13: Response surface plots for spiciness score of sambharam added with water, shallot and ginger

Fig.13a

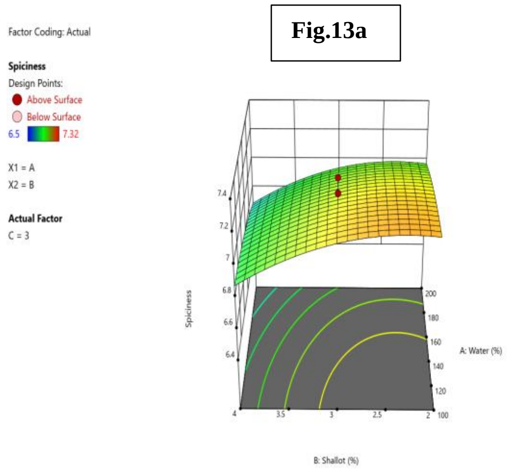


Fig.13b

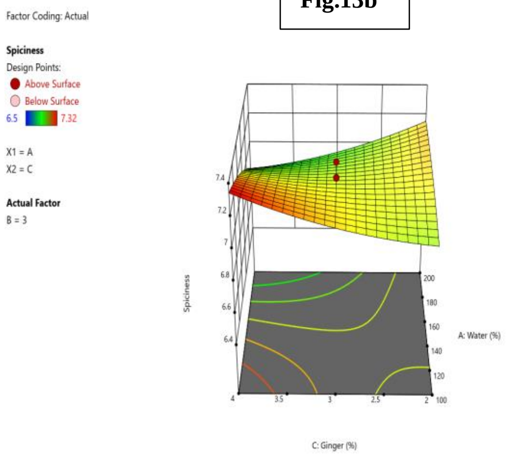


Fig.13c

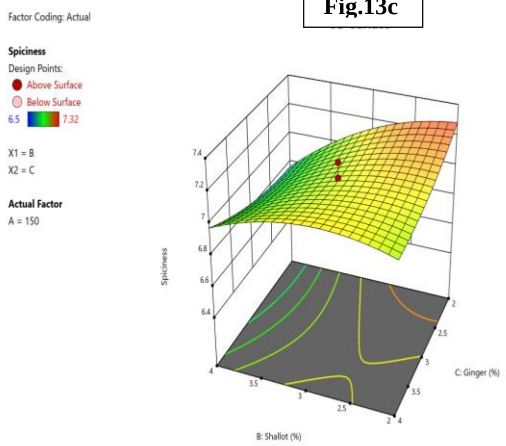


Fig.14: Response surface plots for overall acceptability score of sambharam added with water, shallot and ginger

Fig14a

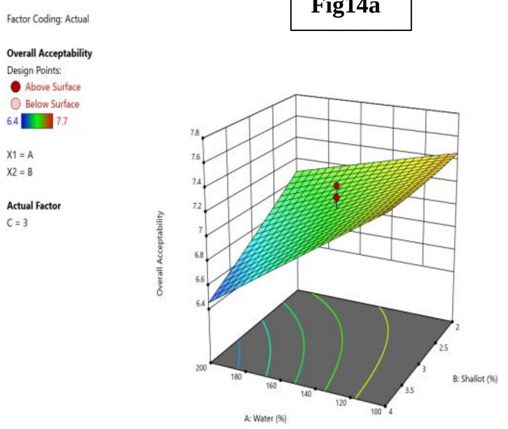


Fig.14b

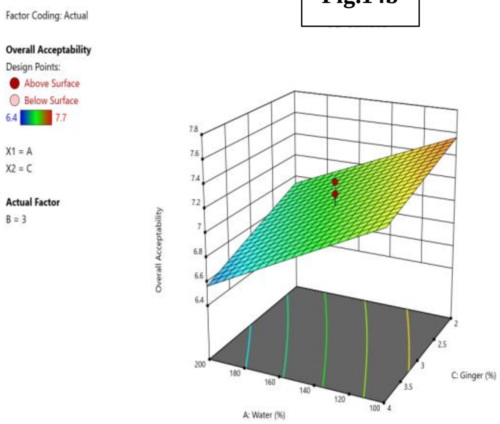
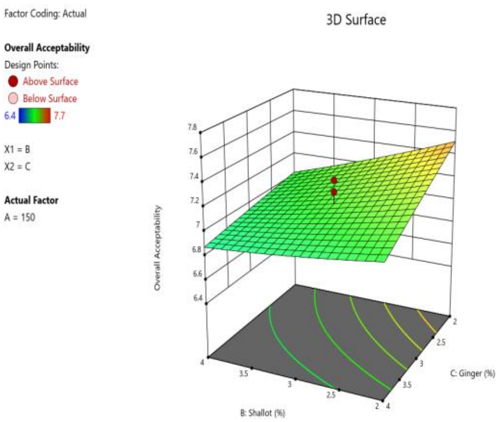


