DEVELOPMENT OF FIBER FORTIFIED REDUCED CALORIE PROBIOTIC SMOOTHIE

Thesis

Submitted to the Guru Angad Dev Veterinary and Animal Sciences University in partial fulfillment of the requirements for the degree of

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in
DAIRY TECHNOLOGY
(Minor Subject: Dairy Engineering)

By

Kusum Lata
(L-2018-D-08-M)

Department of Dairy Technology
College of Dairy Science and Technology
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CERTIFICATE – I

This is to certify that the thesis entitled, **"DEVELOPMENT OF FIBER FORTIFIED REDUCED CALORIE PROBIOTIC SMOOTHIE"** submitted for the degree of **M. Tech.**, in the subject of **Dairy Technology** (Minor Subject: **Dairy Engineering**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Kusum Lata (L-2018-D-08-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

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

(Dr. Rekha Chawla)
Major Advisor
Assistant Professor
Department of Dairy Technology
Guru Angad Dev Veterinary and Animal Sciences University
Ludhiana – 141 004 (Punjab)

CERTIFICATE – II

This is to certify that the thesis entitled, **"DEVELOPMENT OF FIBER FORTIFIED REDUCED CALORIE PROBIOTIC SMOOTHIE"** submitted by **Kusum Lata (L-2018-D-08-M)**, to the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfillment of the requirements for the degree of **M. Tech.**, in the subject of **Dairy Technology** (Minor Subject: **Dairy Engineering**) has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an external examiner.

(Dr. Rekha Chawla)
Major Advisor

(Dr. D.N. Yadav)
External Examiner
Principal Scientist & Head
Transfer of Technology Division
ICAR-CIPHET, Ludhiana, Punjab

(Dr. Sandeep Sodhi Kakkar)
Head
Department of Dairy Technology

(Dr. Sanjeev Kumar Uppal)
Dean, Postgraduate Studies
Guru Angad Dev Veterinary
and Animal Sciences University
Ludhiana, Punjab

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ABSTRACT

In today's fast-moving and busy lifestyle, people are attracted towards functional foods due to their immense health benefits. There are abundant options of functional foods available in market wherein smoothie is one of the potential upcoming products in Indian market. Smoothies are blended drinks consisting of a number of ingredients including fruit (or less commonly vegetables), fruit juice, ice, yogurt, milk. The current work was aimed to develop fiber fortified reduced calorie probiotic smoothie, keeping in consideration the unavailability of such product in Indian counterparts. The study initiated with preliminary trials wherein different raw ingredients like fibers (sunfiber and apple fiber), sugar, fruit pulps were standardised on the basis of sensory acceptability. Two different variants (strawberry and mango were targeted to evaluate. Smoothie was optimized with 4% apple fiber, 10 per cent Sunfiber, 10-12 per cent pulp and 12-15 per cent sugar on the basis of sensory evaluation. The chosen combination of ratios was analysed for its physio-chemical, microbiological parameters and the study was carried further to prepare reduced calorie counterpart of the prepared product replacing the sugar with artificial or natural sweeteners. The product prepared with artificial (neotame) or natural sweetener (stevia) could not fetch desired sensory scores and was apparent from the sensory evaluation scores (8.42 for sucrose vs. 7.71 for reduced calorie on '0'day), which degraded to (7.14 for sucrose vs. 6.21 for reduced calorie on '12th 'day), and could not attract consumer attraction. Both the prepared variants were equally acceptable in terms of sensory evaluation. However, mango smoothie was comparatively more liked by the panellists. There was no syneresis occurred in fiber fortified smoothies of both the variants. However, it was observed in reduced calorie smoothies on 3rd day. The resistance to syneresis in conventional smoothies could be due to the property of water holding capacity of sugar. The probiotic count remained in significant limits during the course of the study. However due to arisen yeast and mold count study, was stopped on 12th day. The product holds great potential and therefore, the product could be transferred for its further commercialization.

Keywords: Smoothie, Probiotic**,** Mango pulp, Strawberry pulp, Dietary Fiber

Signature of Major Advisor **Signature of the Student**

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ABBREVIATIONS

%	:	Per cent
µ	:	Microgram
Cfu	:	Colony Forming Unit
Gm	:	Gram
Gs	:	Gram second
Kg	:	Kilogram
Kg	:	Kilogram
LA	:	*Lactobacillus acidophillus*
MSS	:	Mean sum of squares
NLT	:	Not less than
NMT	:	Not more than
NS	:	Non significant
RC	:	Reduced calorie
SE		Standard error
TDF	:	Total dietary fiber
TS		Total solids
TSS	:	Total soluble sugars
Var.	:	Variety

CHAPTER I

INTRODUCTION

The behaviour of consumers towards food has changed nowadays leading towards more concern about their health prospects. In today's fast-moving and busy lifestyle, people are attracted towards functional foods due to their health benefits. Functional foods are foods that can be satisfactorily demonstrated to beneficially affect one or more target functions in the body, beyond adequate nutritional effects, in a way relevant to an improved state of health and wellbeing and/or reduction of risk of disease' (Henry, 2010). The term 'functional food' was introduced in Japan in mid 1980's for food products fortified with special constituents that possess advantageous physiological effects (Hardy, 2000). The global functional food market was estimated to be worth 174.75 billion USD in 2019 and it is expected to increase 275.77 billion USD by 2025. The global functional drinks market size was estimated to be worth 93.68 billion USD in 2019. These functional commodities especially beverages are prepared mainly by incorporation of the probiotics. It has been reported that probiotics can improve the functioning of immune system, nutrify the gut microflora, promote its development and strengthen its action while also improving the absorption of minerals and vitamins (Abdel-Hamid et al., 2020). However, the health benefits of probiotics majorly rely on the adequate dose administered (Johansson et al., 2015). The recommended dose of orally administered probiotics varies between 10^6 – 10^7 colony forming unit (CFU/mL) of the product and a daily intake of 10^9 CFU depending upon the different application purposes and regulatory limits defined by various countries (Fu et al., 2018). The strains of *Lactobacillus* and *Bifidobacterium* serve as major probiotic source in various functional foods, amongst which yogurt and cheese are the two major dairy products in which probiotics are widely used.

Yogurt is one of the most well-liked dairy products and is a rich source of probiotics. Yoghurt and yoghurt-based drinks and beverages such as smoothies, kefir, koumiss, aryan are loaded with a specific taste and nutrients (Jayathilake, 2019). The addition of fresh or processed fruits such canned pulp, purees, juices, slices etc. in yoghurt is the trendiest way to enhance the phenolic content as well as antioxidant properties of the prepared products.

Addition of fruit-based ingredients also leads to the advantage of the naturally occurring bioactive compounds which are generally used in preparation of functional food due to their health benefits (Sun-Waterhouse et al., 2009). Studies have demonstrated that a diet rich in fruits and vegetables can reduce the risk of heart disease and strokes; prevent cancer, lower blood pressure and digestive problems (Boeing et al., 2012). Furthermore, there is growing interest of incorporating waste generated during fruit processing as functional food ingredient due to the presence of highly valuable bioactive substances (Balasundram et al., 2006). Coherently, due to the increasing incidence and prevalence of several metabolic disorders such as gluten intolerance and obesity, there is also a growing demand for developing fiber enriched foods due to their health benefits.

The incorporation of dietary fibers in food provides several health benefits such as protection against type -2 diabetes and cardiovascular diseases as well enhanced immune system, laxation and weight loss in obese (Mudgil & Barak, 2016). The consumption of fiber can also regulate the impact of satiety and appetite in numerous ways. The gelling effect of soluble viscous fiber can improve gastric distension and reduce the rate of gastric discharging into the intestine, which provides feeling of the fullness (Erickson & Slavin, 2016). Furthermore, the fermentation of fiber in colon improves the growth of hormones which associated with satiety and increased fiber consumption can lead to reversed trends in obesity (Slavin, 2013). Dietary fibers classified on the basis of structure and solubility (Dai & Chau, 2017). On the basis of structure, they are categorized as linear or non-linear molecules and solubility basis they are classified into soluble and non soluble dietary fibers. Insoluble dietary fibers mainly consist of cell wall components (lignin, cellulose, hemicellulose) while soluble dietary fibers consist of non cellulose polysaccharides (pectin, mucilage, gums) (Chawla & Patil, 2010). Soluble fibers dissolved in water which forms viscous gel. They avoid small intestinal digestion and are easily fermented by large intestinal microflora; while insoluble fibers are not water soluble in human gastrointestinal tract and don’t form gel due to their water insolubility and fermentation is limited (Lattimer & Haub, 2010). Amongst abundant options apple is the most commonly processed fruit that produces large quantity of apple pomace which consists majorly of pulp. Furthermore, stem seeds and peels which is obtained as a by product along with little quantity of pulp serve as a by product for production of apple fiber.

Apple pomace is rich source of dietary fiber such as soluble pectin, β glucans, galactomanan gums and insoluble lignin, cellulose and hemicelluloses as well as polyphenolic compounds with antioxidant, antitumor and hepatoprotective, antidiabetic, antiantioxidative, anti-inflammatory cardioprotective effects (Yang et al., 2013; Sun et al., 2017; Zlatanovic et al., 2019). Another most promising dietary fiber is partially hydrolyzed guar gum. It is a water soluble dietary fiber obtained from the endosoerm of *Cyamopsis tetragonolpbus* L. seeds, mainly made up of mannose and galactose with 2:1 ratio and is generally tasteless, colourless and odourless (Yoon et al., 2008). It has low viscosity and can easily be added to food and beverages (Anon 2021a). Partially hydrolyzed guar gum has been in the commercial market as a dietary fiber with the trade name Sunfiber®. The role of dietary fiber on the intestinal microflora system has drawn considerable attention. Wherein consumption of partially hydrolyzed guar gum provides several health benefits such as prebiotic effects with the production of high amount of short chain fatty acids and is also found to be effective in reducing hypergycemia and hyperlipidemia and also aids in preserve satiety (Yasukawa et al., 2019).

To stay fit and healthy food is an essential part of human diet and a balance diet that provides all the basic nutrients in correct quantities is essential to maintain good health. However, no single food contains all the essential nutrients required for the body. Therefore, eating food from each of six groups i.e., grains, vegetables, fruits, milk products, meat and beans is important for prevention and treatment of diseases. However, over the years there has been a drastic change in the eating habits of people. The lack of time and busy schedule has caused the change in diet structure of the people which has led to two prolonged nutritional problems; one emerging from excess and other from deficiency. To cater nutrition deficiency problems, food scientists and nutritionists are developing food products containing all the essential nutrients and are easy to grab and eat. In this category, smoothie could be an alternative grab-and-go breakfast that contains the entire essential nutrient present in abundance.

Smoothies, originally consisting of purely fresh fruits and vegetables, were first introduced in the 1969's in the United States and re-emerged in 2000 (Titus, 2008). Smoothies are blended drinks consisting of a number of ingredients including fruit (or less commonly vegetables), fruit juice, ice, yoghurt, milk and smoothies are characterised by a high nutrient concentration (Watzl, 2008). A smoothie is also an excellent and convenient source to promote the daily consumption of fruits and vegetables wherein, fruits and vegetables are rich source of dietary fibers, vitamins especially A, C and minerals as well as antioxidants etc. (Slavin & Lloyd, 2012). An adequate intake of fruits and vegetables helps in the prevention and alleviation of non communicable diseases such as type II diabetes, cancer, cardiovascular diseases and neurodegenerative diseases (Lapuente et al., 2019).

A healthy smoothie composition is based on its ingredients and their quantity. Many smoothies consist of a small and large portion of fruits and vegetables which are considered as a meal replacement and a suggested way for a healthy diet. Smoothies may be prepared using other ingredients such as milk, fruit juices, chocolates, whey powder, nuts water, crushed ice, sweeteners such as honey, syrup and artificial sweeteners, herbal or nutritional supplements. A smoothie containing dairy ingredients similar to a milkshake; however, the final product mainly consists of less fruit and generally uses yogurt or ice cream.

Functional smoothies, such as those that contain probiotics, have appeared very recently in the market. Most of the breakfasts available in market are either high in one or other components such as sugar, fat and cholesterol while being inadequate in protein, fibre and micronutrients, which lead to coronary heart disease, obesity, hyperglycemia, type II diabetes and other chronic diseases. It was estimated that between 2010 and 2030, there will be a 69% increase in the number of adults with diabetes in developing countries and a 20% increase in developed countries (Shaw et al., 2010). To reduce the calorie intake replacement of natural sugar (sucrose) with low calorie or high–intensity sweetener is a novel way to developing functional beverages. Concern exists that many commercially available smoothies are high in calories and added sugars. Therefore, present study was envisaged to develop probiotic smoothie with reduced calorie having enhanced nutritional attributes.

Objectives:

1. Optimization of ingredients for the manufacture of fiber fortified reduced calorie probiotic smoothie.
2. Storage study of developed product at refrigeration temperature.

CHAPTER II

REVIEW OF LITERATURE

The literature relevant to the various aspects of preparation of functional smoothie with added benefits of dietary fibers and low-calorie sweeteners has been reviewed under the following heads:

2.1 Functional foods

2.2 Probiotics

- 2.2.1 Definition
- 2.2.2 Health benefits of probiotics
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2.6 Smoothie

- 2.6.1 Definition
- 2.6.2 Literature on smoothie

2.1 Functional foods

Functional foods can be described as foods containing adequate levels of biologically active components that provide specific health benefits beyond the basic nutrients they contain (Drozen & Harrison, 1998). Functional foods are not pills or capsules but are consumed as part of a normal daily diet. The functional foods include conventional foods containing naturally occurring bioactive substances (e.g., dietary fiber) or bioactive substances enriched food (e.g., probiotics, antioxidants) or synthesized food ingredients added to traditional foods (e.g., prebiotics) (Syngai et al., 2016) Functional components include probiotics and prebiotics, conjugated linoleic acid, soluble fiber, omega-3-polyunsaturated fatty acids, plant antioxidants, vitamins and minerals, some proteins, peptides and amino acids as well as phospholipids (Bhat & Bhat, 2011).

2.2 Probiotics

2.2.1 Definition

The term "probiotic" was first coined in 1965, by Lilly and Stillwell, a substances secreted by one organism which stimulate the growth of another. According to Food and Agriculture Organization of the United Nations and World Health Organization, Probiotic are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO & WHO 2003) therefore, since past two decades probiotic bacteria have been gradually included in yoghurts and fermented milks because of their potential health benefits. Most commonly used probiotic bacteria are *Lactobacilli* such as *Lactobacillus acidophilus* and *Bifidobacteria* (Daly & Davis, 1998). The strains of bacteria that are commonly used as probiotics are listed in Table 2.1.

The selection of probiotic microbial strains involves screening of various safeties, functional and technological aspects (Saarela et al., 2000). Safety aspects include strain origin such as healthy human gastrointestinal-tract, non-pathogenicity and antibiotic resistance properties. Functional aspects consist of viability and persistence in the gastrointestinal-tract, immune stimulation, antagonistic and antimutagenic properties. Technological aspects include growth of microorganism in milk, sensory properties, stability, phage resistance, viability in processes.

2.2.2 Health benefits of probiotics

The role of probiotics in health maintenance and disease prevention may include improvement in intestinal tract health, stimulation of immune system, synthesis and enhancement of the nutrients bioavailability, reduction in lactose intolerance symptoms, reduction in the occurrence of common infectious diseases and also lowered the risk of certain cancer (Panchal, 2015). Blaabjerg et al. (2017) reported that probiotic consumption is an effective tool in the prevention of antibiotic- associated diarrhoea. Baroja et al. (2007) studied the anti-inflammatory effect of consuming probiotic yoghurt on the inflammatory bowel disease. The results showed that there was a significant reduction of inflammatory bowel disease among the people who consumed yoghurt for one month.

Probiotics have different mechanisms of action although the precise manner in which they exert their effect is not yet fully explained but may associate with altering intestinal pH, development of bacteriocin and short chain fatty acids, stimulation of mucosal barrier function and immunomodulation (Kechagia et al., 2013).

2.2.3 Lactobacillus acidophilus as Probiotic microorganism

Lactobacillus acidophilus (Earlier known as *Bacillus acidophilus*) was first isolated from infant faeces in 1900 by Ernst Moro and in 1970, it was officially acknowledged as *Lactobacillus acidophilus* by Hansen and Mocquat. *Lactobacillus acidophilus* is Gram-positive rod of small size (2–10 µm), grows from 37 to 42 °C optimally (Altermann et al., 2005) but can also grow at high temperatures as 45 °C. The bacterial strains attain its maximum growth rate in slightly acidic media at pH 5.5–6.0 whereas below pH 4.0 growth rate terminates (Shah, 2007). It is a necessary homofermenter which produces lactic acid during carbohydrate fermentation and is one of the least oxygen tolerant *lactobacilli* (Archibald & Fridovich, 1981).

Lactobacillus acidophilus is a probiotic strain widely present in conventional foods such as milk, yogurt, toddler formula as well as dietary supplements (Bull et al., 2013). About 80% of yoghurt is now prepared with *Lactobacillus acidophilus* in United States (Sanders, 2003). Although this microorganism has slow growth in milk, it isn't involved in the fermentation of yoghurt. Therefore, most of the yogurt fermentation is achieved by yoghurt starter culture i.e. *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* whereas *Lactobacillus acidophilus* is later added for the additional probiotic health benefits (Shah, 2000).

Table 2.1: Probiotic microorganism and their application in food products

Probiotic strains	Food product	Title	References
Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus paracasei or *Bifidobacterium* species	Cheedar cheese	Chemical analysis and sensory evaluation of cheddar cheese	Ong et al. (2007)
Lactobacillus paracasei	Chocolate mousse	Potential probiotica and synbiotic chocolate mousse	Aragon- Alegro et al. (2007)
Bifidobacterium breve	Yakult – fermented soymilk	Evaluation of *Bifidobacterium breve* strain Yakult- fermented soymilk as probiotic food	Shimakawa et al. (2003)
Lactobacillus casei, Lactobacillus plantarum, Bifidobacterium longum	Noni juice	Probiotic potemntial of noni juice fermented with lactic acid bacteria and bifidobacteria	Wang et al. (2009)
Lactobacillus bacterium, Lactobacillus reuteri	Cereal based food	Use of mix cultures for the fermentation of cereal based substrate with potential probiotic properties	Kedia et al. (2007)
Bifidobacterium adolescentis, Bifidobacterium infantis, Bifidobacterium brevis, Bifidobacterium longum	Malt- based beverage	Evaluation of *Bifidobacterium* species for the production of potentially probiotic malt- based beverage	Sanchez et al. (2008)
Bifidobacterium pseudocatenulatum	Peanut milk	Survival of *Bifidobacterium pseudocatenulatum G4 during the storage of fermented peanut milk and skim milk products*	Kabeir et al. (2009)
Lactobacillus acidophilus (LA5 and LA 1748) *Lactobacillus rhamnosus, Lactobacillus reuteri*	Maize porridge	Growth and metabolism of selected strains of probiotic bacteria, in maize porridge with added malted barley	Helland et al. 2004
Lactobacillus acidophilus LA5, *Lactobacillus rhamnosus, Lactobacillus plantarum,*	Milk/Carrot juice mix drink	Effect of refrigerated storage on the probiotic survival and sensory properties of milk/carrot juice mix drink	Daneshi et al. (2013)

Probiotic strains	**Food product**	**Title**	**References**
Bifidobacterium lactis BB12			
Lactobacillus helveticus, Streptococcus thermophillus ST5,*Bifidobacterium longum* R0175	Soy and dairy milk	Immunomodulatory properties of fermented soy and dairy milks	Wagar et al. (2009)
Lactobacillus acidophilus, Bifidobacterium bifidium	Probiotic beverage	Development of probiotic beverage from whey and orange juice	Shukla and Kushwaha (2017)
Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus delbrueckii subsp.	Probiotic beetroot drink	Development of probiotic beet root drink	Panghal et al. (2017)
Bifidobacterium lactis BB12, *Lactobacillus plantarum* 299V, *Lactobacillus acidophilus* LA5	Pineapple juice	Probiotic beverage from pineapple juice fermented with *Lactobacillus* and *Bifidobacterium strains*	Nguyen et al. (2019)
Lactobacillus casei	Cashew apple juice	Probiotic beverage from cashew apple juice fermented with *Lactobacillus casei*	Pereira et al. (2011)
Bifidobacterium lactis BB12, *Bifidobacterium longum BB46, Lactobacillus casei, Lactobacillus acidophilus* LA5	Apricot juice	Lactic acid fermentation of apricot juice by mono- and mixed culture of probiotic *Lactobacillus* and *Bifidobacteriums* strains	Bujna et al. (2018)
Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus casei TD4,Lactobacillus casei T4	Cornelian cherry juice	Viability of probioticbacteria and sone chemical and sensory characteristics in cornelian cherry juice during cold storage	Nematollahi et al. (2016)
Lactobacillus plantarum B28	Oat based probiotic drink	Development of new oat based probiotic drink	Angelov et al. (2006)

2.2.4 Health benefits of *Lactobacillus acidophilus* in yoghurt

Lactobacillus acidophilus has been widely studied as probiotic and studies have shown positive health impacts against several diseases such as cold, lactose intolerance, diarrhoea, diabetes inflammation, infection and hypercholesterolemia.

Hilton et al. (1992) analyzed the effect of daily consumption of yogurt containing *Lactobacillus acidophilus* prevents vulvovaginal candidal infections in women. In this study, 33 patients were selected which consumed yoghurt for six months. Authors observed a three-fold reduction in infection when *Lactobacillus acidophilus* containing yoghurt was consumed by patient. This could be due to the yogurt containing probiotic strain *Lactobacillus acidophilus* are protective against mucosal candida infection and their beneficial action by suppressing the growth of candida which can relieve symptoms of vulvovaginal infections in women. Study also suggested that the daily consumption of 8 ounces of *Lactobacillus acidophilus* containing yogurt reduced both candidal colonization and infection.

Sazawal et al. (2006) conducted a Meta analysis study of 34 masked, randomised, placebo-controlled trials to determine the use of probiotics in the prevention of severe diarrhoea and reported that intake of probiotic reduced the risk of severe diarrhoea by 57 per cent and 26 per cent in children and adults, respectively. Authors also reported that the impact of acute diarrhoea was dependent on the age of host and bacterial strains.