Fig.14c



4.2.1. Optimized Solutions and their Validation

Numerical optimization was carried out with the goal to obtain best feasible formulation of water, shallot and ginger in the preparation of *Sambharam*. The required objectives for all factors as well as responses were selected and different weights were designated to each goal to alter the shape of its specific function of desirability. Other factors were placed in range and responses were kept at maximum during the process of optimization. Solution obtained from RSM software is shown in Table 18.

Table 17: Constraints and criteria for optimization of sambharam with different levels of water, shallot and ginger

Constraints	**Goal**	**Lower limit**	**Upper limit**
A: Water	Is in range	100	200
B: Shallot	Is in range	2	4
C: Ginger	Is in range	2	4
Colour and appearance	Maximize	6.41	7.7
Flavour	Maximize	6.4	7.8
Consistency	Maximize	6.3	7.6
Sourness	Maximize	6.5	7.6
Saltiness	Maximize	6.6	7.5
Spiciness	Maximize	6.5	7.32
Overall acceptability	Maximize	6.4	7.7

Table 18: Solution obtained after response surface analysis

Sl.no	Water (%)	Shallot (%)	Ginger (%)	Desirability
1	103.679	2	2	0.910

4.2.2. Verification of the optimum formulations

Sambharam was prepared by using optimized combinations recommended by RSM and evaluated on the basis of sensory attributes. The results obtained were statistically analyzed using t-test with the corresponding predicted value. Closeness between the observed and predicted values is depicted in Table 19. It was noted that the difference between the observed values and predicted values were not significant in terms of all attributes.

Table 19: Verification of the Optimum Formulations

Attributes	Predicted value	Observed value	t-value
Colour and Appearance	7.62	7.40±0.15	2.982^{ns}
Flavour	7.64	7.36±0.19	2.619^{ns}
Consistency	7.65	7.40±0.18	0.898^{ns}
Sourness	7.33	7.43±0.07	-0.594^{ns}
Saltiness	7.50	7.03±0.33	3.856^{ns}
Spiciness	7.19	7.20±0.00	0.425^{ns}
Overall acceptability	7.65	7.56±0.06	1.844^{ns}

4.3. PHYSICO-CHEMICAL ANALYSIS OF *SAMBHARAM*

After optimization the optimized product is subjected to physico chemical analysis. Estimation of moisture, ash, total solids, fat and protein were included in the analysis part.

Table 20: Physico Chemical analysis of sambharam

Parameters	Value
Moisture (%)	93.264
Total Solids (%)	6.7359
Ash (%)	1.4417
Fat (%)	2.35
Protein (%)	2.0988

4.4. MICROBIOLOGICAL ANALYSISM

The factors which affect the microbial quality of *Sambharam* includes, quality of raw materials, hygiene of the equipment as well as personnel hygiene maintained during the preparation. Standard Plate count (SPC) and Coliform count of *Sambharam* were enumerated in the table.

Table 21: Microbiological quality of *Sambharam*

Microbial parameter	Result
Standard Plate Count (SPC)	1.9×10^6 cfu/gm
Coliform Count	Nil

5. DISCUSSION

Sambharam is a healthy refreshing drink. In this current study, attempts were taken to develop a standardized process for the preparation of *Sambharam*. The data obtained during the course of study was statistically analysed and the results are discussed in this chapter.

5.1. SELECTION OF MAXIMUM AND MINIMUM LEVELS OF WATER, SHALLOT AND GINGER FOR THE PREPARATION OF SAMBHARAM

5.1.1. Selection of levels of water in Sambharam

Purified water is used for the preparation of *Sambharam*. Initial and the preliminary trials revealed that lower levels of water in *Sambharam* is acceptable. The minimum and maximum levels of water in *Sambharam* was selected as 100% and 200% respectively.

5.1.2. Selection of levels of shallot in Sambharam

Shallot was added in the *Sambharam* by cutting it into small pieces and grinded it with other ingredients. From the preliminary trials it was understood that lower levels of shallot was acceptable in the product. The minimum and maximum levels of addition of shallot were selected as 2% and 4%, respectively.

5.1.3. Selection of levels of ginger in Sambharam

Ginger was added in the *Sambharam* by cutting it into small pieces and grinded it with other ingredients. From the preliminary trials it was understood that lower levels of Ginger was acceptable in the product. The minimum and maximum levels of addition of Ginger were selected as 2% and 4%, respectively.