Ataie-Jafari et al. (2009) studied the cholesterol-lowering effect of probiotic yoghurt in hypercholesterolemic cases. In this study, authors compared the effect of consuming probiotic yoghurt with the ordinary yoghurt on the serum cholesterol level in slightly to moderately hypercholesterolemic individuals. Researchers reported that probiotic yoghurt prepared from two probiotic strains *Lactobacillus acidophilus* and *Bifidobacterium lactis* had cholesterol-lowering effect in comparison to the ordinary yogurt. Several mechanisms for cholesterol level reduced by consumption of probiotic had been suggested. One of suggested mechanism is the assimilation of cholesterol by probiotics (Pereira & Gibson, 2002). Cholesterol is introduced into the cell membrane of probiotic bacteria. These mechanisms may reduce serum cholesterol level by reducing cholesterol absorption in the intestine (Liong & Saah, 2005; Ooi & Liong, 2010). The cholesterol lowering effect of probiotic can also described by enzymatic deconjugation of bile salts by bile salt hydrolase. It can intervene with bile salt in the enteroheptic circulation. Since cholesterol is the major precursor for the de novo synthesis of new bile acids, this intervention will result in reduced serum cholesterol level (Bagley et al., 2006; Ooi & Liong, 2010).

Leyer et al. (2009) studied the effect of probiotic consumption on cold and influenza like symptoms and their duration in healthy children during winter season. In this study, 326 children of 3-5 ages were treated twice everyday for six months. Researchers reported that the tested probiotic was effective in reducing incidence of fever, cough and rhinorhea cases along with a significant decrease in duration compared to placebo. They also concluded that daily dietary probiotic consumption is a safe and efficient way to decrease the incidence and duration of cough, fever and rhinorhea among children of 3 to 5 ages.

Andreasen et al. (2010) investigated the effect of oral supplementation of probiotic *Lactobacillus acidophilus* on insulin sensitivity and inflammatory response in normal or impaired insulin sensitive people for four weeks. Authors reported that the intake of probiotic *Lactobacillus acidophilus* for four weeks-maintained insulin sensitivities, but did not affect the systemic inflammatory response

Ejtahed et al. (2012) *evaluated the effect of* probiotic *(Lactobacillus acidophilus* and *Bifidobacterium lactis)* and conventional yoghurt on blood glucose and antioxidant status in type 2 diabetic patients. Authors reported that the probiotic yogurt significantly reduced fasting blood glucose ($P < 0.01$) and haemoglobin ($P < 0.05$) and increased total antioxidant status ($P < 0.05$) in comparison with control group. These results suggested that probiotic yogurt is a promising agent for controlling diabetes and consumption of probiotic yogurt improved fasting blood glucose and antioxidant status in diabetic patients of type 2. Larsen et al. (2010) also reported that type 2 diabetes mellitus was related with the compositional changes in the gut microflora and the amount phylum of bacteria was significantly reduced in adults with type 2 diabetes compared to nondiabetic persons.

Pakdaman et al. (2015) examined the effect of *Lactobacillus acidophilus* on lactose intolerance in randomized, double-blind, placebo-controlled and crossover cases. Lactose intolerance is the inability to digest the lactose due to absence of lactase enzyme in gut and symptoms includes nausea, cramps, bloating, gas, and diarrhoea. Studied showed the strain of *Lactobacillus acidophilus* improved the intestinal tract health and had significant reductions in symptoms of lactose intolerance such as cramping and vomiting. Dairy products are rich source of calcium, potassium, vitamin D and vitamin d and proteins, no consumption of such type of food caused several health diseases such as bone fracture, osteoporosis etc. Recent trends aim to alter the natural intestinal flora to create an environment more conductive for breakdown and absorption of lactose by using of probiotic supplement (Shaukat et al., 2010 & Almeida et al., 2012). Alteration of colonic bacteria is thought to be increase the intra- intestinal lactase activity and thus lower the effect of fermentation products (Hertzler & Savaiano, 1996). Several studies reported that cultured yogurt possess considerable enzyme activity primarily due to the lactase produced by lactic acid bacteria such as *Lactobacillus acidophilus and Lactobacillus bulgaricus* (Pakdaman et al., 2015). This type of probiotic culture products beneficial to individuals suffering from lactose intolerance and reduced lactose and lactase produced.

2.3 Dietary fiber

2.3.1 Definition

Dietary fibre term was first introduced by Hipsley in 1953 and defined as a non-digestable constituent of plant cell wall. Later, American Association of Cereal Chemists (AACC) in 2000 defined dietary fibre as the edible parts of plant or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine (AACC 2000).

Dietary fiber is classified into two groups one is water- insoluble (less fermented fibres) such as cellulose, hemicelluloses and lignin while other is water-soluble (well fermented fibres) includes pectin, gums and mucilages (Antia & Abraham, 1997). Different types of fibers and their sources are listed below in table 2.2. Each group has various impacts on physiology (Schneeman, 1987; Ajila & Rao, 2013). The insoluble component is associated with water absorption and intestinal regulation while soluble part is related with the lowering of blood cholesterol and reduction in glucose absorption by small intestine (Yangilar, 2013). The composition of dietary fiber varies from food to food. Cereals are a major source of dietary fiber as it contributes to about 50 per cent of the fiber consumption in western countries, while 30-40 and 16 per cent may be derived from vegetable and fruits, respectively (Dhingra et al., 2012). Diets with high fiber content like those abundant in cereals, fruits and vegetables have a positive health impact because their ingestion has been related to a decrease in the incidence of several types of diseases. This reduced risk of diseases may be attributed to various beneficial effects of consuming of fiber-rich diets, such as an increase in the volume of fecal bulk, a reduction in the time of intestinal transit, cholesterol and glycaemia rates and trapping compounds that may be harmful to human organisms (mutagenic and carcinogenic agents), enhancing the growth of the intestinal flora etc. (Heredia et al., 2002; Beecher, 1999). Diet rich in fibers has been linked with decreased risk of coronary heart disease and mortality rate as well (Streppel et al., 2008).

2.3.2 Fiber enriched products

Several research investigations have been conducted focusing on the development of dietary fiber fortified products. Mudgil et al. (2016b) studied different levels of partially hydrolyzed guar gum as soluble fiber in noodle development and its effect on their textural properties such as hardness, cohesiveness, chewiness and resilience. They found that the addition of partially hydrolyzed guar gum have a significant effect on textural properties of noodle and fortification of noodle with fiber also increased the soluble content as compared to control sample.

Staffalo et al. (2004) examined the effect of various dietary fibers such as inulin, wheat, bamboo, and apple fiber on the sensory and rheological properties of yoghurt. Researchers found that yogurt fortification with fibers showed no syneresis till 21 days storage at 4˚ C. Also, during storage time, water activity, pH, and colour parameters remained steady. Rheological parameters showed significant changes depending on the type of fiber and storage time but sensory analysis had non-significant effects in fortified and control treatments except the apple fiber. Study also revealed that 1.3 per cent dietary fiber fortification of yogurt seems to be an effective way for increase fiber consumption, with higher acceptability among consumers.

Raju & Pal (2014) evaluated the effect of dietary fibers (inulin, soy, oat fiber) on the physicochemical, sensory and textural attributes of *misti dahi*. Authors reported among all the fibers, inulin significantly reduced viscosity and instrumental firmnesss and increased colour intensity, syneresis and work of shear values of fiber fortified *misti dahi*. The reduction in the viscosity of inulin fortified *misti dahi* could be due to the interaction of inulin and milk protein which caused formation of weak gel and firmness was due to the disrupting effect of fiber on the gel, respectively. They also concluded that 1.5 per cent inulin and soy fiber fortification in *misti dahi* had best quality acceptability. Bhaskar et al. (2017) examined the effect of fiber fortification of β- glucan on physico- chemical rheological, textural, colour and sensory attributes of the low fat *dahi.* Authors prepared low fat *dahi* by adding various levels of β- glucan i.e. 0.25, 0.50, 0.70 and 1 per cent in skimmed buffalo milk. Authors reported that 0.5 per cent level of β- glucan was found to be ideal in terms of syneresis and viscosity properties. Results also showed that the fortification with 0.5 per cent β- glucan had a significant effect on instrumental colour values and sensory score in comparison with other level of β- glucan treatment. This could be due to the increased fiber concentration decreased the instrumental colour and sensory scores. The viscosity of *dahi* caused an unstable product with increased concentration of β- glucan which also resulted in decreased syneresis value.

Akalin et al. (2018) examined the effect of five dietary fibers such as apple, orange, oat, bamboo and wheat on the physico-chemical, rheological, textural, sensory attributes and culture viability of probiotic ice cream for 180 days at -18 ˚C storage period. Authors reported that ice cream mix fortified with apple and orange fibres increased the rheological properties and decreased melting in comparison with control and other experimental treatments. They also concluded that wheat fiber had ability to increase rheological, textural, sensory properties as well as probiotic viability in the development of probiotic ice-cream, because wheat extract had significant protective effect of viability of *Lactobacillus acidophilus* under acidic condition whereas, the lowest count observed in the orange fiber because reduction in *Bacillus lactis* caused by high acidity of the orange fiber as well as the presence of inhibitory compounds such as antibacterial polyphenols in orange fiber.

Figueroa and Genevese (2019) prepared apple jellies fortified with 3g/ 100 g of dietary fiber and evaluated their structural and sensory properties with commercial apple jams. For the preparation of apple jellies fiber mix was prepared by adding one part of psyllium and three parts of bamboo, wheat and apple fiber. Authors studied the colour, syneresis, rheological, mechanical sensory attributes of product throughout storage time. Authors reported that viscoelastic properties improved with the addition of fiber and pectic mass fraction. Syneresis decreased with the increase of pectic mass fraction whereas, the effect of fiber addition depends upon the type of fiber. Study indicated that gel with psyllium fiber showed no syneresis but their overall appearance was gummy. These attributes may be associated with three mechanical properties such as lack of fracturability, high cohesiveness and low hardness. Although, gel with apple fiber were too dark, and gel with bamboo and wheat fiber had floury mouthfeel. These organoleptic properties were enhanced by mixing two different types of fiber in the ratio of 1:1. This combination also decreased the viscoelastic properties of the gel. The overall acceptable results were obtained by adding psyllium fiber with another type of fiber, as this combination reverted the mechanical properties of the gel incorporated with psyllium fiber only (showing less "gummy" gel), maintaining the syneresis at 0 g/100 g. Therefore, gel with pectin 0.5 g/ 100 g enriched with psyllium mixed with apple, bamboo or wheat fiber seem to be the most acceptable for further development of healthy products.

Table 2.2: Source and fiber

Dietary fiber	**Source**
Pectin	Middle lamellae of plant cells
β- glucan	Oat and barley
Cellulose	Cell wall of higher plants
Legume fiber	Legume seeds
Oat bran	Outer layer of oat groats
Xanthan gum	*Xanthomonas campestris*
Inulin	Banana, chicory, barley, onion
Oligosaccharides	Enzymatic hydrolysis of inulin
Guar gum or partially hydrolysed guar gum	*Cyamopsis tetragonalobus.*
Gum acacia	*Acacia Senegal*
Apple fiber	Apple pomace

Dietary fiber when added to food products contributes multiple functional properties such as modification and improvement of texture, sensory properties and shelf life of food products. These functional properties occur due to fiber gel forming ability, water binding capacity, fat mimetic, anticlumping, anti-sticking, texturizing and thickening effects (Staffalo et al., 2004; Thebaudin et al., 1997). The physico-chemical properties of dietary fibers are influenced by different physical factors like type of fiber, particle size nature of chain and molecular weight (Kaushik et al., 2016).The average daily dietary intake level in countries varies from 21 – 40 g/ day which have established guidelines and World Health Organization WHO/FAO 2003 and Food and Nutrition Board, Institute of Medicine 2001 has approved that the total fiber intake should be 25 g per day (Nishida et al., 2004). The total dietary intake for men and women has been recommended as 38 and 25 g per day respectively (Yangilar, 2013). Amongst various fibers, sunfiber is one of the promising soluble fibers and has commercial and research applications.

2.3.3 Sunfiber®– Partially hydrolysed guar gum as dietary fiber

Dietary fibers are recognised as essential nutrient components for people's healthy life. Guar gum is one of the most effective soluble dietary fibers, derived from seeds of legume plant known as *Cyamopsis tetragonalobus.* It was first reported in food products in U.S. 1949, Federation of American Societies for Experimental Biology (FASEB, 1973). The major component of guar gum is polygalactomannan which is widely used in food processing as thickener and emulsion stabilizer. The molecular weight of guar gum varies from 2000 to 3000 kDa, which offer relatively high viscosity even at low concentration in aqueous solution. Due to its high viscosity properties, it cannot be effectively used in food products in large quantities. Therefore, in order reduce viscosity the recommended way is partially hydrolyzation of guar gum by using β- endo- mannanase by which the molecular size becomes one tenth of the original length of guar gum (Yoon et al.,2008). Partially hydrolysation of guar gum mainly made for the nutritional purposes i.e., for manufacturing of fiber supplemented food products such as bread, cookies, noodles, flavoured milk, yogurt etc. (Mudgil et al., 2018). Partially hydrolyzed guar gum is fully water soluble, clear solution, low viscous, colourless, tasteless, stable at low pH, resistant to heat, acid, salt, digestive enzymes and is also known as a prebiotic fiber (Li, 2018; Niv et al., 2016). Partially hydrolyzed guar gum named as Benefiber® in USA and has self affirmation on GRAS status of standard grade of partially hydrolyzed guar gum from May, 1995. Now, partially hydrolysed guar gum is known as Sunfiber® and used in various food products, beverages as well as in medicinal foods as safe, natural and functional dietary fiber around the world (Yoon et al., 2008).

Alam et al. (2000) evaluated the effect of benefiber supplemented oral rehydration solution in the diagnosis of severe diarrhoea in children. In this study a blind, randomized control trials were conducted in 150 male children of age 4-18 months who had severe diarrhoea. Authors gave the benefiber supplemented oral rehydration solution to diarrhea affected children and measure the outcome such as duration of diarrhea, quantity of stool output and reported that benefiber supplemented oral rehydration solution decreases the duration of diarrhea and reduced stool output in children, suggested it as antidiarrheal remedy against severe diarrhea. Minekus et al. (2005) examined the effect of partially hydrolysed guar gum on the bioaccesssabilty of fat and cholesterol. In this study, 3 per cent sunflower oil, 4 per cent egg yolk containing yogurt evaluated with 3 per cent and 6 per cent partially hydrolysed guar gum. The results were compared to the control treatment (without partially hydrolyzed guar gum). Investigations were performed in multi-compartmental model of the gastrointestinal tract, designed to examine the digestion and bioaccessibility of fat. The results indicated that partially hydrolyzed guar gum reduced bioaccessibility of both fat and cholesterol in concentration dependent mode. Study also indicated that partially hydrolysed guar gum lowers the bioaccessibility via depletion flocculation process.

Polymeros et al. (2014) evaluated the effect of guar gum on colonic transit time and long-term constipation syndrome. In this study, 49 patients were selected and treated with 5 mg dose of partially hydrolysed guar gum daily for 4 weeks. Authors reported that intake of partially hydrolysed guar gum for 4 weeks increases colon transit time in patients with long term constipation, particularly in slow transit patients and enhance other symptoms like frequency of bowel movement.

Rao et al. (2015) investigated the effect of post meal perceivable satiety and subsequent energy intake with ingestion of partially hydrolysed guar gum. In this study, three different treatments were evaluated: in treatment 1, 12 people consumed partially hydrolysed guar gum along with breakfast, lunch and an evening snacks; in treatment 2, 24 people consumed 2 g of partially hydrolyzed guar gum or dextrin along with yoghurt as breakfast for two weeks; in treatment 3, 6 people consumed 6 g of each either partially hydrolysed guar gum or indigestible dextrin or inulin along with lunch. In all the treatments different satiety parameters were calculated on visual analogue scales before and after the ingestion of partially hydrolysed guar gum. The incorporation of partially hydrolyzed guar gum showed a significant, severe (treatment 1 and 3) and long-term satiation effects in comparison to control or other types of soluble fiber. Treatment 2 also showed that the prolonged ingestion of partially hydrolysed guar gum may significantly decrease the energy intake from entire day snacking. Study also concluded that partially hydrolysed guar gum is an ideal natural soluble fiber for delivering severe and long-term satiety effects for easy appetite management.

Kapoor et al. (2016) examined the effect of partially hydrolysed guar gum on postprandial hypergylcaemia, hyperlipidaemia and incretins metabolic hormones. In this study, 12 people consumed 6 g of partially hydrolysed guar gum along with each meal for 12 months. Authors reported that the consumption of partially hydrolysed guar gum reduced the postprandial hyperglycaemia significantly. The level of high-density lipoprotein was also increased significantly, while low density lipoprotein, plasma leptin, high sensitive C reactive protein and fasting glucagon like peptide were lowered. In fact, after three months supplementation of partially hydrolysed guar gum 3 out of 6 glucose intolerant individuals became normal glucose tolerant. Study also proven that the incorporation of partially hydrolysed guar gum in diets potentially impact on the health profile of metabolites that are responsible for glycemia, hyperinsulinaemia, hyperlipidaemia aspects. Another widely studied fiber is apple fiber which is the combination of soluble and insoluble fibers and has several research applications.

2.3.4 Apple fiber

Apple fiber is a by product of apple pomace produced by the processing of apples. Apple fiber is excellent source of soluble and insoluble dietary fibers. Apple is one of the most commonly and regularly consumed fruit throughout the year. Apples are perceived as nutritious food and various products are available in market. About 33 per cent of harvested apples are processed into vinegar, jam, jellies, ciders, juices, concentrate, alcoholic beverages, sauces, canned apples, frozen apple slices, dried apple flavour compound, pectin (Sharma et al., 2016a). During the processing of apples about 25 percent of wastes such as skin, pulp, peel, core, stem, soft tissues are generated and this waste is called as apple pomace. Apple pomace have high nutritional components such as polyphenol and dietary fiber therefore, it is widely used as food ingredient substitute (Skinner et al., 2018). Apple fiber has wide range of application and it is mainly used as an ingredient to increase the fiber content of food products. Apple fiber is a good bulking agent as well as fat replacer. Kohajdova et al. (2011) evaluated the effect of replacement of wheat flour with apple fiber levels as 0, 5, 10 and 15 per cent on the baking and sensory properties of cookies. Authors found that addition of apple fiber to cookies negatively influenced specific volume, volume index and also decreased thickness and width of cookies. They also reported that addition apple fiber resulted in significant decreased lightness and increased cookies redness. Sensory evaluation indicated that apple fiber enriched cookies had fruity, sweet taste and odour and firmer texture in comparison to control samples. Study also showed that apple fiber at 10 per cent level in cookies was overall acceptable.

Kayacier et al. (2014) manufactured wheat chips enriched with apple fiber and researchers evaluated the effect of processing variables such as fiber range, temperature and time on physico-chemical, textural and sensory characteristics of chips using response surface methodology. Response surface methodology was carried out to verify the optimization levels of processing variables. Authors reported that the addition of apple fiber raised the dry matter, ash content colour values of sample; whereas increased frying temperature reduced the hardness of product and overall acceptability for apple fiber enriched chips.

Zbikowska and Kowalska (2017) prepared apple fiber cookies by adding apple fiber as a fat replacer. In this experiment, authors studied two different fat content of trans- fatty acid isomers one is less than 1g TFA/100 g FA and another is greater than 58 g TFA/ 100 g FA. Based on the physical properties of bakery products, it could not be used beyond 24 per cent. The effect of apple fiber on the quality of product was dependent on the type of fat removed. Authors reported that a significant reduction in the quality of cookies occurred in combination with increased replacement of fat with a high trans fatty acids content. However, the replacement of fat with trans fatty acid < 1g/100 g fatty acid by fiber caused no significant reduction in the quality of product.

2.3.5 Diesol™

Acacia gum or gum arabic is a natural dietary fiber produced from the branches and stem of *Acacia Senegal or Acacia Seyal*. It is a polysaccharide with high molecular weight made up of D- galactopyranose, D-glucuronic acid, L – rhamnopyranose and L- arabofuranose. Gum acacia is natural soluble dietary fiber which is fermented in large intestine and widely used by the food industry for the various applications such as coating, emulsification, encapsulation etc.

Diesol™ is soluble dietary fiber blend produced from several varieties/species of acacia trees. It provides all natural soluble dietary fiber depend on 100 per cent gum arabic, which consist 90 per cent soluble fiber with prebiotic effect (Anon, 2021b). It is available as light brown colour powder with free-flowing property having a viscosity of 10 cps in a 5 per cent solution that can be effectively incorporated in high and low fiber beverages, ice cream, sauces, soups, yogurt, cheese, cake, snacks, bar etc. (Ambuja & Rajkumar, 2018). Fermentation of diesol has prebiotic effect and it produces short chain fatty acids which help in lowering of cholesterol (Kravtchenko, 1997). The consumption of diesol™ reduced the risk of several diseases such as obesity, hypertension, diabetes etc. and also observed the maximum acceptability in human body with zero adverse effects. (Anon, 2021b). Castellani (2005) reported that gum acacia helps in reduction of the glycaemic index of food products.

Sidhu and Bawa (2004) evaluated the effect of addition of 0.1 – 0.5 per cent gum acacia on bread manufacturing process. In this study, Farinograph, Viscoamylograph, Rheofermentrometer, Test baking and Instron procedures were examined. Authors concluded that gum acacia can be added up to 0.3 per cent levels to examine the rheological, baking, gas formation, gas retention and bread firmness characteristics of medium protein wheat.

Chawla et al. (2015) prepared functional *dado burfi* with a combination of diesol™ and oat fiber. Authors studied the effect of temperature on functional *doda burfi* at temperature 4 and 30˚C, and evaluated their sensory, textural properties for 30 days. Authors found that sensory and textural attributes scores for functional *doda burfi* reduced at both temperatures, but most of the reduction in sensory scores reported at temperature 30˚C in comparison with 4˚C. They also reported that prepared functional *doda burfi* provided fifty per cent of the daily need of the dietary fiber and product was shelf stable at room temperature for about 12 -15 days.