5.2. OPTIMIZATION OF QUANTITY OF WATER, SHALLOT AND GINGER BY RESPONSE SURFACE METHODOLOGY

5.2.1. Effect of Different Levels of Water, Shallot and Ginger on the Sensory Attributes of Sambharam

The minimum and maximum levels of water, shallot and ginger were selected as 100 per cent and 200 per cent, 2per cent and 4 per cent, 2 per cent and 4 per cent, respectively, from the statistical analysis of preliminary trial data. On feeding these levels to RSM software, the output showing upper and lower limits of ingredients and the central composite rotatable design of three factors constituting 20 runs including lower and upper limits were obtained. The responses of the sensory attributes are exhibited in the table 14.

5.2.1.1. Diagnostic Check of the Quadratic Model

In the quadratic model recommended by RSM, F values for all the sensory characteristics were greater than tabled F-values ($p<0.01$) showing the developed model is significant. The coefficient of determination (R^2) for colour and appearance, flavour, consistency, sourness, saltiness, spiciness and overall acceptability was found to be 0.9892, 0.9557, 0.9612, 0.9793, 0.9826, 0.9659 and 0.9468 respectively, revealed that the quadratic model indicated more than 80 per cent of the deviation in the data. The test of the lack of fit was found to be non-significant in all the cases and it was obvious that the model is valid for forecasting the sensory properties of *sambharam* prepared with any group of variables within the limit appraised.

5.2.1.2. Effect on Colour and Appearance

The sensory scores obtained for the colour and appearance of *Sambharam* ranged from 6.41 to7.7. Trial no. 1 and 14 have got the maximum score while minimum score obtained for the trial no. 5. By successive regression analysis, quadratic model for colour and appearance was obtained. The effect of three factors (water, shallot and ginger) on colour and appearance is detailed in the table 15. The F value for the colour and appearance in the table was more than the tabled value. Acceptance of the response, colour and appearance to guide the design is indicated by the value obtained for the determination coefficient (R^2) 0.9892 with satisfactory precision 43.11. Since the F value for lack of fit test was non-significant, it was clear that the model is authentic enough for forecasting the *sambharam*. The p values for the factors indicated that there is significant impact for water, shallot and ginger on the colour and appearance score of *Sambharam*. The colour and appearance value for ginger 2% level is considered as good according to sensory core. When the level increases the score decreases. Gaur *et al.* (2019) stated that addition of 3% level of ginger juice in herbal milk will significantly affect the colour and appearance of the product. Jadhav *et al.* (2017) found that the score for general appearance increased up to 5% addition of ginger juice and, thereafter, score was declined simultaneously. Sharma *et al.* (2020) studied the addition of ginger powder in chhana whey beverage and found that treatment with 0.3% ginger powder was superior among all other treatments under study. These two studies shows results similar to our findings. Simon *et al.* (2018) found no difference in the sensory evaluation of colour value of different treatments with ginger, It is contradictory to our findings.

5.2.1.3. Effect on Flavour

The sensory scores obtained for the flavour of *Sambharam* ranged from 6.4 to 7.8. Trial no. 1 have got the maximum score while minimum score obtained for the trial no. 18. By successive regression analysis, quadratic model for flavour was obtained. The effect of three factors (water, shallot and ginger) on flavour is detailed in the table 15. The F value for the flavour in the table was more than the tabled value. Acceptance of the response, flavour to guide the design is indicated by the value obtained for the determination coefficient (R^2) 0.9557 with satisfactory precision 18.25. Since the F value for lack of fit test was non-significant, it was clear that the model is authentic enough for forecasting the *Sambharam*. The p values for the factors indicated that there is significant impact for water, shallot and ginger on the flavour score of *Sambharam*. 2% level of ginger is considered as good for the flavour score. Gaur *et al.* (2019) found that the flavour scores improved significantly by the addition of ginger juice being highest for 3% level of ginger juice in accordance with the study of development of herbal milk using ginger juice, tulsi juice and turmeric. It is similar to our findings. Agrawal *et al.* (2016) reported that use of ginger juice in ice cream increased its flavour preference, when added up to 4% only. It is contradictory to our findings. Addition of the juice at 5% level led to decrease in flavour score. Pinto *et al.* (2004) reported flavour preference for ginger juice when added at the rate of 4%, 3%, 2% and 1% in ice cream when judged against vanilla as control. In another study, Pinto *et al.* (2010) reported increase in flavour when treated ginger shreds were added up to 6%. Sharma *et al.* (2020) reported that the flavour score for chhana whey beverage incorporated with ginger was increased with the addition of ginger powder in the range from 0.1%- 0.3%. Simon *et al.* (2018) reported that addition of ginger juice at the rate of 4 per cent was found to improve the flavour score.