2.4 Sweeteners

2.4.1 Definition

Sweeteners are food additives, which imitates the effect of sugar on taste (Chattopadhay et al., 2014). Therefore, they are also known as sugar substitute.

2.4.2 Types of sweeteners

There are two types of sweeteners available in the market one is natural sweetener and another is artificial sweetener.

2.4.2.1 Natural sweeteners

Natural sweeteners are sweetening agents extracted naturally from plants and have high nutritional value. The main component of natural sweetener is mono- or disaccharides. Natural sweeteners include stevia, agave nector, honey, brown rice syrup, maple syrup, molasses, sorghum syrup etc. (Marcus, 2013).

2.4.2.2 Artificial sweeteners

Artificial sweeteners are the sugar substitutes that are chemically derived and have no or little nutritional value. They are also known as non saccharides/ synthetic/ high intense sweeteners. Artificial sweeteners are neotame, acesulfame-K, aspartame, saccharin, sucralose, tagatose (Bukhamseen & Novotny, 2014). However, sweeteners derived from natural source have high caloric value which caused diabetes, cardiovascular diseases, and obesity. There has been a gradual increase in the number of diabetic people globally. India becomes the world's diabetic country in the world. In 2017, 72 million cases of diabetes were reported and this number will be double, reaching 134 million by 2025 (Cho et al., 2018). The increased health awareness among people, there is a high demand for the sugar substitutes which provide lesser or no calories and have good sweetening ability.

The consumption of stevia has numerous health benefits. The benefits are anti-caries, anti- hyperglycaemic, anti hypertensive, anti-cancer (Gandhi et al., 2018). Several studies have been conducted to replace the sugar with the low-calorie sweeteners. Ozdemir et al. (2015) prepared ice cream using stevia as sweetener. In this research, authors studied the effect of stevia addition on physical, chemical and sensory properties of ice cream. Authors prepared four different ice cream samples using sucrose and stevia as sweeteners such as the plain + sucrose, cocoa + sucrose, plain + stevia and cocoa + stevia. The sample with added stevia and cocoa were liked more over others due to highest overrun ratio, high viscosity and longest melting point.

Rocha and Bolini (2015) studied the impact of different sweeteners on the sensory profile and acceptance by descriptive analysis. Passion fruit juice prepared with sucralose, stevia, sucrose, aspartame, neotame and cyclamate/saccharin in 2:1 ratio. Results indicated that aspartame and sucralose were the good sucrose substitutes in juice as both sweeteners showed a high intensity of passion fruit flavour and had no bitter taste, bitter after taste and metallic taste in passion fruit juice.

Sharma et al. (2016b) developed ready-to- serve- lemon beverage with honey as a natural sweetener and analyzed their physico-chemical properties under different storage conditions for six months. The results indicated that honey lemon ready-to-serve drink had high ascorbic content and antioxidant activity than the control samples at initial stage. The results also showed a better sensory score and overall acceptability even after six months of storage period.

Moriano and Alamprese (2017) examined the effect of honey, trehalose and erythritol on the characteristics of artisanal ice cream. Authors developed ice cream by replacement of sucrose partially (50 %) or fully (100%) with alternative sweeteners and milk-based sucrose sweetener ice cream was developed as control sample. In comparisons to control sample, the honey containing ice cream mix indicated a significant reduction in soluble solids and apparent viscosity whereas, extrusion time was significantly greater. The complete replacement of sucrose with trehalose and erythritol resulted in high extrusion temperature, high firmness and low melting rate than the control sample. The results of this investigation provided a direction for the fortification of ice cream with honey, trehalose and erythritol.

2.4.3 Stevia- a natural sweetener

Stevia is a natural sweetener extracted from the leaves of *Stevia rebaudiana,* a plant native to the areas of South America and also known as steviol glycosides. Among the 230 varieties, rebaudiana and phlebophylla are two species which produces sweet steviol glycosides (Brandle & Telmer, 2007). Stevia leaves contain eleven diterpene glycosides, such as stevioside, rebuadioside A-F, dulcoside etc.

(Gandhi et al., 2018). The sweetest glycosides present in stevia leaves are stevioside and rebaudioside which are 250-300 times sweeter than sucrose. The Market products of Stevia contain steviol glycosides as stevioside or rebaudioside- A. Stevia is stable at high temperature and wide range of pH therefore it can be widely used in food industry. Stevia provides zero or no calories and thus ideal for the people who are suffering from phenylketonuria, diabetes and obesity (Figlewicz et al., 2009). Atteh et al. (2008) reported that stevia improves the kidney functions by increasing the water excretion. Another studies showed that consumption of stevia enhanced the cellular immunity (Sehar et al., 2008). Stevia has been tried widely to sweeten various products such as yogurt, soft drinks, soy sauce, ice cream, traditional sweets etc.

Lisak et al. (2011) examined the effect of stevia on the quality of strawberry flavoured yogurt. Authors prepared strawberry flavoured yoghurt with different levels of sucrose, stevia and equal proportion of both sweeteners at three different concentrations i.e., 3, 4.5 and 6 per cent in 100 g yogurt. They studied the viscosity and sensory attributes of yogurt. The study indicated that the strawberry yogurt prepared with sucrose found to be sweeter followed by combination of sucrose and stevia while stevia sweetened yogurt had least sweetness. The acceptable level by panellists for single or combination of sweeteners for yogurt was 4.5 g / 100 g. The study also observed that the sucrose yogurt has low apparent viscosity in comparison to the product prepared with stevia and combination of stevia and sucrose.

Giri et al. (2014) studied the effect of partial replacement of sugar with stevia on the quality of kulfi. Authors prepared dietetic kulfi in which 50, 60 and 70 per cent sugar was replaced with 0.05, 0.06 and 0.07 per cent of refined stevia extract powder respectively. They reported that above 50 per cent sugar replacement resulted in bitterness, lack of brownish appearance and presence of icy texture. This could be due to reduction in sugar level by 60 and 70 per cent resulted in increased addition of stevia which caused bitterness in the product at higher level of sugar replacement, and also at higher sugar replacement there was an increase in free moisture content resulted in formation of large ice crystals which decrease melting rate in kulfi.

Alizadeh et al. (2014) developed a low-calorie soft ice cream by using mixture of sucrose and stevia. Authors investigated the effect of stevia on physico-chemical, sensory, rheology and glycemic index of soft ice cream and reported that replacement of sucrose with stevia resulted in low viscosity and brix with a high overrun as well as melting rate. The study also showed there was a significant reduction in caloric value in stevia added soft ice cream in comparison to control sample.

Karp et al. (2016) studied the effect of sucrose replacement with stevia on the quality attributes of muffin. The colour, texture, cooking yield, browning index, sensory scores of stevia added muffins were studied. The study indicated that the optimization with 25 per cent stevia as a sucrose replacement in muffin was acceptable. In addition, the study indicated that a reduction in sucrose above 50 per cent had a negative effect on the quality and sensory attributes of muffins.

2.4.4 Neotame as artificial sweetener

Neotame is non calorie sweetener which is a derivative of dipeptide of amino acids such as aspartic acid and phenylalanine. Neotame have high degree of sweetness and is produced by N-alkylating aspartame. It is 7000 to 13000 times sweeter than sucrose and 30 to 60 times sweeter than aspartame. It is an odourless white to off white powder and is easily soluble in alcohol while water solubility at ambient is 13g/L (Tiefenbacher, 2017). It is used in food and beverages such as carbonated soft drinks, candies, tabletop sweeteners, frozen dessert yogurt etc. Due to its high heat stability, it is widely used in baking and cooking of foods. The USFDA approved neotame as general purpose sweetener in 2002 (USFDA, 2002). The Joint Expert Committee on Food Additives defined an acceptable daily intake (ADI) of 2mg/ kg body weight/day (JECFA, 2002), it comes in market under the brand name Newtame®.

Hernández-Morales et al. (2007) studied the effect of the partial substitution of sucrose by neotame on the sensory and consistency characteristics of plain yoghurt. Authors recorded the sweetness profile of 25 per cent sucrose substituted by neotame yogurt comparable to that of the sucrose sweetened control sample.

Castro et al. (2014) developed xoconostle (sour fruit) snack using neotame sweetener. The objective of investigation was to examined the total phenolic content, antioxidant activity of fruits, dehydration techniques and sensorial attributes of the final product. Researchers reported that sweetened xoconostle snack had lower total phenolic content and antioxidant activity in comparison to control samples, whereas it had no impact on dehydration time. The sensorial analysis indicated that neotame at the concentration of 32mg/L was found acceptable in sweetened xoconostle snack.

Azevedo et al. (2015) examined the effect of high intensity sweeteners in espresso coffee. In this investigation researchers studied the ideal and equivalent sweetness and time intensity profile of sweetener in espresso coffee for sucralose, neotame, aspartame, stevia and a combination of cyclamate and saccharin at ratio of 2:1. Authors reported that the concentration of sucrose found optimum by consumers at 12.5 per cent in coffee. The equivalent sweetness indicated that neotame had highest sweetening effect in espresso coffee in comparison to control sample followed by sucralose, aspartame, stevia and combination of cyclamate and saccharin (2:1).

Kumari et al. (2016) studied the stability of aspartame and neotame in yoghurt. The comparative stability of aspartame and neotame was monitored in yoghurt during its processing, fermentation and storage. There was non-significant effect on the stability of either aspartame or neotame during storage (4–7 °C/15 days). The results indicated that neotame was more stable than aspartame under both pasteurisation and fermentation conditions; however, during storage, both sweeteners exhibited excellent stability.

2.5 Fruit pulps

India is the largest producer of fruits. These are mainly used as functional food ingredients for the preparation of several food products. Fruits are also rich source of vitamin C, potassium, carbohydrate and dietary fibers. Their regular consumption helps in the reduction of risk of various diseases such as cardiovascular cancer, alzheimer, cataract etc. (Kaur et al., 2001). Traditionally fruits, as foodstuff were available for a limited period, and when ripe, were sometime difficult to collect, preserve and transport. When ripe they had a short period of acceptability before senescence intervenes. Thus, many fruits consumed in today’s world are processed, frozen, canned or dried (Slavin & Lloyd 2012). In this study, we studied the mango and strawberry fruit pulps due to their good nutrient profile and health benefits**.**

2.5.1 Mango pulp

Mango (*Mangifera indica* L.) is known as “the King of fruits” because of its exotic flavour and mouth-watering taste. India is the largest producer of mango. Mango production in country was estimated to 20.95 million tonnes in 2018-19 (Anon, 2021c). In India mangoes are mostly grown in tropical and subtropical areas from sea level to an altitude of 1,500m. Mangoes grow best at temperature of 27 ˚C.

India is popular global exporter of fresh mangoes. The country exported 46510.27 million tonnes of mangoes globally which was worth of Rs. 60.26 USD millions during 2018-19. In India largest portion of mangoes consumed directly as fresh or table use. As mango is seasonal crop and fruits are highly perishable therefore, fruit is processed into pulp industrially and preserved for long terms ready to use products such as mango canned slices, frozen pulp, purees, bars etc. while homogenised pulp is used in the preparation of bakery filling, puddings, various drinks, ice cream and smoothies. Mango fruit also used in the preparations of various processed commodities such as jam, jellies, marmalade, etc. The nutrient composition of ripe mango is given in table 2.3.

Table 2.3: Nutrient composition of ripe mango

Fresh mango	**Nutrient Contents per 100 g**
Calories (cal)	62.1- 63.7
Moisture (g)	78.9- 82.8
Protein (g)	0.36- 0.40
Fat (g)	0.30- 0.53
Ash (g)	0.34- 0.52
Carbohydrate (g)	16.20- 17.18
Dietary fiber (g)	0.85-1.060
Calcium (mg)	6.1-12.80
Phosphorous (mg)	5.5- 17.90
Iron (mg)	0.20-0.63
Vitamin A (mg)	0.135- 1.872
Thiamine (mg)	0.020- 0.073
Riboflavin (mg)	0.025- 0.068
Niacin (mg)	0.025- 0.707
Ascorbic Acid (mg)	7.8- 172.0
Tryptophan (mg)	3-6
Methionine (mg)	4
Lysine (mg)	32-37

Source: Tharanathan et al. (2006)

Various researches have been undertaken utilizing this amazing and nutritious fruit utilizing the benefits in the product developed. Likewise, Parab et al. (2014) developed mango bar from frozen alphonso mango pulp and studied their physico-chemical analysis and sensory score at 0, 30, 60, 90 days of storage period. Researchers examined the data by using Factorial Completely Randomized Design (FCRD). They reported that moisture, non–enzymatic browning, acidity and reducing sugars had increased trend while total soluble solids, total sugars and β- carotene were reduced in mango bar during 90 days of ambient storage. Increase in moisture content was due to the absorption of moisture from the atmosphere by the package at ambient condition. Increase in reducing sugar was due to acid hydrolysis of sucrose during storage whereas decrease in the total sugar and total soluble solids was due to the significant increase in the moisture. The reduction of β- carotene content of mango bar was due to non- oxidative changes which altered the β- carotene content, colour as well as reduced the flavour and nutritive value of the product whereas, increase in the non-enzymatic browning of might be due to the decrease in sulphur dioxide.

Kadam et al. (2017) prepared *khoa burfi* with fortified with alphonso mango pulp. Authors prepared mango burfi from buffalo milk using 15 per cent alphonso pulp, 5 percent sugar, and 0.15 per cent turmeric powder (w/v of milk respectively) and added all ingredients at pat formation stage of *khoa* making. The Study showed that the product was stable for 6 days at ambient temperature which was 3 times shelf life of control burfi.

Jaglan et al. (2018) developed novel herb supplemented fruit based-soy milk fortified-yogurt. Authors prepared yogurt by using 10 per cent soy milk, 15 per cent alphanso mango pulp, 1 per cent *tulsi* leaf extract and analyzed their physio- chemical and sensory attributes. The study showed that the novel product had high protein content and antioxidant activity while fat, carbohydrate content was lower and novel product also had good consumer acceptability.

2.5.2 Strawberry pulp

Strawberry (*Fragaria x ananassa*) is typically a spurious fruit of the family *rosaceae* that grows as small sweet red fruit. It is one of the world's most commonly consumed fruit due to its great organoleptic characteristics, nutrition and vitamins. These fruits are also containing phenolic compounds which are mostly natural antioxidants. All these compounds are essential for the health especially; the strawberry phenolics are known best for their antioxidant and anti- inflammatory activity and also have anti microbial, anti-allergy and anti-hypertensive effects, as well as ability to suppress the activities of certain physiological enzymes and receptors (Giampieri et al., 2013).The top strawberries producing countries are United States followed by China and Spain. In India, it is grown commercially in Himachal Pradesh, Uttarakhand, Uttar Pradesh, Maharashtra, West Bengal, Nilgiri Hills, Delhi, Punjab, Haryana, and some areas of Rajasthan. The major centres of strawberry cultivation in India are Nainital and Dehradun districts of Uttarakhand, Mahabaleswar in Maharashtra, Kashmir valley, Bangalore and Kalipong in West Bengal. Strawberry fruit is used in various processed products like chewing gum, ice cream, soft drinks, confectionary, yogurt, fruit juices, jam and jellies etc. in order to enhance their shelf life and increase their market worth. The nutrient composition of fresh strawberry is given below in table 2.4.

Table 2.4: Nutrient composition of fresh strawberries

Fresh strawberry	Nutrient Contents per 100 g
Water (g)	91.1
Total soluble solids (g)	10
Energy (Kcal)	35
Fat (g)	0.17
Protein (g)	0.64
Carbohydrate (g)	7.63
Sugar (g)	4.89
Thiamine (mg)	0.025
Riboflavin (mg)	0.016
Niacin (mg)	0.038
Vitamin C (mg)	56
Dietary fiber (g)	1.8

(Source: USDA, 2019)

Bajwa et al. (2003) prepared ice cream using different concentration of strawberry pulp viz., 10 %, 15 %, 20 and 25 %. Authors analyzed the effect of strawberry pulp on the physico- chemical and sensory attributes of ice cream during 0, 10, 30, 40 days of storage. They reported that the addition of strawberry pulp in fresh ice cream and storage ice cream had a significant effect on the moisture, ash total solids, milk solid not fat, sucrose ascorbic acid, pH, meltdown, overrun. The fresh prepared ice cream also showed a significant difference in protein, fat, lactose and non-significant difference for storage treatments. Sensory evaluation showed that the addition of 15 per cent strawberry pulp in ice cream had scored highest followed by ice cream with 20 per cent strawberry. The study also showed that ice cream could be prepared with exotic flavour and taste using strawberry pulp.

Sonawane et al. (2007) examined the effect of various levels of strawberry pulp and sugar on the chemical composition of *Shrikhand* during storage. In this study, three levels of strawberry pulp i.e., 10, 15 and 20 per cent and two levels of sugar i.e., 30 and 40 per cent on the weight basis of *chakka* were incorporated in the preliminary trials along with the control sample. Study indicated that the moisture content during storage of *shrikhand* at ambient temperature was higher compared to refrigeration storage. The acidity of *shrikhand* was also increased rapidly at ambient temperature in comparisons to samples stored at refrigeration temperature.

Balthazar et al. (2019) developed a fermented beverage of semi-skimmed sheep milk fortified with strawberry pulp, potato starch, inulin or both. Authors reported the survival of *L. plantarum* after in vitro simulated digestion, viability of lactic acid bacteria, nutritional profile and bioactivity compounds during storage. Researchers reported that lactic acid bacteria were viable throughout the storage period but *L. plantarum* showed good viability. This could be due to the addition of potato starch; inulin or both did not affect the *L. plantarum* survival during in vitro digestion. The study also concluded that developed strawberry added fermented semi skimmed sheep milk beverage is excellent source of minerals and proteins.

2.6 Smoothie

2.6.1 Definition

Smoothies are blended beverages containing fruit, fruit juices, yogurt, milk or honey. Smoothies can be considered as a typical example of so-called super foods, which are defined as natural foods, considered as especially beneficial due to their nutrient profile and health- protecting qualities which are created by fruit components (Medina, 2011). These are categorized under food beverages prepared by blending different dairy and non-dairy-based ingredients. On the basis of ingredients and functionality, it is classified as fruit-based, fruit and dairy-based and functional beverages. Smoothies are defined by higher contents of dietary fibers, higher energy value, vitamin C and other substances with antioxidative properties as compared to fruit-based juices (Ruxton, 2008; Watzl, 2008). Smoothies are commonly found on the market in the form of ready- made or made-to-order drink, snack and meal alternative. Smoothies are nutrient dense and it contains different nutrients such as carbohydrate (sugars), vitamins and minerals needed by the body for healthy living (Aderinola, 2018).

2.6.2 Literature on smoothie

Balaswamy et al. (2013) developed smoothie from selected fruit pulp and juices. Authors used fruit pulp from mango, papaya, sapota, banana and juices from green grapes and pineapple blended in different ratios (10-60 per cent). The prepared smoothie packed in glass bottle after thermal treatment lasted for six months. Authors reported increase in reducing sugar and total phenols, whereas drop in acidity and total carotenoids was recorded during storage. Increase in reducing sugar and total phenols may be due to the release of monomers by hydrolysis of polysaccharides in the presence of citric acid and the release of bound phenols from the cell wall and dissociation of dimers into monomers during storage, respectively. Drop in acidity and total carotenoids may be due to the variation in quantity of fruit pulps and reaction of organic acids with sugar and oxidation of polyphenols during storage, respectively.

Sun-Waterhouse et al. (2014) prepared smoothie with high concentrations of apple polyphenols i.e.,500 mg per 300 ml beverage, apple fiber (each soluble and insoluble fiber) of two different particle sizes one is 250 µm and other ranges from 300- 700 µm and carboxymethylcellulose (CMC) as stabilizers (0.05-2.00 %). The results indicated that the particle size of apple fiber, simulated beverage consumption temperature and CMC concentrations had an impact on the viscoelastic properties, phase stability and polyohenols extractability of developed smoothie. 1- 1.5 % concentration of carboxymethylcellulose found to be acceptable in order to achieve relatively high polyphenols extractability, consistency and flow in smoothies and viscosity of smoothie increases with increasing apple fiber particle size. The increase in reducing sugars and in polyphenols might be due to the release of bound phenols from cell wall and dissociation of dimers into monomers during storage.

Rani et al. (2016) studied the effect of different ingredients viz., mango pulp, flour, sugar and pectin on the sensory characteristics of milk-based breakfast smoothie. Authors reported that milk beyond 30 per cent resulted in coagulation of milk proteins and separation of watery layer. This is because above 30 per cent samples were coagulated during pasteurization at 90 C for 5 min. Therefore, the proportion of milk, honey, carrot juice, sugar, pectin were found optimum at 30 %, 3 %, 3 %, 9-11 % and 0.2 -0.4 per cent respectively.

Nowicka et al. (2016) studied the sensory attributes and changes of physicochemical properties during storage of smoothie prepared from selected fruit. Authors prepared the smoothie by mixing sour puree with apple, pear, quince and flowering quince juices. Phenolic compounds, antioxidant activity, physical parameters and sensory attributes studied before and after six months of storage period. After six months storage, authors found there was a protective effect on flowering quince juice and quince juice addition on the polyphenolic content, antioxidant activity. They reported that the greater stability of polyphenol compounds in prepared product was related with higher antioxidant activity and positive co-relation of polymeric procyanidins.

Picouet et al. (2016) studied the effects of thermal and high-pressure treatments on the microbiological, nutritional and sensory quality of a multi-fruit smoothie. Authors studied the effect of the application of two stabilization treatments mild heating (MH; 80 °C for 7 min) and high-pressure processing (HPP; 350 MPa for 5 min) on multi-fruit smoothies were compared on a wide range of quality parameters immediately after treatment and during a refrigerated storage of 21 days. They reported that HPP provided smoothies with better sensory properties and higher nutritional quality than MH. The study also found that HPP better preserved the original colour and flavour of the multi-fruit smoothies and overall, HPP could be an effective alternative to thermal processing for the production of a high-quality multi-fruit smoothie.

Mehta et al. (2017) developed high protein, high fiber smoothie as a grab-and-go breakfast option using response surface methodology on the basis of sensory (color and appearance, flavor, consistency, sweetness and overall acceptability) and physical (expressible serum and viscosity) properties of the product. Smoothie was optimized with 1.8% (w/w) soy protein isolate, 166.8 ppm sucralose and 0.5% (w/w) pectin with acceptable quality.

Ribeiro et al. (2017) developed banana, strawberry and jucara smoothie by using experimental mixture design. In this study jucara ratio kept constant while varied the banana: strawberry ratio and analyzed the antioxidant capacity and sensory acceptability. Results indicated that formulation of smoothie with equal amount of banana and strawberry pulps provided good consistency and flow, intermediate value of antioxidant capacity as well as good sensory acceptability.

Saranyambiga et al. (2017) prepared synbiotic smoothie utilizing jamun and yogurt. The inclusion level of jamun: yoghurt ratio at 20:80 per cent was found optimum by sensory analysis. Jamun juice exerted maximum prebiotic effect on *L. plantarum*. *L. plantarum* was examined for its probiotic properties, microencapsulated in jamun juice and incorporated into smoothie to make it synbiotic.

Aderinola (2018) studied the effects of pumpkin leaves on the chemical composition and antioxidant properties of smoothies. The aim of the study was to evaluate the effect of the addition of fluted pumpkin leaves on the crude protein content and antioxidant capacities of smoothies made from a blend of pineapple, banana and apple. Pumpkin leaves were added at the rate of 1.5, 3.0 and 4.5% to the different ratios of the fruit blends (smoothies) and analyzed for proximate, antioxidant (FRAP and DPPH), mineral, vitamin, physicochemical and quality acceptability. The study showed the addition of fluted pumpkin leaves significantly improved the protein, carbohydrate and minerals especially calcium and potassium. The addition of the leaves however did not improve the antioxidant abilities of the smoothies compared to the control sample. The nutrient composition of the samples indicated that it is a nutrient dense non-alcoholic beverage which can serve as a healthy alternative to carbonated drinks.

Gallino et al. (2019) studied the development and characterization of probiotic fermented smoothie beverage. Authors first prepared thirty-six formulations using yoghurt and different pulp. Six formulations were selected for further study. These formulations included probiotic yoghurt with the addition of mango pulp, grape, red fruits (containing 8 and 10% sucrose), mango/ passion fruit, and red fruits/açaí, using a yogurt/pulp ratio of 60/40 (w/w) and evaluate the microbiological quality, pH, acidity, syneresis, probiotics viability, sensory acceptance and consumers' preference. The beverages presented adequate hygienic-sanitary quality, with an initial probiotics viability around 7 log CFU/mL. Researchers observed an increase in acidity and a decrease in pH, as well as a higher rate of syneresis and a slight decrease in probiotic viability in all formulations throughout 30 days of storage. The probiotic smoothie made with red fruit pulp (10% sugar) was selected due to its better sensory acceptance and maintenance of probiotic counts to confer health benefits.

Teixeira et al. (2019) prepared a fruit smoothie with solid albumen of green coconut and studied their composition. Smoothie formulation were made by keeping constant concentration of solid albumen i.e., 20 per cent and varied the concentration of acerola (West Indian cherry) pineapple and coconut water pulp, which represented 80 per cent of total weight of the product. Authors examined the vitamin C content, antioxidant properties and overall sensory acceptability. The results indicated that the formulation with high concentration of acerola pulp had higher value of bioactive compounds but were not sensory acceptable. The final optimized formulation contained 52.8 % pineapple, 27.2% acerola and 20 % of solid albumen of green coconut which was also sensorially acceptable with score of 7 i.e., good. The study also represented that this new type of beverage formulation provides good nutritional value and a source of vitamin C that can add value to a co-product of the beverage industry.

CHAPTER III

MATERIALS AND METHODS

This chapter includes all the experimental details employed in the present investigation entitled "Development of fiber fortified reduced calorie probiotic smoothie" and contains information pertaining to the raw materials utilized in research. Also, along with methodologies adopted and analytical procedures (sensory, physico-chemical, microbiological and statistical), equipment's and instruments have been illustrated in detailed herein.

The present investigation was divided into preliminary trials for screening of optimized levels of dietary fiber, two different fruit pulps (strawberry, mango) and sweeteners consisting of synthetic and natural origin. Also, standardization of technology at laboratory scale, the storage study of the developed optimized product, costing and consumer survey was undertaken to complete the potential perspectives of the study. All experimental samples of investigation were prepared and analyzed in the Food Technology Laboratory, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The details of materials and methods followed are presented herein.

3.1 PROCUREMENT OF RAW MATERIALS

Selection of ingredients

Selection of good quality raw material is very important factor for any research investigation followed by good manufacturing practices. Hence, premium quality ingredients were selected for use in the present study listed as under.

3.1.1 Raw milk

Fresh, clean and good quality mixed milk was obtained from Experimental Dairy plant, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. Mixed milk was obtained from plant which was further standardized to desired SNF and fat ratio employing cream separation (Cowbel cream separator, capacity 120 Kg per hour). A final composition of 3% fat & 8.5 % SNF was maintained in milk for the probiotic preparation of yogurt.

3.1.2 Fruit pulp

3.1.2.1 Strawberry pulp

Frozen strawberry pulp (Jain farm fresh Lot no. 9SWS5BH103) was purchased from the local market of Ludhiana.

3.1.2.2 Alphonso Mango pulp

Alphonso Mango pulp (AAMRUS Lot no. 8SAM1BH052) was purchased from the local market of Ludhiana.

3.1.3 Sweeteners

Free samples of Stevia (Rebaudioside- A 97 % Lot no. RA20190725) and Neotame (Lot no. HT190718A) sweeteners were provided by Sweetener India®, Ghaziabad Uttar Pradesh.

3.1.4 Dietary fibers

3.1.4.1 Apple fiber

Free samples of apple fiber (Lot no. 07524181030) were provided by Rettenmaier India Pvt. Ltd. Thane, Maharashtra.

3.1.4.2 Sunfiber®

Free samples of Sunfiber® (Lot no. 909071) were provided by Taiyo Kagaku India Pvt. Ltd. (TKI) in Aurangabad, Maharashtra.

3.1.4.3 Diesol ™

Free samples of Diesol™ (Lot no. 204003) were provided by Drytech Processes India Pvt. Ltd. Mumbai, Maharashtra as a free sample.

3.1.5 Commercial yoghurt Starter culture

Commercial starter cultures STA IDC 70 (Lot no. C242126A) (*S. thermophilus and L. delbrueckii ssp. bulgaricus*) was purchased from Centro Sperimentale del Latte in freeze dried form and stored as per the recommendation of the manufacturer.

3.1.6 Probiotic culture

Thermophilic strain of probiotic culture *Lactobacillus acidophilus* NCDC- 15 was procured from National Collection of Dairy Cultures (NCDC) of Dairy Microbiology Division, National Dairy Research Institute (NDRI) Karnal.

3.1.6.1 Culture maintenance and propagation

Probiotic culture procured from NCDC was maintained in sterilized skim milk for propagation. The fresh skim milk 100 ml was taken into 250 ml conical flasks and plugged with non- absorbent cotton plugs. The flasks were transferred in autoclave and sterilized at 15 psi pressure for 15 minutes. The culture was propagated by inoculating sterilized skim milk @ 3 per cent level, in laminar air flow chamber (Make: NSW INDIA). Thereafter, the flasks were incubated at 37˚ C for 6 hours and stored at refrigeration temperature. The sub- culturing was done at regular intervals to maintain culture activity.

3.1.7 Refined table sugar

Good quality food grade refined sugar was procured from domestic market, Ludhiana**.**

3.1.8 Dietary fiber kit

Dietary fiber kit purchased from the Sigma Life Sciences TDF100A-1KT (Lot no. #SLCB6004).

3.1.9 Flavour and colour

Mango and strawberry flavour and colour were obtained from International Flavours and Fragrances India Pvt. Ltd., Chennai.

3.1.10 Chemicals

The chemicals used in the investigation of various physico-chemical and microbiological parameters were of analytical grade (AR).

3.1.11 Microbiological media

Dehydrated media, viz potato dextrose agar (PDA), nutrient agar (NA) and MRS agar and MacConkey agar procured from Hi-Media Laboratories, Mumbai were used for microbiological analysis.

3.1.12 Packaging material

Smoothie was packed in glass bottles and stored at refrigeration temperature for further analysis at regular intervals of 3 days till spoilage.

3.2 Methodology opted

Various technological and analytical methods adopted in the preparation and testing of raw materials and final product (smoothie) have been outlined in following section as here under.

3.2.1 Preparation of probiotic yogurt

Probiotic yoghurt was prepared by the standardization of milk (3 per cent fat and 8.5 per cent SNF) followed by heating at 90˚C for 5 minutes was performed. The milk was cooled to 42 ˚C before inoculation. Active culture of *Lactobacillus acidiphillus* was added @2 % (w/w) whereas commercial yogurt starter culture was added @ 0.02 per litre as per the recommendation. The inoculated mix was then mixed thoroughly and dispensed in 50 mL polystyrene cups with lids and incubated at 42°C either for 4-5 hours or until the pH dropped to 5.0±0.1. The fermentation was stopped by transferring the cups immediately to refrigerator maintained at 4±1°C. The product making experiment was triplicate. Samples of inoculated mixes (0 h) were removed prior to incubation for enumeration of the viable counts, measuring pH, and acidity determining lactic acid content. All samples were analysed for changes in pH, lactic acid content, and probiotics count as well as viability of starter cultures every hour till pH dropped to 5.0±0.1.

3.2.1.1 Fermentation kinetics

Samples were removed at 0, 1, 2, 3, 4, 24h and analysed for pH, titratable acidity and viability of probiotic bacteria.

3.2.1.2 pH

The pH values of the samples during fermentation were monitored using a digital pH analyzer.

3.2.1.3 Titratable acidity

The titratable acidity (TA) was determined after mixing a sample with 10 ml of hot (65±1°C) deionised water and titrated with 0.1 M NaOH using 1% (w/v) phenolphthalein as an indicator. The results were expressed as per cent lactic acid (Dave & Shah 1997).

3.2.1.4 Viability of starter bacteria

Viability of starter and probiotic culture was monitored in the fermented milk at 42°C at 0, 1, 2, 3, 4, 5, 24h. Serial dilutions were carried out using sterile saline solution. For the individually fermented blend, the lactobacilli colony counts, both starter and probiotic, were determined by enumeration on MRS agar, respectively (Sarvari et al., 2014). The counts were expressed as log colony forming units (CFU) per gram of fermented milk.

3.2.2 Preparation of fiber fortified probiotic smoothie

3.2.2.1Preparation of sugar powder

The finely powdered table sugar (sucrose) was prepared by using mixer grinder.

3.2.2.2 Addition of fiber for fortification

Fortification was done using addition of different fibers in different ratio. Three different fibers i.e., diesol, sunfiber, apple fiber were selected and added to smoothie at different levels in preliminary trials. For effective dissolution lukewarm water (40˚C) was added to dissolve the fiber make smooth slurry and then added to blended yogurt.

3.2.2.3 Addition of pulp

Two different fruit pulps, viz. mango pulp and strawberry were selected and added to smoothie at various concentrations in preliminary trials. Out of all screened levels, optimized level of pasteurized pulp i.e. mango @ 10% and @12% strawberry was incorporated in blended yogurt.

3.2.3 Process flow chart for fiber fortified Mango probiotic smoothie

Standardization of milk (3% fat and 8.5 % SNF)

↓

Pasteurization of milk at 90º C/ 5min

↓

Cooling at 42º C

↓

Inoculation of milk with starter culture (0.02g/L) and probiotic culture (*Lactobacillus acidophilus*) @ 2%

↓

Incubation the mixture at 42º C for 4-5 hours

↓

Storage at refrigeration temperature 5º C

↓

Stir & blend obtained yoghurt

↓

Addition of standardized levels of apple fiber, sunfiber, pasteurized mango pulp and sugar

↓

Mixing and blending

↓

Packaging

↓

Storage (5º C)

Fig. 3.1: Process flow diagram for preparation of fiber fortified mango probiotic smoothie

Note: In reduced calorie mango smoothie sugar was fully replaced with blend of artificial and natural sweeteners.

3.2.4 Process flow chart for fiber fortified Strawberry probiotic smoothie

Standardization of milk (3% fat and 8.5 % SNF)

↓

Pasteurization of milk at 90º C/ 5min

↓

Cooling at 42º C

↓

Inoculation of milk with starter culture (0.02g/L) and probiotic culture (*Lactobacillus acidophilus*) @ 2%

↓

Incubation the mixture at 42º C for 4-5 hours

↓

Storage at refrigeration temperature 5 ºC

↓

Stir & blend prepared yoghurt

↓

Addition of standardized levels of apple fiber, sunfiber, pasteurized strawberry pulp and sugar

↓

Mixing and blending

↓

Packaging

↓

Storage (5º C)

Fig. 3.2: Process flow diagram for preparation of fiber fortified strawberry probiotic smoothie

Note: In reduced calorie smoothie preparation sugar was fully replaced with blend of artificial and natural sweeteners.

3.3. Sensory evaluation of smoothie

The smoothie samples were evaluated for their acceptability at various stages during storage studies like checking acceptability in terms of percentages of fiber, pulp etc. Sensory evaluation was carried out by presenting approximately 50 ml of smoothie sample to seven trained panellists, selected from the faculty of Dairy Technology Division. Nine-point hedonic scale was followed to carry out the evaluation of samples for sensory attributes such as colour and appearance, consistency, sweetness, flavour and overall acceptability (Amerine et al.,1965; Shone et al.,1979). The nine-point hedonic scale includes various scales of grading i.e. liked extremely (9), liked very much (8), liked moderately (7), liked slightly (6), neither liked nor disliked (5), disliked slightly (4), disliked moderately (3), disliked very much (2), disliked extremely (1) (Lawless & Heymann, 1998).

3.4 Analytical methods

3.4.1 Physico-chemical analysis

Various tests conducted for physico- chemical analysis for the raw materials and fiber fortified reduced calorie probiotic smoothie during its storage are described in the following section.

3.4.1.1 Analysis of raw materials

3.4.1.1.1 Fat content

Fat content of mixed mil and skim milk was estimated using Gerber method as per IS: 1981**.**

3.4.1.1.2 SNF content

SNF content in mixed milk and skim milk was estimated using following Richmond formula as per IS: 1981**.**

$$SNF = 0.25 \times (Fat + CLR) + 0.44$$

3.4.1.2 Physico-chemical tests for smoothie

The brief description of different methods used to examine physico-chemical properties of smoothie is given below.

3.4.1.2.1 Crude fat

Crude fat in products was determined by ether extraction method as per AOAC (1995). Five g moisture-free sample was quantitatively transferred to extraction thimble and extracted in Soxplus apparatus (SCS 08R) with petroleum ether at 150-200˚C for 90 min. The extracted fat was collected in previously weighed and dried extraction beakers. Fat extracted was dried in oven at 100˚C for 30 min, desiccated, cooled and weighed.

$$\text{Fat \% (by weight)} = \frac{W1 - W}{W2} \times 100$$

Where,

W = Weight of extraction beaker,

W1= Weight of extraction beaker + extracted fat after drying, and

W2 = Weight of Sample.

3.4.1.2.2 pH

pH of smoothie was determined by using digital pH meter at 20 ± 0.1˚C. The pH meter was first calibrated using standard buffers of pH 4.0 and 9.2 and standardized using pH buffer of 7.0 at 20.0 ± 0.10C. The pH was measured in triplicates and after each reading the electrode was dipped into distilled water and wiped off.

3.4.1.2.3 Titratable acidity

The titratable acidity of the products were estimated by adopting the method as described by (AOAC, 1975) for cheese with modifications.

About two g of sample was taken in 250 ml conical flask and mixed homogenously by adding 20 ml hot distilled water (65˚C). This was followed by addition of 10 ml of 0.1 N sodium hydroxides and 1 ml of 0.5% phenolphthalein indicator. The mixture was titrated against 0.1 N HCl with continuous stirring till the pink colours disappeared completely. Then acidity was calculated as % of lactic acid.

$$\text{Titratable acidity (as \% Lactic acid)} = 9NV/W$$

Where,

V = Titrate value in mL

N = Normality of the alkali solution

W = Weight in g of smoothies taken for the test.

3.4.1.2.4 Viscosity

The rheological properties of samples were measured at refrigeration temperature (5±1°C) by a rotational viscometer (Model LV DV2T, Brookfield Engineering, Inc., USA) equipped with spindle model LV-(02)62. Before measurements, smoothie samples were gently stirred with a spatula for homogeneity. Samples were sheared at 10 rpm for 3 minutes and 5 readings were recorded at the interval of 30 seconds and the apparent viscosity was recorded in centipoises (cP). Readings recorded after 30 seconds of shearing were taken for average viscosity calculation.

3.4.1.2.5 Total solids

Due to presence of good amount of carbohydrate & fiber, total solids (TS) content of smoothie’s samples was determined by the gravimetric method as described in (AOAC, 1995) suggested for condensed milk samples. A dry, empty and clean aluminium dish was weighed with cover. Metal dish containing about 25 g of the prepared acid washed sand with lid open and a stirring rod was heated, in the oven at 98-100°C for about two hours. The dish with lid on was allowed to cool in an efficient desiccator for 30 to 40 minutes. The sand was tilted to one side of the dish and about 4-5 g of the material was weighed accurately. About 5 ml of distilled water was added in the dish and the sand was thoroughly mixed with the sample by stirring with the glass rod, smoothing out lumps and spreading the mixture over the bottom of the dish. The rod was left in the mixture. The dish was placed on a boiling water bath for 20 minutes, carefully stirring the mixture from time to time, then the bottom of the dish was wiped and the dish was transferred along with the glass rod to the 'well-ventilated' oven at 98-100°C. After one and half hour, the dish was removed, placed in an efficient desiccator, allowed to cool and weighed. The dish was replaced in the oven for a further period of 1 hour at 98-100°C, removed from desiccator, cooled and weighed again. The process was repeated for heating, cooling and weighing after every 1 hour, till consecutive weighing agreed to within 0.5 mg.

$$\text{\% TS in smoothie} = \frac{W3-W1}{W2-W1} \times 100$$

Where,

W1 = Weight of empty dish + sand + glass rod

W2 = W1 + weight of sample

W3 = W1 + weight of sample after drying

3.4.1.2.6 Total sugars

Phenol–Sulfuric Acid Method (Dubois et al., 1956) is the most widely used colorimetric method to date for determination of carbohydrate concentration in aqueous solutions (Dubois et al., 1956). The basic principle of this method is that carbohydrates, when dehydrated by reaction with concentrated sulfuric acid, produce furfural derivatives. Further reaction between furfural derivatives and phenol develops detectible color. The standard procedure of the method is as follows- A 1 mL aliquot of a sample solution is mixed with 1 mL of 5% aqueous solution of phenol in a test tube. Subsequently, 5 mL of concentrate sulfuric acid is added rapidly to the mixture. After allowing the test tubes to stand for 10 min, they are vortexed for 30 s and placed for 20 min in a water bath at room temperature for color development. Then, light absorption at 490 nm is recorded on a spectrophotometer. Reference solutions are prepared in identical manner as above, except that the test solution is replaced by distilled water.

3.4.1.2.7Total Soluble Solids (TSS)

The total soluble solids of the optimized beverage were calculated in terms of degree Brix using a hand refractometer. Refractometer is an analog instrument for measuring substances dissolved in liquid by noting the refractive index of the respective liquid. It works on the principle that the light travelling through the sample is either passed through to the reticle or totally internally reflected. The net effect is that a shadow line forms between the illuminated area and the dark area. It is where this shadow line crosses the scale a reading is taken. Because refractive index is very temperature dependent, it is important to use a refractometer with automatic temperature compensation. Compensation is accomplished through the use of a small bi-metallic strip that moves a lens or prism in response to temperature changes. Further, the refracted angle is then viewed by the user through a magnifying eyepiece. The scale most commonly used is referred to as the Brix scale.

Firstly, the 0-32˚ Brix range refractometer was calibrated using distilled water. Then a sample of fiber fortified probiotic smoothie was placed between a measuring prism and a small cover plate, and reading was noted according.

3.4.1.2.8 Instrumental Colour measurement

Colour of smooothie samples were measured using Colour Flex Colorimeter (Hunterlab, Reston, Virginia) supplied along with the universal software Easy Match QC (version 4.62) and the results were expressed in terms of CIE-LAB system. The colorimeter was equipped with dual beam xenon flash lamp as source of light. Prior to analyzing the samples, the instrument was calibrated with standard black glass and white tile as specified by the manufacturer of the equipment. Data was received through the software in terms of values for L* (lightness, 0 (black) to 100 (white)); a* (redness, +60 (red) to -60 (green)) and b* ((yellowness, +60 (yellow) to -60 (blue)) (Bindu et al., 2007).

The sample of smoothie was placed in the transmission port of the optical unit of a color difference meter. Light energy from a controlled source was directed through the specimen into an integrating sphere. Phototubes are positioned at the top of the sphere to view the interior of the sphere. Electrical signals proportional to light quantities present are directed by cable to the measuring unit, where they are read directly as L*, a* and b* colour values.

3.4.1.2.9 Serum separation

Serum separation of smoothie was measured by centrifugation method as defined by (Keogh & Kennedy, 1998) with slight modifications. Ten ml of sample was filled in 15 ml centrifuge tubes and centrifugated in a centrifuge at 2000 rpm for 10 minutes. The volume of clear whey measured and expressed as ml of whey off per 10 ml of sample.

3.4.1.2.10 Dietary Fiber content

Total dietary fiber content in the product was determined using Total Dietary Fiber Assay Kit supplied by Sigma Aldrich Inc. (TDF-100A). It uses a combination of enzymatic and gravimetric methods, based on (AOAC, 2000). The principle involves gelatinization of samples with heat stable α-amylase and enzymatic digestion with protease and amyloglucosidase to remove the protein and starch present in the sample. Ethanol is added to precipitate the soluble dietary fiber. The filtered residue is washed with ethanol and acetone and weighed after drying. Half of the residue is analyzed for protein and the other half for ash. Total dietary fiber is the weight of the residue less the weight of the protein and ash.