5.2.1.4. Effect on Consistency

The sensory scores obtained for the consistency of *Sambharam* ranged from 6.3 to 7.6. Trial no.1 have got the maximum score while minimum score obtained for the trial no.18. By successive regression analysis, quadratic model for consistency was obtained. The effect of three factors (water, shallot and ginger) on consistency is detailed in the table 15. The F value for the consistency was more than the tabled value. Acceptance of the response, consistency to guide the design is indicated by the value obtained for the determination coefficient (R^2) 0.9612 with satisfactory precision 18.83. Since the F value for lack of fit test was non-significant, it was clear that the model is authentic enough for forecasting the *Sambharam*. The p values for the factors indicated that there is significant impact for water, shallot and ginger on the consistency score of *Sambharam*. The consistency doesn't influence the consistency of the Sambharam. But, water is the reason for consistency. When water increases the product gets loose. 100% level is considered as good. Sharma *et al.* (2020) reported that the consistency of chhana beverage become thicker as level of ginger powder increased, it is contradictory to our findings. David (2014) reported that the scores for body and texture of ice cream did not differ significantly when ginger juice is incorporated at the rate of 2%, 4% and 6%, it is similar to our findings.

5.2.1.5. Effect on Sourness

The sensory scores obtained for the sourness of *Sambharam* ranged from 6.5 to 7.6. Trial no. 6 and 17 have got the maximum score while minimum score obtained for the trial no.18. By successive regression analysis, quadratic model for sourness was obtained. The effect of three factors (water, shallot and ginger) on sourness is detailed in the table 15. The F value for the sourness in the table was more than the tabled value. Acceptance of the response, sourness to guide the design is indicated by the value obtained for the determination coefficient (R^2) 0.9793 with satisfactory precision 21.10. Since the F value for lack of fit test was non-significant, it was clear that the model is authentic enough for forecasting the *Sambharam*. The p values for the factors indicated that there is significant impact for water, shallot and ginger on the sourness score of *Sambharam*. The value for sourness changes with levels of water. Singh and Kumar (2013) suggested that the Ginger and Mint extract increases the growth of yoghurt culture and they recommended that herbal extract can be used as growth promoter for yoghurt culture.

5.2.1.6. Effect on saltiness

The sensory scores obtained for the saltiness of *Sambharam* ranged from 6.6 to 7.5. Trial no. 16 have got the maximum score while minimum score obtained for the trial no.12. By successive regression analysis, quadratic model for saltiness was obtained. The effect of three factors (water, shallot and ginger) on saltiness is detailed in the table 15. The F value for the saltiness in the table was more than the tabled value. Acceptance of the response, saltiness to guide the design is indicated by the value obtained for the determination coefficient (R^2) 0.9826 with satisfactory precision 29.98. Since the F value for lack of fit test was non-significant, it was clear that the model is authentic enough for forecasting the *Sambharam*. The p values for the factors indicated that there is significant impact for water, shallot and ginger on the saltiness score of *Sambharam*. The values for saltiness influences the level of water used to prepare sambharam. Phan *et al*. (2008) and Bae et al. (2017) suggested that the lowering of salt and increasing the taste of cheese is to increase water and to decrease the content of fat.

5.2.1.7. Effect on spiciness

The sensory scores obtained for the Spiciness of *Sambharam* ranged from 6.5 to 7.32. Trial no.9 have got the maximum score while minimum score obtained for the trial no.17. By successive regression analysis, quadratic model for Spiciness was obtained. The effect of three factors (water, shallot and ginger) on Spiciness is detailed in the table 15. The F value for the Spiciness in the table was more than the tabled value. Acceptance of the response, Spiciness to guide the design is indicated by the value obtained for the determination coefficient (R^2) 0.9659 with satisfactory precision 18.73. Since the F value for lack of fit test was non-significant, it was clear that the model is authentic enough for forecasting the *Sambharam*. The p values for the factors indicated that there is significant impact for water, shallot and ginger on the Spiciness score of *Sambharam*. Score for spiciness depends on the level of water added. High spiciness score obtained for the water having low concentration. Rodríguez *et al.* (2016) reported that both water and the soft drink are polar substances; therefore, they were less effective easing the hotness caused by the sauce.