Fritted crucibles of porosity #2 (coarse 40 -60 µ) were washed and heated in muffle furnance at 525 ˚C for one hour and cooled. They were then soaked and washed in water followed by air drying. Celite (0.5g) was added to each crucible and dried at 130 in a hot air oven to constant weight (for one hour). The crucibles ere then cooled in a desiccator and weighed to the nearest 0.1 mg. This was recorded as 'W_1' (celite +crucible weight). These crucibles were stored in the desiccator until needed.

Samples and blanks to be tested were run in quadruplicate so that duplicate protein and ash values were available for improved accuracy. 1g sample of sample were weighed into tall form beakers in such a way that sample weights did not differ by more than 20 mg. The weights were recorded 0.1 mg. 50 ml of phosphate buffer (pH 6.0) was added to each beaker. Using micropipette 0.10 ml α-amylase product (product code A3306) as dispensed into each beaker and mixed well. Beakers were covered with aluminium foil and placed in a water bath. Contents were incubated for 15 min after the internal temperature reached 95 ˚C and beakers were agitated gently at 5 min intervals. They were then allowed to cool to room temperature. The pH of the solutions were adjusted to 7.5± 0.2 by adding 10 ml of 0.275 N NaOH. Again pH was checked and adjusted using NaOH or HCl. 50 mg/ml solution of protease (Product code P3910) was prepared immediately before use phosphate buffer (0.08 M, pH 6.0). Using micropipette accurately 0.1 ml of this solution (5 mg protease) was added into each beaker. After covering with aluminium foil, the beakers were kept in a 60 ˚C water bath. They were incubated for 30 min after the internal temperature of the beakers reached 60 ˚C with continuous agitation. After cooling the solutions to room temperature, the pH was adjusted to 4.0 using 0.325 M HCl (~ 10 ml). Again 0.1 ml of amyloglucosidase (Product code A9913) was added to each beaker with a micropipette. After covering the beakers were again kept for incubation in a water bath at 60 ˚C for 30 min after the internal temperature reached 60 ˚C. Four volumes of 95 % ethanol were added to each beaker and kept overnight at room temperature to allow complete precipitation.

The bed of celite was wetted and redistribute in each crucible using 78 % ethanol. Using gentle suction, the celite was drawn onto frit as an even mat and maintaining that suction the precipitate and suspension were transferred from each beaker to its respective crucible. The residue was washed with three 20 ml portion of 78 % ethanol, two 10 ml portion of 95 % ethanol and two 10 ml portion of acetone. The crucibles containing residues were dried overnight in an oven set at 105 ˚C. The crucibles were cooled in a desiccator, weighed to nearest 0.1 mg. This weight was recorded as 'W_2' (residue + celite + crucible weight).

The residues from two samples and two blanks were analyzed for protein by Kjeldahl nitrogen analysis as specified in the (AOAC, 1997). The residues in crucibles from the remaining two samples and two blanks were ignited for 5 hours at 525˚C in muffle furnance. After cooling in a desiccator, they were weighed to nearest 0.2 mg and the weight recorded as 'W_3' (Ash + Celite + Crucible Weight).

Calculation:

$$\text{Residue weight} = W_2\text{-}W_1$$

$$\text{Ash weight} = W_3\text{-}W_1$$

$$B = [R_{BLANK} - P_{BLANK} - A_{BLANK}]$$

$$\%\ TDF = [R_{SAMPLE} - P_{SAMPLE} – A_{SAMPLE} - B)/SW] \text{ X } 100$$

Where,

TDF = Total dietary fiber

R = Average residue weight (mg)

P = Average protein weight (mg)

A = Average ash weight (mg)

SW = Average sample weight (mg)

3.4.2 Microbiological analysis

Fresh and stored smoothies were analyzed for the lactic count, coliform count and yeast and mould count in accordance with method described in IS:SP:18 (1981).

3.4.2.1 Preparation of media

All media were prepared from dehydrated media obtained from Hi-media laboratory Mumbai and sterilized by autoclaving at 121˚ C for 15 min.

3.4.2.2 Dilution blank

The dilution blanks of 9 ml portions were made from (0.85-0.90 % sodium chloride) solution in culture tubes. The tubes were plugged with cotton and autoclaved at 121˚ C for 15 minutes.

3.4.2.3 Sampling of smoothie

Sample of smoothie was mixed thoroughly to make the contents homogenous. One ml of representative sample was taken and transferred to the tube containing 9 ml sterile saline. Several serial dilutions were then made in 9 ml dilution blanks.

3.4.2.4 Probiotic count

The count was enumerated using MRS agar. Media was rehydrated by dissolving 67.15 g of dry media in 1000 ml distilled water. The mixture was then boiled to dissolve the media completely. It was then filled in conical flask and the mouth of the conical flask was closed with cotton plugs. The conical flasks were then sterilized by autoclaving at 15 psi pressure (121˚ C) for 15 minutes.

One ml of the diluted sample (suitable dilution) was transferred in each of the triplicate petri dishes. Then in each petri dish 15-20 ml of the melted agar (at 45˚ C) was poured, and the contents were mixed well by rotating in a clock wise and anticlockwise position slowly. The contents were allowed to solidify at room temperature. The plates were then inverted and incubated at 37±1˚ C for 2 to 3 days.

3.4.2.5 Yeast and mould count

The count was enumerated using potato dextrose agar (PDA). Media was rehydrated by dissolving 44 g of dry media in 1000 ml distilled water. The mixture was then boiled to dissolve the media completely. It was then filled in conical flask and the mouth of the conical flask was closed with cotton plugs. The conical flasks were then sterilized by autoclaving at 15 psi pressure (121˚ C) for 15 minutes. One ml of 10% tartaric acid was added to lower the pH of the media to 3.5.

One ml of the diluted sample (suitable dilution) was transferred in each of the triplicate petri dishes. Then in each petri dish 15-20 ml of the melted agar (at 45˚ C) was poured, and the contents were mixed well by rotating in a clock wise and anticlockwise position slowly. The contents were allowed to solidify at room temperature. The plates were then inverted and incubated at 25±2˚ C for 2 to 3 days.

3.4.2.6 Coliform count

The count was enumerated using MacConkey agar. Media was rehydrated by dissolving 40.07 g of dry media powder in 1000 ml distilled water. The mixture was then boiled to dissolve the media completely and then cooled to 45˚ C. It was then filled in conical flask and the mouth of the conical flask was closed with cotton plugs. The conical flasks were then sterilized by autoclaving at 15 psi pressure (121˚ C) for 15 minutes. One ml of the diluted sample (1:00 dilution) was transferred in each of the triplicate petri dishes. Then in each petri dish 15-20 ml of the melted agar (at 45˚ C) was poured, and the contents were mixed well by rotating in a clock wise and anticlockwise position slowly. The media was allowed to solidify and then incubated after inverting the plates at 37±1˚ C for 2 days.

3.5 HPLC analysis of neotame sweetener

3.5.1 Materials for HPLC analysis

The glassware from Borosil Glass Works Ltd. (India) and plasticwares including polypropylene tubes (Corning, USA), micropipette tips (Imperial Bio Medic, India), microfuge tubes, PTFE syringe filters 0.45 µm (Randisc™), ultrasonic cleaner bath (titus™) and disposal syringes (Dispovan) were used in the present study. Chromatographic auto sampler glass vials (amber coloured) were purchased from Agilent Technologies Ltd. (USA). HPLC grade water, acetonitrile, zinc sulphate, potassium ferrocyanide were purchased from Hi Media. Solid phase extraction cartridge Discovery™ DSC 18Lt SPE Tube bed wt. 1g, volume 6ml (Product code 52616) was purchased from Sigma-Aldrich. Free sample of neotame standard (Lot no. H809349211) was provided by NuraSweet Co., 1762 Lovers Lane, Augusta GA 30901.

3.5.2 Chromatographic conditions for neotame analysis

HPLC analysis conditions for the extraction of neotame from the smoothie sample was optimized by Kumari et al., 2016 was used.

Table 3.1: Chromatographic conditions for neotame analysis

HPLC analysis conditions:	
Analytical equipment	Agilent 1260 infinity HPLC system operated via OpenLAB EzChorme software
Guard column	Water ® Spherisorb® C-18 (250*4.6mm , 5µm)
Mobile phase	Water: Acetonitrile (60:40)
Flow rate	1 ml/ min
Run time	20 min
Column heater temperature	40˚C
Sample volume	20µl
UV detector wavelength	210 nm

3.5.3 Plotting of standard curve and determination of detection limit

Standard curves for neotame were plotted. Curves were made by injecting 5, 10, 25, 50, 100 ppm of standard solution of neotame. Five points standard curve was then drawn by plotting concentration against peak area.

3.5.4 Peak identification and confirmation

The detection of the peak in the unknown sample was achieved by through the comparing the retention times of reference standards. Identification was done from the peak areas of the reference standards. The criteria used for confirmation of the presence of sweetener in the sample were overlapping of standard spectrum and sample spectrum for a sweetener.

3.5.5 Sample preparation

3.5.5.1 Extraction of neotame from sample

20 gm of neotame sweetened smoothie was accurately weighed in 100 ml beaker and added 50 ml of HPLC water to it. Mixture was vortexed for 2 min and kept in an ultra sonication bath at 40 ˚C for 20 minutes. 5ml of Carrez solution no.1 (dissolving 3.6 g potassium ferrocyanide in 100 ml water) was added and mixed followed by addition of 5 ml of Carrez solution no. 2 (dissolving 7.2 g of zinc sulphate in 100 ml water). The solution was shaken vigorously and allowed to stand for 10 min and final volume made up to 100 ml with HPLC water. Further, the mixture was filtered through Whatman no. 1 filter paper. The filtrate was introduced into the SPE cartridge for pre- purification.

3.5.5.2 Solid phase extraction of neotame

Additional purification was carried out using a SPE C18 cartridge. 2ml of filtrate was introduced in SPE cartridge previously activated with 3 ml methanol and 20 ml HPLC water as flavourings and fat could not be separated by Carrez clarification. The flow rate through cartridge was less than 1 ml/ min. The cartridge was then washed with 10 ml of HPLC water to remove impurities. Neotame absorbed on the cartridge was eluted with 3 ml of methanol. Further, elute was concentrated to dryness by nitrogen drying. The residue was dissolved in 1 ml of methanol and filtered through a syringe filter of 0.45µm and then aliquot of 0.22 µl the filtrate was injected into the HPLC.

3.5.5.3 Recovery experiments

The recovery percent of neotame was calculated against the standardized chromatographic conditions. Standard solutions of sweeteners were given similar treatments as used for the sample preparation to obtain maximum recovery. Area of sweetener extracted from the product was compared with area of the standard.

Recovery percentage was calculated using the following formula:

$$\textbf{Percent Recovery} = \frac{X}{Y} \times 100$$

Where,

X = Peak area of sweetener after recovery from Product

Y = Peak area of standard sweetener

3.6 Storage studies

The fiber fortified reduced calorie probiotic smoothie samples along the control sample (without addition of fiber) were stored at 4±1˚ C. The samples were analyzed for the sensory, physico-chemical and microbiological analysis on alternate days.

3.7 Cost estimation

Cost of the smoothie sample was calculated for one batch 100 litres taking into considerations all fixed made.

3.8 Statistical analysis

The data of the samples obtained from various experiments were statistically analyzed by Analysis of Variance using statistical software SPSS 16 (IBM) for Windows Evaluation Version. A probability level of $p \leq 0.05$ was used for testing the s statistical significance of all experimental data.

CHAPTER IV

RESULTS AND DISCUSSION

This chapter deals with the results obtained during the present investigation on the development of fiber fortified reduced calorie probiotic smoothie and also studied various physical and chemical properties during storage at refrigeration temperature. Raw materials (e.g., probiotic yogurt, apple fiber, sunfiber, sugar, mango pulp, strawberry pulp, stevia and neotame etc.) were utilized for fiber fortified reduced calorie probiotic smoothie. The first phase of this study dealt with preparation of probiotic yogurt and further optimized the formulation and processing parameters of probiotic smoothie by adding various fibers, fruit pulp for developing the fiber fortified probiotic smoothie with desired sensory characteristics. In the second phase, the level of optimized sugar was completely replaced with combination of natural and artificial sweeteners and also evaluates its impact on sensory, physicochemical and microbiological characteristics throughout the storage period was evaluated. Results obtained during the course of present investigation in preparation of smoothie with enhanced overall functional attributes are presented in this chapter in a tabulated form, figures and graphs along with relevant discussion.

4.1 Preparation of probiotic yogurt

With the aim of developing probiotic smoothie, strain of *Lactobacillus acidophilus* (NCDC 15) was selected as a probiotic source. For preparation of probiotic yogurt milk was standardized to a level of 3% fat and 8.5 % SNF content. It was heated at 90˚C for 5 minutes and cooled to 42˚C. Further, yogurt culture was added @ 0.02% per litre and active culture of probiotic was added @ 2%. The mixture was incubated at 42˚C for 4-5 hour until pH drop to 5±0.1˚C.

The changes in pH, acidity and probiotic viability of probiotic strain during manufacturing of yogurt have been depicted in Figure 4.1. it can be seen from the figure that the initial count of fermentation was 6.37 log cfu/ ml at 0 h with an initial acidity 0.17% LA, which increased significantly ($p<0.05$) to 8.67 log cfu/ ml with 0.56% LA after 4 h. However, after 24 h incubation, count significantly increased with value of 9.07 log cfu/ ml. After 24 h incubation viability of probiotic count increased almost 2 to 2.5 log cycles. Hull et al. (1984) also observed a similar trend in yogurt wherein *L. acidophilus* multiplied during manufacture and reached to 10^7 viable organisms per ml in the finished product. From the Table 4.1, the change in pH and titratable acidity has been shown to possess an inverse relation with each other and was quite obvious as well.

Table 4.1: Fermentation time, pH, acidity and probiotic viability of probiotic strain (n=3)

Fermentation time (per hour)	pH	Acidity (%LA)	Probiotic count (log cfu/mL)
0 hour	6.64±0.16[a]	0.17±0.08[d]	6.37±0.08[e]
1 hour	6.50±0.17[a]	0.22±0.02[d]	6.63±0.03[e]
2 hour	6.20±0.20 [a]	0.26±0.01[d]	7.26±0.12[d]
3 hour	5.69±0.16[b]	0.43±0.00[c]	8.01±0.26[c]
4 hour	5.07±0.12[c]	0.56±0.01[b]	8.67±0.27[b]
24 hour	3.42±0.05[d]	1.94±0.06[a]	9.07±0.06[a]

Value presented in Mean ± SE
Values with different superscripts within the same column differ significantly ($p<0.05$)

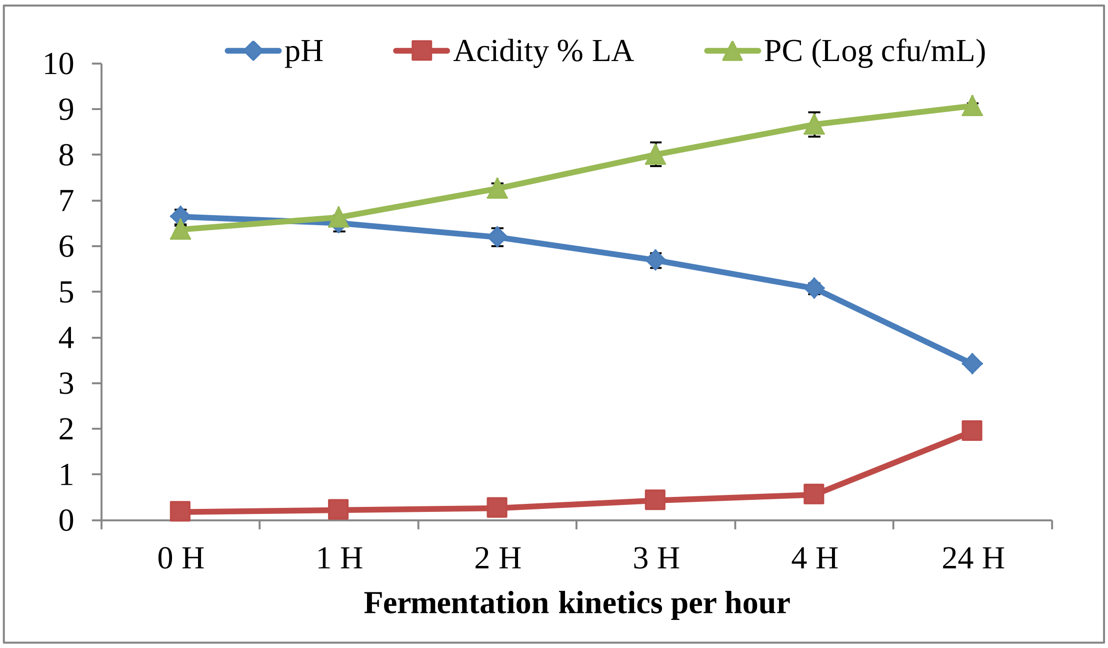


Fig. 4.1: Fermentation time, pH, acidity and probiotic viability of probiotic strain

At 0 h, highest pH of 6.64 and lowest titratable acidity of 0.17 % LA was observed while, at 24 h of incubation lowest pH level of 3.42 and maximum titratable acidity of 1.94 % LA was observed. The similar trend of results was observed by Mazloomi et al., 2011 in the preparation of low-fat probiotic yogurt. Sookolinska et al. (2004) also reported that the pH value of milk decreased during the manufacturing process of yogurt, from the time it was inoculated with bacterial culture to the time when it was fully set and is due to the lactic strain ability to ferment lactose into lactic acid with an increase in acidity and a decrease in pH of fermented milk. From Table 4.1, it can be seen that in initial 1-2 hours of fermentation the pH was minimum (6.64-6.20) with a titratable acidity (0.17-0.26) was observed. This could be due to the late lag or early log phase of bacterial growth. The another reason for the slow decline of pH could be buffering capacity of product and increased level of titratable acidity due to the production of organic acids (Sarvari et al., 2014).

4.1.2 Preliminary Trials

The objective of this phase was to standardize formulation prepared by blending different levels of fiber sources, fruit pulps and sugar to produce probiotic smoothie. The levels for fat (3 per cent) and SNF (8.5 per cent) of milk were kept constant for optimization of the product. Though, various previous studies have been already conducted on yogurt-based beverages. However, this particular preparation was involving fiber and combination of natural and artificial sweeteners. Therefore, some preliminary trials were necessary to derive some conclusion regarding per cent age of fiber and pulp to be used for smoothie preparation. Considering previous studies, and to enrich product with fiber, fruit pulp and additional fiber addition was thought off. Two different fruit pulps (mango and strawberry) and dietary fibers (Sunfiber®, apple fiber and diesol™) were used in preparation of two different types of smoothies. During preliminary trials, the minimum and maximum levels of fruit pulps and dietary fibers were tried to incorporate in probiotic smoothie as shown in Table 4.1.1.

Table 4.1.1: Different combination of fibers, pulps and sugar concentration in smoothie preparation

Ingredients (%)	CS	CM	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Apple fiber	-	-	8	8	8	4	4	4	4	4	4	4
Sunfiber®	-	-	4	6	8	10	10	10	10	10	10	10
Strawberry pulp	15	-	12	12	12	12	9	10	12	-	-	-
Mango pulp	-	10	-	-	-	-	-	-	-	10	12	15
Sugar	12	12	15	15	15	15	15	15	15	12	10	10

CS= control strawberry, CM= control mango

4.1.2.1 Screening of Dietary fiber, fruit pulp and sugar for the development of fiber fortified smoothie

Trials were carried out with dietary fibers levels ranging from 4-10 per cent (diesol, apple fiber and sunfiber), sugar (10-15) and 9- 12 per cent mango pulp and strawberry pulp as shown Table 4.1.1. The products with different levels of both pulps and dietary fibers were subjected to sensory analysis using 9-point hedonic scale. The smoothie samples (40–50 ml) were served to semi-trained panellists selected from the faculty and students of College of Dairy Science and Technology, GADVASU, Ludhiana. These trials served as a basis for further optimization of the product. The sensory responses for the smoothie sample as shown in Table 4.1.2 & 4.1.3.