5.2.1.8. Effect on Overall acceptability

The sensory scores obtained for the Overall acceptability of *Sambharam* ranged from 6.4 to 7.7. Trial no.9 have got the maximum score while minimum score obtained for the trial no.13. By successive regression analysis, quadratic model for Overall acceptability was obtained. The effect of three factors (water, shallot and ginger) on Overall acceptability is detailed in the table 15. The F value for the Overall acceptability in the table was more than the tabled value. Acceptance of the response, Overall acceptability to guide the design is indicated by the value obtained for the determination coefficient (R^2) 0.9468 with satisfactory precision 15.64. Since the F value for lack of fit test was non-significant, it was clear that the model is authentic enough for forecasting the *Sambharam.* The p values for the factors indicated that there is significant impact for water, shallot and ginger on the Overall acceptability score of *Sambharam*. Low levels of water (100%), ginger (2%) and shallot (2%) influences the overall acceptability score. Jadhav *et al.* (2017) found that the score for overall acceptability increased up to 5% addition of ginger juice and, thereafter, score was declined simultaneously. Pinto *et al.* (2004) reported flavour and overall acceptability preference for ginger juice when added at the rate of 4%, 3%, 2% and 1% in ice cream when judged against vanilla as control. He reported overall acceptability score in ice cream prepared with 4% ginger juice was higher than rest of treatments. Palthur *et al*. (2014) reported that overall acceptability of the ginger flavoured herbal milk was found to be good and recommended for market exploration. Sharma *et al.* (2020) reported that the overall acceptability of chhana whey beverage sample significantly influenced due to the addition of ginger powder in chhana whey. These studies have results similar to our findings.

5.2.2. Optimized Solutions and their Validation

The constraints and criteria that had been generated by RSM for the optimization of sambharam is given in the table 16. During the optimization process all the factors were kept in range and the sensory scores were kept at maximum. The suggested solution for the preparation of sambharam is presented in the table 18. The optimum values selected were 103.679% for water, 2% for shallot and 2% for ginger and the solution got a desirability of 0.910. The predicted values for sensory score responses, colour and appearance, flavour, consistency, sourness, saltiness and overall acceptability of *sambharam* were 7.62, 7.64, 7.65, 7.33, 7.50, 7.19 and 7.65, respectively.

5.2.3. Verification of Optimum Formulations

The sambharam was prepared by the addition of the ingredients at the level recommended by RSM. The obtained scores were subjected to statistical analysis and the result is shown in the table 19. It is observed that there is no significant difference between the predicted and observed value with regard to all sensory attributes.

5.3. PHYSICO-CHEMICAL ANALYSIS

Physico-chemical analysis of optimized product was analysed. It includes estimation of Moisture, Total Solids, Ash, Fat and Protein. The optimized product have got 93.26 per cent for Moisture, 6.73 per cent for Total Solids, 1.44 per cent for Ash, 2.35 per cent for Fat and 2.09 per cent for Protein. As there is no control sample available, so, comparative analysis is not possible with the optimized product.

5.5. MICROBIOLOGICAL QUALITY

Microbiological quality of the optimized product was analysed by carryout Standard Plat Count (SPC) and got count 1.9×10^6 cfu/gm and no count were obtained for coliform test.

6. CONCLUSION

This study aimed to standardization the *Sambharam* by the addition of water, shallot and ginger (Chilly and Curry leaves). The addition of such spices helps in improving the health benefits and overall quality of the product.

Central Composite Rotatable Design of RSM was used to optimize the various levels of water, shallot and ginger on sensory characteristics such as colour and appearance, flavour, consistency, sourness, saltiness, spiciness and overall acceptability all had coefficients of determination (R^2) of 0.98, 0.95, 0.96, 0.97, 0.98, 0.96 and 0.94 respectively, indicating that the fitted quadratic model explained more than 80 per cent of the variation in the experimental data. The model is satisfactorily accurate for forecasting the sensory properties of *Sambharam* made with any combination of the variables within the range estimated.

Sambharam was prepared by adding water, shallot and ginger at optimized levels of 100%. 2% and 2% respectively while other ingredients such as chilly, curry leaves and salt are at fixed level to the curd. The highest desirability of 90 per cent was obtained with the levels 103.679 per cent for water, 2 percent for shallot and 2 per cent for ginger.

The optimized *Sambharam* was evaluated for its physico-chemical and microbiological attributes. The optimized *Sambharam* was found to have 93.26 per cent moisture, 6.73 per cent total solids, 1.44 per cent ash, 2.35 per cent fat and 2.09 per cent protein. The microbiological quality of the optimized product was also evaluated and got 1.9×10^6 count for Standard Plate Count (SPC) and no count for coliform test.

The ingredients used to make *sambharam* have no adverse effect on human health. So, the hypothesis was null which means our assumptions were true.

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