Table 4.1.2: Screening of dietary fibers on the basis of sensory attributes for fiber fortified strawberry smoothie (n=7)

Treatment	CS	T1	T2	T3	T4
CA	8.28±0.01^{a}	5.92± 0.22^{d}	6.57± 0.17^{c}	5.64± 0.26^{d}	7.57± 0.27^{b}
Consistency	7.07±0.02^{a}	6.21± 0.14^{b}	6.28± 0.18^{b}	6.35± 0.14^{b}	7.57±0.20^{a}
Sweetness	8.42±0.17^{a}	7.07± 0.20^{b}	7.00± 0.21^{b}	7.00± 0.15^{b}	7.71±0.35^{b}
Flavour	8.14±0.14^{a}	5.65± 0.20^{c}	6.78± 0.18^{b}	5.92± 0.25^{c}	7.64± 0.29^{b}
OA	8.31±0.09^{a}	6.42± 0.20^{c}	6.78± 0.10^{c}	6.21± 0.14^{c}	7.92± 0.33^{b}

Values presented are Mean± SE

Values with different superscripts within the same row differ significantly ($p<0.05$)

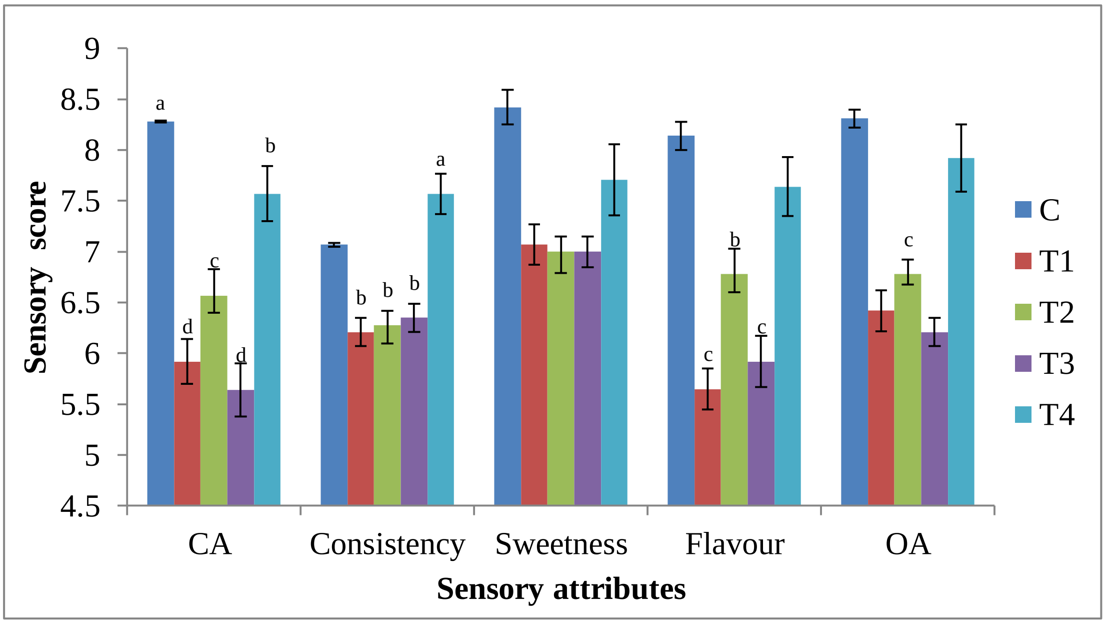


Fig. 4.1.2: Effect of dietary fibers concentration on sensory attributes for fiber fortified strawberry smoothie

Table 4.1.3: Screening of pulp on the basis of sensory attributes for fiber fortified strawberry smoothie (n=7)

Treatment	CS	T5	T6	T7
CA	8.35±0.10[a]	7.92± 0.13[b]	7.92± 0.22[b]	8.00± 0.14[b]
Consistency	7.10±0.17[b]	7.50± 0.26[ab]	7.45± 0.16[ab]	8.00± 0.26[a]
Sweetness	8.31±0.14[a]	7.78± 0.18[a]	7.92± 0.20[a]	8.21± 0.14[a]
Flavour	8.07±0.13[a]	7.71± 0.24[a]	8.07± 0.04[a]	8.20± 0.24[a]
OA	8.42±0.13[a]	7.78± 0.26[b]	7.92± 0.17[ab]	8.20± 0.21[ab]

Values presented are Mean± SE

Values with different superscripts within the same row differ significantly ($p<0.05$)

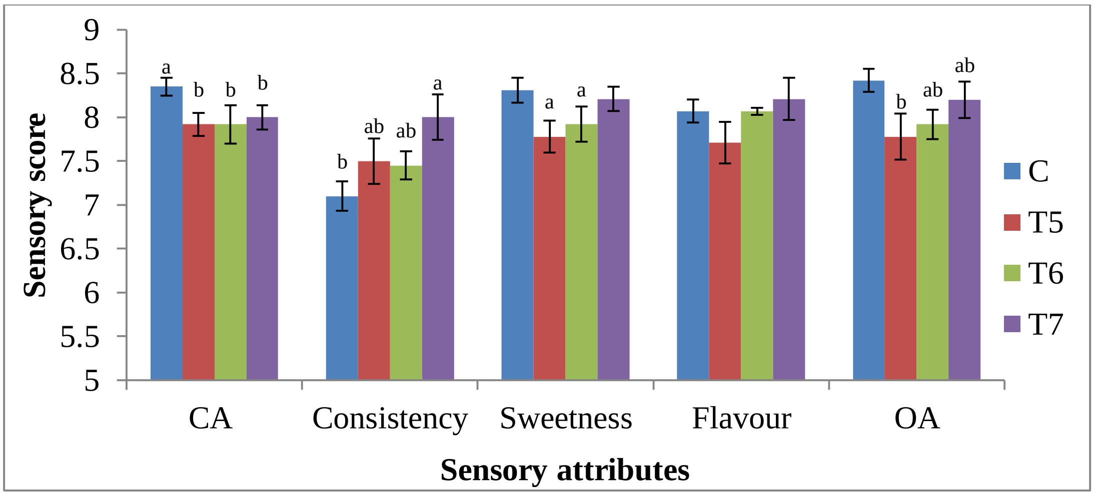


Fig. 4.1.3: Effect of different pulp concentration on sensory attributes of strawberry smoothie

It is apparent from Table 4.1.2 & 4.1.3 fiber fortified probiotic strawberry smoothie prepared from apple fiber, sunfiber, strawberry and sugar (4, 10, 12 and 15 per cent) fetched highest in comparisons to control sample. Fiber fortified smoothie score for flavour (8.20), consistency (8.00) and overall acceptability was (8.20) but lowest score obtained in colour and appearance of the product as compared to control sample with significant difference ($p<0.05$). This might be due to the brown colour of apple fiber which influenced negatively impact when its concentration in smoothies was increased. Moreover, consumer indicated that the colour of both control smoothies (strawberry and mango) was natural colour which decreased the consumer liking on the 9-point hedonic scale. Apple fiber fortified smoothie when added beyond 4 per cent affected colour, flavour and consistency negatively. Similar results were reported by Issar et al. (2017) in apple pomace fiber enriched yogurt. A significant difference ($p<0.05$) in consistency of control and fiber fortified smoothie was also observed. This might be due to the addition of fiber which increased the consistency in smoothie.

In perspective of sugar content in the formulation, the sweetness of control smoothie sample was sweeter than the fiber fortified samples. These results are in accordance with Hoppert et al., 2013 who reported that insoluble dietary fiber could be used to lower the perceived sweetness in dietary fiber enriched yogurt as compared to control sample. The addition of sunfiber in smoothies increased viscosity but had no effect on the sensory characteristics of fiber fortified smoothies. This might be due to the properties of fiber which is colourless, odourless and tasteless (Mudgil et al., 2014). The addition of diesol due to its soluble nature with apple and sunfiber was also tried but found not compatible with sensory analysis.

Table 4.1.4: Screening of fruit pulp, sugar on the basis of sensory attributes of fiber fortified probiotic mango smoothie (n=7)

Attributes	Control (M)	M1	M2	M3
CA	8.54±0.13^{a}	7.35±0.21^{c}	8.00±0.14^{b}	7.21±0.14^{c}
Consistency	7.00±0.18^{c}	7.70±0.20^{b}	8.10±0.13^{a}	7.80±0.15^{b}
Sweetness	8.41±0.12^{a}	6.92±0.13^{d}	8.20±0.10^{b}	7.35±0.14^{c}
Flavour	8.32±0.17^{a}	7.42±0.20^{c}	8.14±0.17^{ab}	7.57±0.22^{bc}
OA	8.42±0.13^{a}	6.92±0.13^{b}	8.35±0.18^{a}	7.35±0.17^{b}

Values presented are Mean± SE

Values with different superscripts within the same row differ significantly ($p<0.05$)

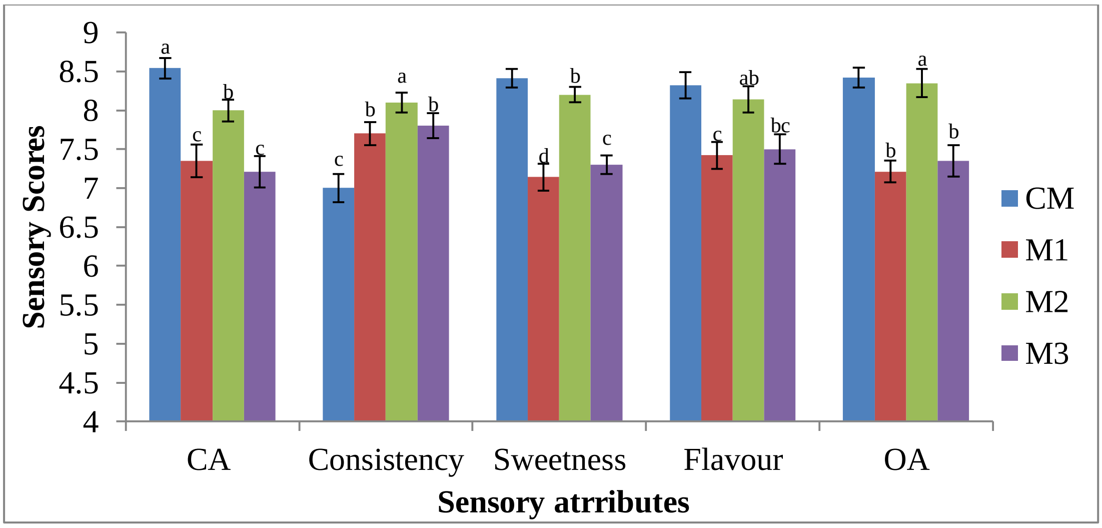


Fig. 4.1.4: Effect of different pulp concentration on sensory attributes of mango smoothie

It was apparent from the Table 4.1.4 that the fiber fortified probiotic mango smoothie prepared from apple fiber, sunfiber, mango pulp and sugar with (4, 10, 10 and 12 per cent) obtained highest score after control samples for flavour (8.14), consistency (8.07) and overall acceptability (8.35). In comparison to both fibers fortified smoothies (strawberry and mango) mean score for overall acceptability for mango smoothie added with 12 per cent (w/w) sugar and 10 per cent (w/w) mango pulp was 8.35, which showed liking of mango smoothie amongst sensory panellists. Sample with 10 per cent added sugar received lowest score of 6.92 indicating consumers disliking towards less sweet product. There was significant difference observed in sweetness when different concentration of sugar used ($p<0.05$) observed between other treatments as shown in Table 4.1.4. Based on sensory scores, two levels of dietary fibers 4 and 10 per cent (apple fibers and sunfiber) and individual fruit pulps levels 10 and 15 per cent (mango and strawberry) were selected for the development of product**.** The overall acceptability of smoothie was determined on the basis of the average of the total score obtained for different sensory attributes viz. colour & appearance, consistency flavour and sweetness.

4.1.2.2 Selection of levels of ingredient on the basis of sensory evaluation

The final selection of raw ingredients on the basis of sensory and functional evaluation entities after preliminary trials is summarised below in Table 4.1.5.

Table 4.1.5: Final optimization after preliminary trials

Ingredients	Range evaluated (%)	Selected (%)	Reason for selection
Apple fiber	4-8	4	Above 4 per cent floury taste of fiber occurred
Sunfiber ®	6-10	10	10 per cent gave best consistency in combination with apple fiber
Sugar	10-15	12-15	In case of mango smoothie sugar with 12 per cent level sugar while 15 per cent level of sugar provide good intensity of sweetness to product.
Mango pulp	10-12	10	High sweetness intensity above 10 per cent.
Strawberry pulp	10-12	12	Above 12 per cent pulp increase acidity of final product was observed.

The results obtained through the preliminary trials and sensory analysis was taken for further studies and proximate analysis was done for optimized product. Table 4.1.6 & 4.1.7 shows the proximate composition of fiber fortified mango and strawberry probiotic smoothies, along with its control counterpart.

Table 4.1.6: Proximate analysis for fiber fortified strawberry probiotic smoothie

Attributes	**Control**	**Fiber fortified strawberry smoothie**
Sensory scores		
Colour and appearance	$8.35±0.10^{a}$	$8.00±0.14^{b}$
Consistency	$7.10±0.17^{b}$	$8.00±0.26^{a}$
Sweetness	$8.31±0.14^{a}$	$8.21±0.14^{a}$
Flavour	$8.07±0.13^{a}$	$8.20±0.24^{a}$
Overall acceptability	$8.42±0.13^{a}$	$8.20±0.21^{ab}$
Physico-chemical analysis (%)		
Fat	$2.20±0.10^{a}$	$1.58±0.10^{b}$
Total soluble solids (TSS) ˚Brix	$23.16±0.16^{b}$	$26.16±0.16^{a}$
Total solids	$26.64±0.08^{b}$	$34.06±0.05^{a}$
Total sugar	$22.54±0.06^{b}$	$24.44±0.14^{a}$
Titratable acidity	$3.316±0.00^{a}$	$3.386±0.04^{a}$
Total Dietary fibers	$2.016±0.05^{b}$	$16.58±0.17^{a}$
Instrumental colour		
L*	$65.38±0.17^{a}$	$51.48±0.01^{b}$
a*	$17.11±0.00^{a}$	$16.86±0.01^{b}$
b*	$9.08±0.01^{b}$	$21.86±0.00^{a}$
Microbiological analysis		
Probiotic count (log cfu/ml)	$8.49±0.02^{a}$	$8.42±0.04^{a.}$
Yeast and Mold	Nil	Nil
Coliform	Nil	Nil

Values presented are Mean ±SE
Values with different superscripts within the same row differ significantly ($p<0.05$).

Table 4.1.7: Proximate analysis for fiber fortified mango probiotic smoothie

Attributes	Control	Fiber fortified mango smoothie
Sensory Scores		
Colour and appearance	8.54±0.13^{a}	8.00±0.14^{b}
Consistency	7.00±0.18^{b}	8.10±0.13^{a}
Sweetness	8.41±0.12^{a}	8.20±0.10^{b}
Flavour	8.32±0.17^{a}	8.14±0.17^{ab}
Overall acceptability	8.42±0.13^{a}	8.35±0.18^{a}
Physico-chemical analysis (%)		
Fat	2.47±0.17^{a}	1.71±0.07^{b}
Total soluble solids (TSS) ˚Brix	23.16±0.16^{b}	27.33±0.33^{a}
Total solids	25.20±0.16^{b}	29.08±0.03^{a}
Total sugar	21.97±0.04^{b}	25.34±0.16^{a}
Titratable acidity	3.100±0.01^{a}	3.180±0.06^{a}
Total Dietary fibers	2.539±0.06^{b}	17.38±0.06^{a}
Instrumental colour		
L*	77.11±0.02^{a}	57.91±0.02^{b}
a*	5.63±0.00^{b}	9.38±0.01^{a}
b*	48.61±0.00^{a}	45.39±0.00^{b}
Microbiological analysis		
Probiotic count (log cfu/ml)	8.56+0.04^{a}	8.52+0.06^{a}
Yeast and Mold	Nil	Nil
Coliform	Nil	Nil

Values presented are Mean ±SE
Values with different superscripts within the same row differ significantly ($p<0.05$).

4.2 Screening of sweeteners for the preparation of fiber fortified reduced calorie probiotic smoothie

To reduce the calorie intake of health-conscious segment, reduced calorie smoothie preparation was developed employing natural and artificial sweetener. On the basis of sucrose equivalence, two different levels of individual and three different levels of combination sweeteners (stevia and neotame) were selected on aqueous solution (w/w) with a reference sample sweetened with the optimum concentration of sucrose 12 and 15 per cent for mango and strawberry preparation of fiber fortified probiotic smoothie. Sensory evaluation of these products was carried out by semi-panellists from the faculty of College of Dairy Science and Technology on the basis of 9-point hedonic scale.

4.2.1 Preliminary screening of sweetener in smoothie

Different levels of (aqueous medium w/w) stevia (0.07- 0.14 per cent) and neotame (0.001- 0.004 per cent) were used for the preparation of smoothie. The best level of each sweetener was selected after comparing the sensory scores.

Table 4.2.1: Different combination of sweeteners for the preparation of reduced calorie smoothie

Treatments	Stevia (%)	Neotame (%)	Stevia + Neotame (%)
S1	0.07	-	-
S2	0.14	-	-
N1	-	0.002	-
N2	-	0.004	-
SN1	-	-	0.07±0.002
SN2	-	-	0.03±0.004
SN3	-	-	0.03±0.002
SNM1	-	-	0.03±0.002
SNM2	-	-	0.03±0.001

Table 4.2.2: Screening of sweetener and its level for replacement of sugar (individual and in combination) in strawberry smoothie (n=7)

Treatment	CA	Consistency	Sweetness	Flavour	OA
S1	6.57±0.20^{bc}	6.79±0.14^{b}	5.86±0.18^{cde}	6.14±0.09^{cd}	6.14±0.09^{de}
S2	6.50±0.18^{c}	6.86±0.09^{b}	5.43±0.17^{e}	5.86±0.18^{d}	5.71±0.14^{ef}
N1	6.90±0.13^{bc}	7.06±0.03^{b}	6.71±0.14^{b}	6.86±0.09^{b}	6.64±0.14^{ab}
N2	7.04±0.25^{bc}	7.09±0.22^{b}	6.18±0.11^{c}	6.60±0.15^{bc}	6.46±0.11^{cd}
SN1	6.64±0.19^{bc}	7.00±0.10^{b}	5.93±0.22^{cd}	6.39±0.17^{bc}	6.11±0.10^{de}
SN2	6.90±0.13^{bc}	6.83±0.14^{b}	5.64±0.13^{de}	6.29±0.24^{cd}	5.36±0.28^{f}
SN3	7.14±0.10^{b}	7.04±0.13^{b}	7.07±0.18^{b}	6.81±0.11^{b}	7.03±0.05^{b}
Control	8.00±0.21^{a}	8.14±0.14^{a}	8.17±0.08^{a}	8.07±0.13^{a}	8.28±0.10^{a}

Values presented are Mean± SE
Values with different superscripts within the same column differ significantly ($p<0.05$)

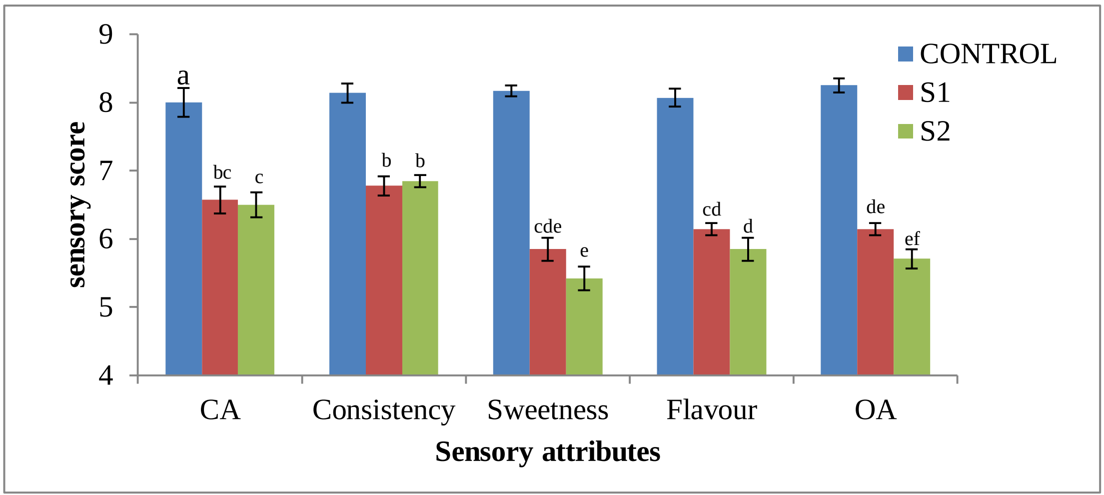


Fig. 4.2.1(a): Effect of stevia on the sensory attributes of strawberry smoothie

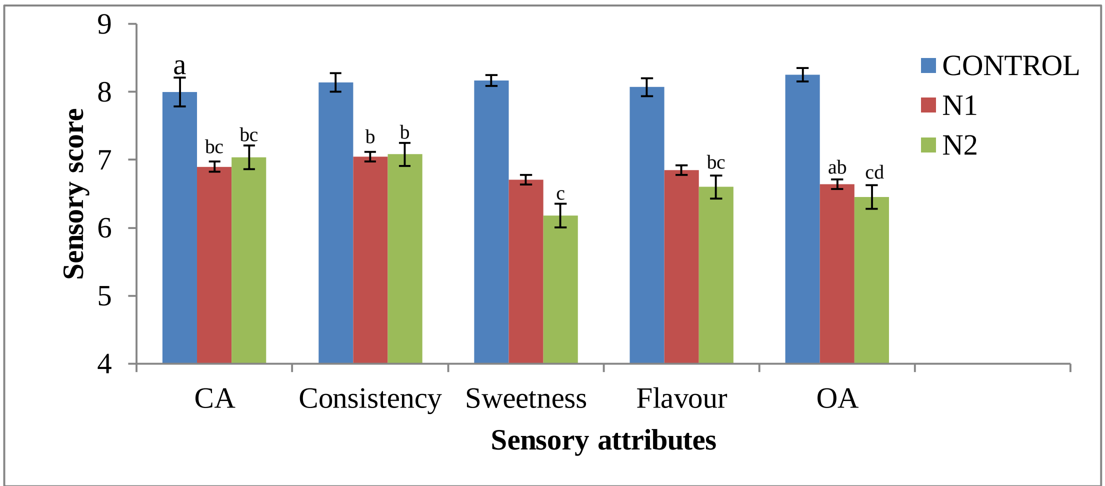


Fig. 4.2.1(b): Effect of neotame on the sensory attributes of strawberry smoothie

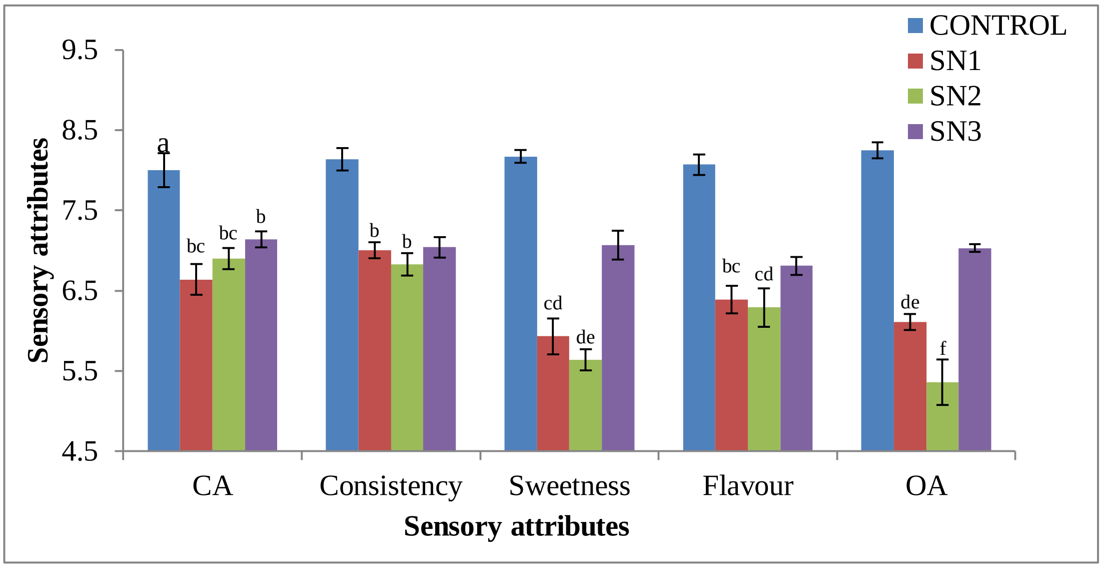


Fig. 4.2.1(c): Effect of different concentration of stevia and neotame (blend) on the sensory attributes of strawberry smoothie

Sensory analysis revealed that sweetener when used alone such as stevia @ 0.07 per cent, 0.14 per cent and neotame @ 0.002 per cent, 0.004 per cent in smoothie, resulted in significantly lower sensory scores, for sweetness and overall acceptability. Smoothie sweetened with stevia had lowest acceptance regarding the attributes flavour, sweetness, consistency and overall acceptability. Increasing the concentration of stevia more than 0.07 per cent gave bittered aftertaste and the same was observed by (Moussa et al., 2003). Stevia imparted undesirable properties in beverages such as bitter aftertaste and metallic flavour whereas, smoothie sweetened with neotame imparted the sweetest aftertaste. Similar, results were reported by Porto-Cardoso and Bolini (2008) in peach nectar sweetened with different sweeteners. Massoud and Amin (2005) also reported the unpleasant attributes of stevia sweeteners in fruit drinks. Hence, sensory analysis of individual sweeteners did not led to acceptance of sweeteners to be used alone in smoothie development. Therefore, further combination of sweeteners stevia and neotame 0.07 & 0.002, 0.03 & 0.004, 0.03 & 0.002, 0.03 & 0.002 and 0.03 & 0.001 per cent was tried strawberry and mango smoothie respectively. Schiffman et al. (2007) recommended that in order to increase the synergistic taste, blend of sweeteners have become an important aspect in the development of food and beverages.

Table 4.2.3: Screening of sweetener for the replacement conventional sugar with combination of both sweeteners in mango smoothie on the basis of sensory analysis (n=7)

Sensory attributes	Control	SNM1	SNM2
CA	7.85±0.14 [a]	6.64±0.17 [b]	7.14±0.22 [b]
Consistency	8.07±0.13 [a]	7.00±0.18 [b]	7.00±0.10 [b]
Sweetness	8.00±0.10 [a]	5.92±0.27 [c]	7.10±0.13 [b]
Flavour	8.14±0.17 [a]	6.38±0.17 [b]	6.85±0.15 [b]
OA	8.21±0.20 [a]	6.11±0.10 [c]	7.02±0.05 [b]

Values presented are Mean± SE
Values with different superscripts within the same row differ significantly ($p<0.05$)

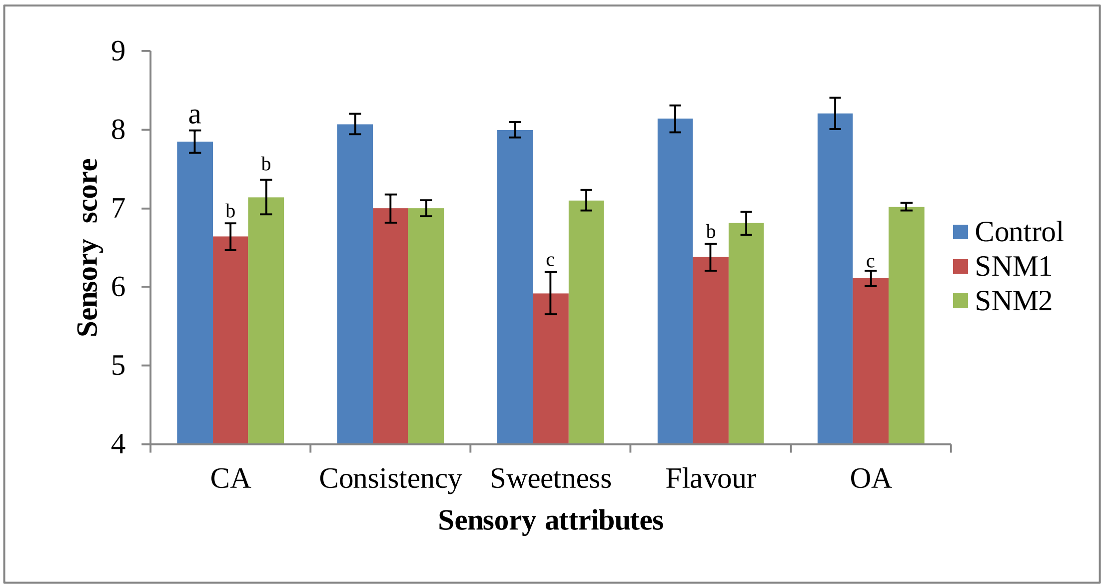


Fig. 4.2.2: Effect of different concentration of blend (stevia and neotame) on the sensory attributes of mango smoothie

Minimum and maximum levels of both sweeteners were tried for the replacement of 12 per cent and 15 per cent sugar. Table 4.2.2 and 4.2.3 depicted 15 per cent sugar replacement with 0.07 per cent stevia in combination with neotame @ 0.002 per cent gave more bitter after taste and sweet after taste which decreased the sweetness scores (5.93) and overall acceptance by the sensory panellists. Employing 0.03 per cent stevia with combination of neotame level 0.004 per cent impart sweeter after taste which also decreased the sweetness score (5.64) and over all acceptability (5.36). There was a significant difference observed in sweetness when different concentration of sweetener blends used for replacement of sugar ($p<0.05$). In reduced calorie strawberry smoothie the blend of sweetener @ 0.03 & 0.002 fetched highest score in sweetness (7.07) as compared to other treatments as shown in Table 4.2.2. In reduced calorie mango smoothie the blend of sweetener @ 0.03 & 0.001 fetched highest score in sweetness (7.1) as compared to other treatments as shown in Table 4.2.3. From sensory analysis of combination of both sweeteners, two optimized levels of sweetener i.e. stevia and neotame (0.03 & 0.002 per cent) for replacement of 15 per cent sugar in strawberry smoothie was selected. In mango smoothie 0.03 &0.001 per cent concentration of stevia and neotame (blend) was selected for the replacement of 12 per cent sugar.

4.2.2 Analysis of neotame in reduced calorie fiber fortified probiotic smoothie employing HPLC

Following conditions were maintained for analysis of neotame listed vides section 3.5.2. To quantify the sample standard curve of 5, 10, 25, 50 and 100ppm was drawn at 210 nm. The calibration curve of neotame was linear over the range of 5-100 ppm. The regression equation and correlation coefficient obtained are depicted in Fig 4.2.3.

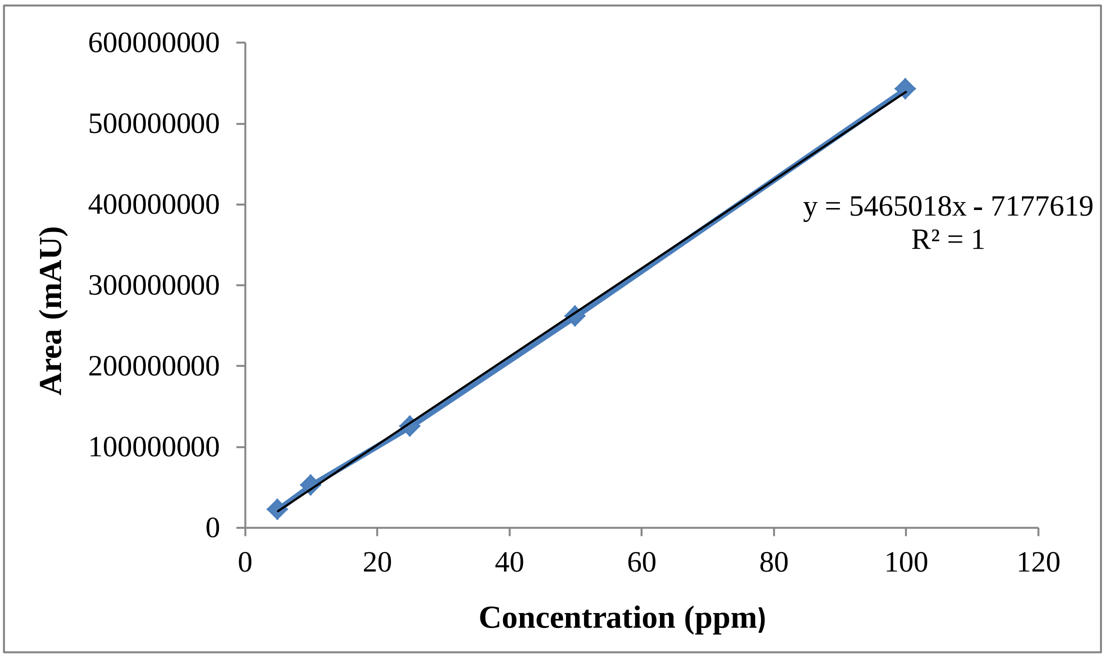


Fig. 4.2.3: Standard curve of neotame

The regression equations and correlations coefficient obtained are depicted in the Table 4.2.4. The correlation coefficient of the calibration curve was better than 0.99. The correlation coefficient of 1 for neotame showed linearity of the method used.

Table 4.2.4: Regression equation and correlation coefficient of neotame

Sweetener/Degradation product	Regression equation	R^2 Correlation Coefficient
Neotame	5465018x - 7177619	1

Table 4.2.5: Retention time and area of neotame standard at 210 nm

Sweetener/Degradation product	Retention time (min)	Area/50ppm
Neotame	8.4	261146124

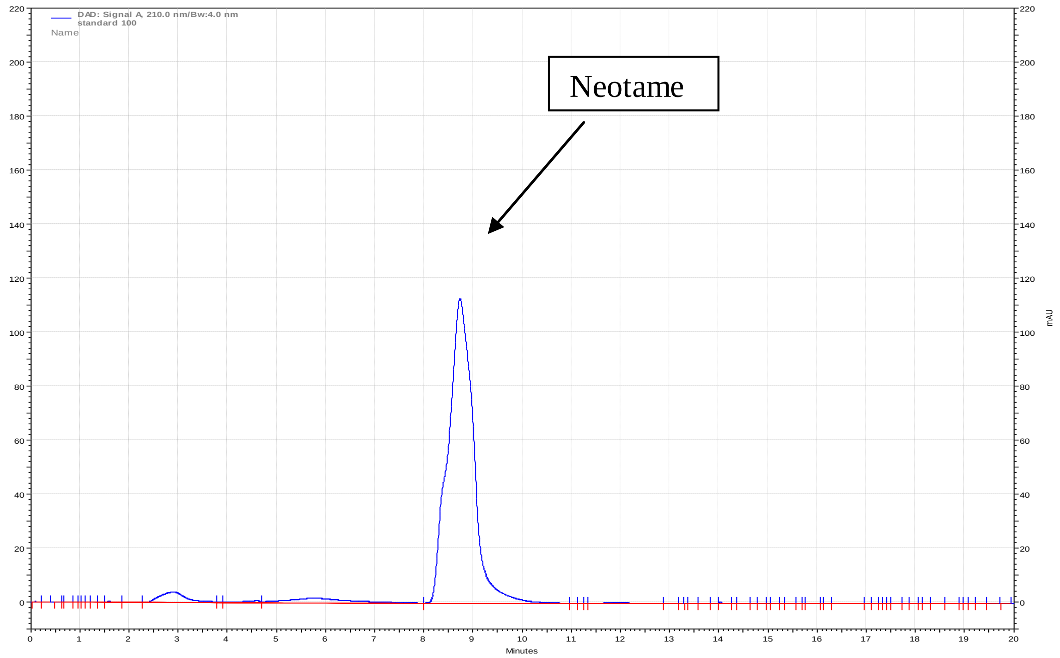


Fig. 4.2.4: **HPLC chromatogram of neotame standard at 50ppm**

4.2.3 Recovery of neotame in strawberry and mango smoothie

Recovery obtained in smoothie was depicted in Table 4.2.6. Results are in accordance with (Kumari et al., 2016; Yang & Chen 2010) who reported 94.00-98.5 per cent recovery in yogurt and flavoured milk.

Table 4.2.6: Recovery of neotame in strawberry and mango smoothie

Product	% Recovery of neotame
Neotame sweetened Strawberry smoothie	96.02±0.66
Neotame sweetened mango smoothie	96.53±0.46

Values are presented as means ± SE (n = 3).

Table 4.2.7: Proximate analysis for reduced calorie fiber fortified strawberry probiotic smoothie

Attributes	Fiber fortified strawberry probiotic smoothie	Reduced calorie strawberry probiotic smoothie
Sensory analysis		
Colour and appearance	8.00 ± 0.20^{a}	7.14 ± 0.10^{b}
Consistency	8.14 ± 0.14^{a}	7.04 ± 0.13^{b}
Sweetness	8.17 ± 0.08^{a}	7.07 ± 0.18^{b}
Flavour	8.07 ± 0.13^{a}	6.81 ± 0.11^{b}
Overall acceptability	8.28 ± 0.10^{a}	7.03 ± 0.05^{b}
Physico-chemical analysis (%)		
Fat	1.58 ± 0.10^{b}	1.98 ± 0.06^{a}
Total soluble solids (TSS) ˚Brix	26.16 ± 0.16^{a}	17.00 ± 0.28^{b}
Total solids	34.06 ± 0.05^{a}	21.72 ± 0.15^{b}
Total sugar	24.44 ± 0.14^{a}	5.50 ± 0.08^{b}
Titratable acidity	3.386 ± 0.04^{a}	3.312 ± 0.01^{a}
Total Dietary fibers	16.58 ± 0.17^{a}	16.09 ± 0.07^{a}
Instrumental colour		
L*	51.48 ± 0.01^{b}	55.17 ± 0.01^{a}
a*	16.86 ± 0.01^{b}	17.59 ± 0.00^{a}
b*	21.86 ± 0.00^{a}	20.15 ± 0.01^{b}
Microbiological analysis		
Probiotic count	$8.49 + 0.07^{a}$	$8.42 + 0.05^{a}$
Yeast and Mold	Nil	Nil
Coliform	Nil	Nil

Values presented are Mean ±SE

Values with different superscripts within the same row differ significantly ($p < 0.05$).

Table 4.2.8: Proximate analysis for reduced calorie fiber fortified mango probiotic smoothie

Attributes	Fiber fortified mango probiotic smoothie	Reduced calorie mango probiotic smoothie
Sensory analysis		
Colour and appearance	7.85±0.14[a]	7.14±0.22[b]
Consistency	8.07±0.13[a]	7.00±0.10[b]
Sweetness	8.00±0.10[a]	7.10±0.13[b]
Flavour	8.14±0.17[a]	6.85±0.15[b]
Overall acceptability	8.21±0.20[a]	7.02±0.05[b]
Physico-chemical analysis (%)		
Fat	1.71±0.17[b]	2.01±0.11[a]
Total soluble solids (TSS) ˚Brix	27.33±0.33[a]	16.83±0.16[b]
Total solids	29.08±0.03[a]	22.03±0.08[b]
Total sugar	25.34±0.16[a]	9.85±0.06[b]
Titratable acidity	3.180±0.06[a]	3.123±0.02[a]
Total Dietary fibers	17.38±0.06[a]	17.07±0.12[a]
Instrumental colour		
L*	57.91±0.02[b]	61.50±0.00[a]
a*	9.38±0.01[a]	8.64±0.02[b]
b*	45.39±0.00[a]	42.01±0.02[b]
Microbiological analysis		
Probiotic count	8.52±0.10[a]	8.49±0.11[a]
Yeast and Mold	Nil	Nil
Coliform	Nil	Nil

Values presented are Mean ±SE

Values with different superscripts within the same row differ significantly ($p<0.05$).

Mango probiotic smoothie

Fiber fortified mango probiotic smoothie

Fiber fortified reduced calorie mango probiotic smoothie

Strawberry probiotic smoothie (Control)

Fiber fortified Strawberry probiotic smoothie

Fiber fortified reduced calorie strawberry probiotic smoothie

4.3 Storage study

Storage study is essential aspect to determine the shelf life of the product throughout storage time. The objective of the storage study is to evaluate whether the product would be safe, acceptable and stable throughout the storage. Fermented milk-based products are relatively more susceptible to quality deterioration during storage due to numerous microbiological, sensorial as well as physicochemical reaction occurring in product. In the present study, product was subjected to sensory, physicochemical and microbiological evaluation at intervals of three days, till spoilage of product i.e., increase in microbiological count beyond limits or drop in probiotic count to a desired level where, product cannot be termed as probiotic.

4.3.1 Changes in sensory attributes during storage

The sensory evaluation was carried out to perceive any changes in the sensory attributes during storage by selected panellist from the department on the basis of 9-point hedonic scale. Table 4.3.1 & 4.3.2 shows the sensory scores of control (without fiber), fiber fortified and reduced calorie strawberry and mango probiotic smoothie throughout the storage period.

Colour and appearance

Each smoothie had three variants viz., control, fiber fortified with sugar and reduced calorie fiber fortified smoothie. The comparison between the three variants of smoothie, control sample was the most appreciated by panellists for colour and appearance and there was a significant difference ($p<0.05$) in C&A of the control and prepared smoothie variants.

In strawberry based smoothie, the mean score for colour and appearance was affected with addition fiber which reduced the overall colour and appearance scores as compared to control sample in both the variants of smoothies. At zero day the mean score of for fiber fortified strawberry probiotic smoothie was 7.42 which decreased to 7.07 on the last day (12^{th}) of storage whereas, in case of reduced calorie fiber fortified strawberry fiber fortified smoothie score was 7.28 at zero day and 6.92 at last day of storage (Table 4.3.1).

In mango-based smoothie, the colour and appearance varied from 8.0-7.0 during storage of fiber fortified mango probiotic smoothie and in case of reduced calorie fiber fortified mango probiotic smoothie varied from 7.78-6.50 (Table 4.3.2). The samples with lower colour and appearance were due to the addition of apple fiber which was brown in colour and masked the natural colour of strawberry pulp as well as mango pulp. Hoppert et al., 2013 also reported yogurt fortified with visible dietary fiber decreases the consumer acceptance due to an unfamiliar appearance.

Consistency

The average consistency score for the three variants of each smoothie are showed in Table 4.3.1 & 4.3.2. Data indicated that consistency score of control samples were different from the fiber added smoothie during storage.

In strawberry based smoothie, fiber fortified smoothie consistency score ranged from the 8.21-7.78 whereas, in reduced calorie fiber fortified the consistency score ranged 7.78- 7.35. The control has consistency score of 7.28 at initial day which decreased to 6.78 on 9th day of storage. This might be due to thinning of product (control) at lower temperature. But, there is no significant ($p>0.05$) difference observed in the consistency score of fiber fortified and reduced calorie smoothie during storage.

In mango-based smoothie, fiber fortified smoothie consistency score at zero day was 8.54 which decreased to 7.71 during storage period whereas in reduced calorie smoothie it was varied 8.28-7.35 during storage. Control sample and fiber fortified smoothie had a significant ($p<0.05$) difference in consistency as shown in Table 4.3.1 & 4.3.2.

Sweetness

The results for sweetness score decreased with increased storage time. However, sweetness was mainly affected by the addition of sweeteners in smoothie. The score for sweetness were high in both control smoothie as compared to fiber fortified and fiber fortified sweetener sweetened smoothie. This might be due to the lingering after taste of neotame in smoothie, where in fiber fortified sugar variant fiber probably masked the perceived intensity of the sweetness.

In strawberry based smoothie, the sweetness score for control, fiber and reduced calorie was varied 8.00-7.64, 7.71-7.54 and 6.71-5.64 respectively. There was a significant decrease in mean scores of sweetness ($p<0.05$) among fiber fortified and reduced calorie probiotic smoothie during storage days as shown in Table 4.3.1.

Table 4.3.1: Effect of storage on the sensory attributes of fiber fortified and reduced calorie fiber fortified strawberry probiotic smoothie (n=7)

Sensory attributes	Product	Days				
		0	3	6	9	12
CA	CS	8.00±0.23^{aA}	7.92±0.15^{aA}	7.71±0.13^{aA}	7.71±0.12^{aA}	-
	FSS	7.42±0.23^{aA}	7.42±0.15^{aB}	7.42±0.13^{aAB}	7.14±0.12^{aB}	7.07±0.12^{aA}
	RCS	7.28±0.23^{aA}	7.21±0.15^{aB}	7.07±0.13^{aB}	6.92±0.12^{aB}	6.92±0.12^{aA}
Consistency	CS	7.28±0.18^{aB}	7.21±0.19^{aB}	7.00±0.14^{aB}	6.78±0.12^{aC}	-
	FSS	8.21±0.18^{aA}	8.14±0.19^{aA}	8.00±0.14^{aA}	7.85±0.12^{aA}	7.78±0.14^{aA}
	RCS	7.78±0.18^{aAB}	7.50±0.19^{aB}	7.42±0.14^{aB}	7.42±0.12^{aB}	7.35±0.14^{aA}
Sweetness	CS	8.00±0.22^{aA}	7.64±0.16^{aA}	7.64±0.16^{aA}	7.64±0.17^{aA}	-
	FSS	7.71±0.22^{aA}	8.00±0.16^{aA}	7.92±0.16^{aA}	7.78±0.17^{aA}	7.54±0.10^{aA}
	RCS	6.71±0.22^{aB}	6.54±0.16^{abB}	6.32±0.16^{abB}	6.14±0.17^{bB}	5.64±0.10^{cB}
Flavour	CS	8.00±0.20^{aA}	8.00±0.21^{aA}	7.91±0.13^{aA}	7.28±0.17^{bA}	-
	FSS	7.78±0.20^{aA}	8.01±0.21^{aA}	7.97±0.13^{aA}	7.71±0.17^{aA}	7.21±0.10^{bA}
	RCS	6.57±0.20^{aB}	6.47±0.21^{aB}	6.42±0.13^{abB}	6.00±0.17^{abAB}	5.78±0.10^{bB}
OA	CS	8.07±0.21^{aA}	7.82±0.21^{abA}	7.71±0.14^{bA}	7.64±0.13^{bA}	-
	FSS	7.78±0.21^{aA}	7.78±0.21^{aA}	7.71±0.14^{aA}	7.42±0.13^{abA}	7.14±0.07^{bA}
	RCS	6.92±.021^{aB}	6.71±0.21^{aB}	6.64±0.14^{abB}	6.32±0.13^{abAB}	6.04±0.07^{bB}

Effect of storage on sensory attributes of strawberry smoothie.

*a_d Different letters in same row denote significant differences ($p<0.05$) for storage period among days.
A_C different letters in same column denote significant difference between control and fiber and reduced calorie smoothie ($p<0.05$)

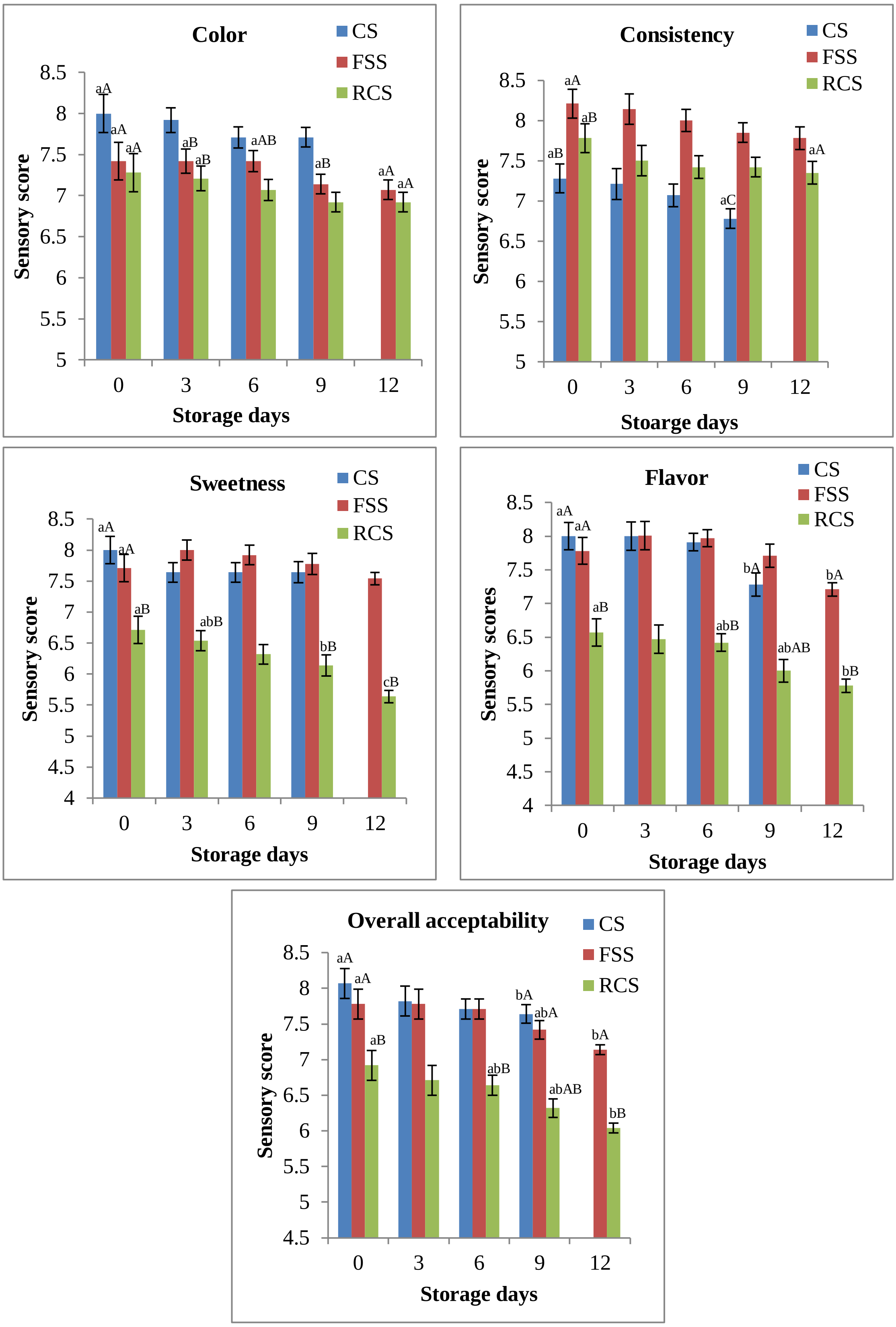


Fig. 4.3.1: Effect of storage on the sensory attributes of fiber fortified and reduced calorie fiber fortified strawberry probiotic smoothie

Table 4.3.2: Effect of storage on the sensory attributes of fiber fortified and reduced calorie fiber fortified mango probiotic smoothie (n=7)

Sensory attributes	Product	Days				
		0	3	6	9	12
CA	CM	8.57 ± 0.14^{aA}	8.50 ± 0.17^{aA}	8.35 ± 0.14^{aA}	7.78 ± 0.20^{bA}	-
	FSM	8.00 ± 0.14^{aAB}	8.07 ± 0.17^{aAB}	7.50 ± 0.14^{bcB}	7.42 ± 0.20^{cAB}	7.00 ± 0.16^{cA}
	RCM	7.78 ± 0.14^{aB}	7.78 ± 0.17^{aB}	7.42 ± 0.14^{aB}	7.00 ± 0.20^{bB}	6.50 ± 0.16^{bB}
Consistency	CM	7.85 ± 0.13^{aB}	7.71 ± 0.15^{abB}	7.42 ± 0.14^{bcB}	7.28 ± 0.21^{cA}	-
	FSM	8.54 ± 0.13^{aA}	8.50 ± 0.15^{aA}	8.07 ± 0.14^{abA}	7.85 ± 0.21^{bA}	7.71 ± 0.18^{bA}
	RCM	8.28 ± 0.13^{aA}	8.28 ± 0.15^{aAB}	7.85 ± 0.14^{abAB}	7.50 ± 0.21^{bA}	7.35 ± 0.18^{bA}
Sweetness	CM	8.21 ± 0.13^{aA}	8.14 ± 0.16^{aA}	7.78 ± 0.15^{abA}	7.57 ± 0.19^{bA}	-
	FSM	8.48 ± 0.13^{aA}	8.14 ± 0.16^{abA}	7.92 ± 0.15^{abA}	7.78 ± 0.19^{bA}	7.14 ± 0.20^{cA}
	RCM	7.42 ± 0.13^{aB}	7.35 ± 0.16^{aB}	6.92 ± 0.15^{abB}	6.50 ± 0.19^{bB}	5.92 ± 0.20^{cB}
Flavour	CM	8.21 ± 0.13^{aA}	8.14 ± 0.11^{aA}	7.75 ± 0.19^{bA}	7.50 ± 0.21^{bA}	-
	FSM	8.24 ± 0.13^{aA}	8.14 ± 0.11^{aA}	7.78 ± 0.19^{abA}	7.64 ± 0.21^{bA}	6.92 ± 0.22^{cA}
	RCM	7.50 ± 0.13^{aB}	7.50 ± 0.11^{aA}	7.07 ± 0.19^{abB}	6.42 ± 0.21^{bcB}	6.14 ± 0.22^{cB}
OA	CM	8.28 ± 0.11^{aA}	8.21 ± 0.12^{aA}	7.85 ± 0.14^{bA}	7.57 ± 0.15^{cA}	-
	FSM	8.42 ± 0.11^{aA}	8.42 ± 0.12^{aA}	7.90 ± 0.14^{bA}	7.71 ± 0.15^{bA}	7.14 ± 0.13^{cA}
	RCM	7.71 ± 0.11^{aB}	7.57 ± 0.12^{aB}	7.07 ± 0.14^{bB}	6.64 ± 0.15^{bcB}	6.21 ± 0.13^{cB}

Effect of storage on sensory attributes of mango smoothie.
*a_c. Different letters in same row denote significant differences ($p<0.05$) for storage period among days.
A-C different letters in same column denote significant difference between control, fiber and reduced calorie smoothie ($p<0.05$)

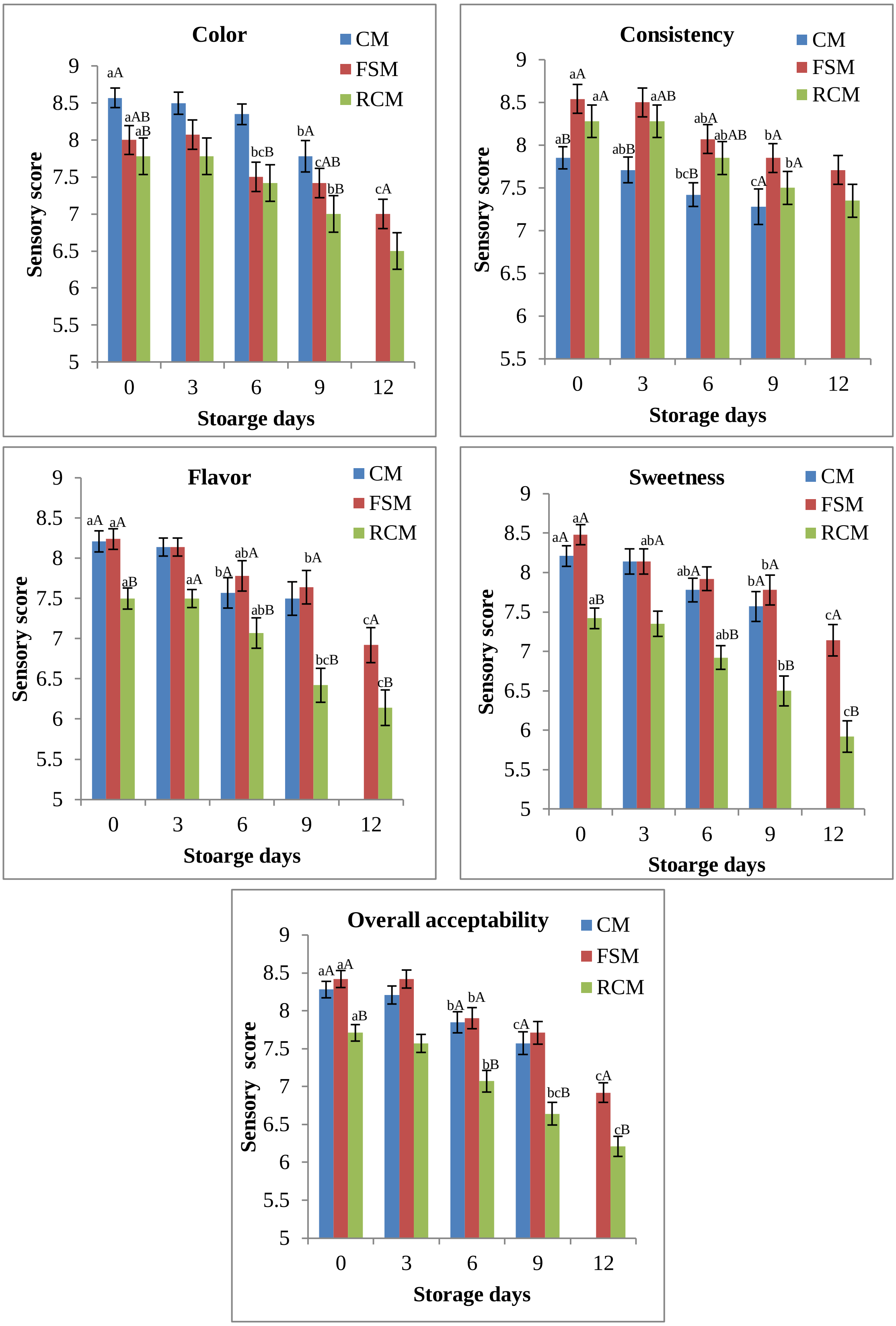


Fig. 4.3.2: Effect of storage on the sensory attributes of fiber fortified and reduced calorie fiber fortified mango probiotic smoothie

In mango-based smoothie, the sweetness mean score was ranged 8.21-7.57, 8.48 -7.14 and 7.42-5.92 for control; fiber fortified and reduced calorie fiber fortified probiotic smoothie respectively. There was a significant difference ($p<0.05$) observed in sweetness during storage days of fiber fortified and reduced calorie fiber fortified probiotic smoothie as shown in 4.3.2.

Flavour

Fresh samples of smoothies were characterized by pleasant flavour and sweet taste except sweetener sweetened smoothie. The flavour score for the control and fiber fortified smoothie was similar at zero day showed in Table 4.3.1& 4.3.2. After 3rd day of storage all the samples were showed decrease in the flavour scores. The decrease in flavour scores occurred due to post acidification caused by the bacteria and production of off flavour due to multiplication of yeast and mold and other spoilage bacteria.

In strawberry based smoothie, flavour score for the control and Fiber fortified smoothie and reduced calorie was ranged 8.00-7.28, 7.78-7.21 and 6.57-5.78 respectively. In fiber fortified and reduced calorie there was as significant ($p<0.05$) difference in sweetness.

In mango-based smoothie, flavour score for the control, fiber fortified and reduced calorie smoothie was ranged 8.21-7.50 and 8.24-6.92 and 7.50-6.14 at during storage period. However, reduced calorie smoothie resulted in significantly ($p<0.05$) lower sensory score as compared to fiber fortified smoothie.

On the sensory score basis, it was also found that fiber fortified smoothie and sweetener sweetened smoothie remained acceptable for 12 days whereas control sample was spoiled after 9th day of storage.

Overall acceptability

There were non-significant differences observed between control and fiber fortified smoothie but a significant effect ($p<0.05$) observed in reduced calorie smoothie at zero day. This was due to the addition of sweeteners in smoothie which caused the lingering after taste and decreased the overall acceptability.

In strawberry based smoothie, the overall acceptability for the control, fiber fortified, reduced calorie was varied from the 8.07-7.64, 7.78-7.14 and 6.92-6.04 respectively.

In mango-based smoothie, the overall acceptability for the control, fiber fortified and reduced calorie was varied from the 8.28-7.57, 8.42-7.14 and 6.92-6.04 respectively.

During refrigeration storage, the overall acceptability scores significantly decreased with increase in storage period. After 12 days of storage, fiber fortified smoothie was also rejected as they get spoiled.

4.3.2 Physico- chemical changes during storage

pH

The pH of control, fiber fortified and sweetener sweetened smoothie did not varied significantly amongst each other during storage as shown in Table 4.3.3 & 4.3.4.

In strawberry based smoothie, the FSS and RCS smoothie pH was 4.21 at zero day and decreased significantly ($p<0.05$) to 3.65 and 3.65 respectively at 15th day of storage.

In mango-based smoothie, the fiber fortified and reduced calorie smoothie pH was 4.12 and 4.10 at zero day, which decreased significantly ($p<0.05$) to 3.70 and 3.65 respectively during storage period**.**

A significant decrease ($p<0.05$) in pH was observed over storage time in fiber fortified and reduced calorie which was due to the production of organic acids by lactic acid bacteria. A similar trend observed by Gallina et al. (2019) in the development of probiotic smoothie and Garcia-Perez et al. (2005) in orange fiber fortified yogurt.

Acidity

The acidity of control, fiber fortified and sweetener sweetened smoothie did not vary significantly amongst each other during storage as shown in Table 4.3.3 & 4.3.4. Acidity of sample had significant effect ($p<0.05$) during storage days which might be due to the microbial growth. In strawberry based smoothie, the acidity of the fiber fortified and reduced calorie smoothie was 3.367 and 3.322 at zero day, which increased significantly ($p<0.05$) to 3.965 and 3.972 respectively at 15th day of storage.

In mango-based smoothie, the acidity of the fiber fortified and sweeteners sweetened smoothie was 3.485 and 3.50 at zero day, which increased ($p<0.05$) to 4.190 and 4.198 respectively at 15th day of storage

There was a slow rate of acidification which may be due to the higher total solid content, which restrict the microbial growth by increasing osmotic pressure and decreasing water activity. Similar results were reported by Saranyambiga et al. (2017) in the development of synbiotic smoothie**.** Results were also in accordance with the Raju and Pal (2014).

Viscosity

A high statistical difference in viscosity of smoothie was observed after every alternate day of storage for control as well as fiber added smoothie. It was clear from Table 4.3.3 & 4.3.4 that viscosity of fiber fortified smoothie was significant more ($p<0.05$) as compared to reduced calorie smoothie and control smoothie. This might be due to the higher total solid content in fiber fortified smoothie (33.94%).

In strawberry based smoothie the viscosity of control sample (CS) decreased with increasing storage days. The mean score for the strawberry control smoothie was 960.6 cp at zero day which decreased to 886.8 cp on the 9th day of storage. However, in the fiber fortified smoothie viscosity at zero day was 1470 cp which further increased significantly ($p<0.05$) upto 9th day of storage and on 12th and 15th day of storage it was decreased to 2595.2 cp. In reduced calorie smoothie viscosity at zero day was 1228.2 which also increased significantly ($p<0.05$) upto 6th day of storage and after that it gradually decreased on 9th, 12th and 15th day of storage. The fiber fortification in smoothie may interact with protein portion of smoothie; stabilize the serum which increased the viscosity of sample as compared to control sample (without fiber). Similar results were reported by Mudgil and Barak (2016a) in development of functional buttermilk.

In mango-based smoothie, spindle no. LV- 64 was used during storage for the viscosity analysis because of the high viscosity of smoothie. The mean score value of viscosity for the fiber fortified smoothed varied from 9113cp -7585 cp. In reduced calorie mango smoothie was ranged in 8653-7152 cp during storage period. After 3rd day there was a significant decrease in viscosity of fiber fortified and reduced calorie probiotic smoothie. This might be due to the reaction occurred due to the action of spoilage microorganism which led to breakdown of casein network (Kiruthika et al., 2018).

Serum separation

The serum separation in the control sample increased with increasing storage days. Both fiber fortified and sweetener sweetened smoothie had lowest syneresis during storage period as shown in Table 4.3.3 & 4.3.4.

In strawberry based smoothie, the mean score value of syneresis in control was 3.26 ml at zero day which increased 5.20 ml on 15th day. Wherein, fiber fortified smoothie syneresis was 0 ml upto 6th day and after 6th day it increased slightly ($p>0.05$) during storage period. In reduced calorie smoothie syneresis was also nil at zero day but after 3rd day it increased significantly ($p<0.05$) from 0.23- 1.30 ml during storage period. The results were in accordance with (Garcia-Perez et al., 2005; Damian & Olteanu 2014).

In mango based smoothie, the mean score value for control mango smoothie ranged 2.8-4.12 ml/10 during storage period. However, in fiber fortified and reduced calorie smoothie there was no syneresis occurred throughout the storage product. Fiber fortification in smoothie also stabilized the aqueous phase and contributed to low syneresis and high viscosity in smoothie. Similar, results were observed in fiber fortified yogurt during 21 days of storage by (Stafolo et al., 2004).

Total solids

Statistical non-significant difference was observed in total solids of product during storage of smoothie at refrigeration temperature. In strawberry based smoothie, among smoothie variants such as control (without fiber), fiber fortified (fiber and sugar) and reduced calorie smoothie (sweeteners blend) had non-significant difference ($p>0.05$) in total solid content during storage as shown in Table 4.3.3.

In mango-based smoothie, total solid content in control (without fiber), fiber fortified (fiber and sugar) and reduced calorie smoothie (sweeteners blend) also had non- significant difference ($p>0.05$) as shown in Table 4.3.4.

Total Dietary fiber

Total dietary fiber had non-significant ($p>0.05$) effect during storage period. In strawberry based smoothie, TDF content varied from 16.58-16.40 and 16.07 -16.00 per cent in fiber fortified and reduced calorie smoothie respectively as shown in Table 4.3.3.

In mango based smoothie, TDF content in fiber fortified and reduced calorie smoothie varied non significantly ($p>0.05$) from 17.31-17.26 and 17.25- 17.08 per cent in fiber fortified and reduced calorie smoothie respectively during storage as shown in Table 4.3.4.

Table 4.3.3: Effect of storage on physico-chemical properties of fiber fortified and reduced calorie fiber fortified strawberry probiotic smoothie (n=3)

Parameters	Strawberry smoothie	Days					
		0	3	6	9	12	15
pH	CS	4.22±0.01^{aA}	4.19±0.00^{aA}	4.09±0.01^{bA}	4.00±0.01^{cA}	3.79±0.01^{dA}	3.60±0.01^{eB}
	FSS	4.21±0.01^{aA}	4.17±0.00^{bAB}	4.04±0.01^{cB}	3.95±0.01^{dA}	3.70±0.01^{eB}	3.65±0.01^{fA}
	RCS	4.21±0.01^{aA}	4.16±0.00^{bB}	4.07±0.01^{cAB}	3.97±0.01^{dA}	3.72±0.01^{eB}	3.65±0.01^{fA}
Acidity (%LA)	CS	3.319±0.02^{eA}	3.335±0.01^{eB}	3.476±0.01^{dA}	3.548±0.00^{cB}	3.787±0.01^{bB}	3.900±0.00^{aA}
	FSS	3.367±0.02^{dA}	3.400±0.01^{dA}	3.520±0.01^{cA}	3.603±0.00^{cA}	3.844±0.01^{bA}	3.965±0.01^{aA}
	RCS	3.322±0.02^{dA}	3.379±0.01^{dAB}	3.486±0.01^{cA}	3.588±0.00^{cA}	3.827±0.01^{bA}	3.972±0.01^{aA}
Serum separation (ml)	CS	3.26±0.19^{fA}	3.63±0.04^{eA}	3.93±0.03^{dA}	4.30±0.05^{cA}	4.80±0.05^{bA}	5.20±0.07^{aA}
	FSS	NIL	NIL	NIL	0.26±0.05^{aC}	0.3±0.05^{aC}	0.32±0.05^{aA}
	RCS	NIL	0.23±0.04^{dB}	0.50±0.03^{cB}	0.9±0.05^{bB}	1.1±0.05^{aB}	1.30±0.05^{aB}
Total solids	CS	26.38±0.07^{aB}	26.35±0.03^{aB}	26.32±0.03^{aB}	26.31±0.02^{aB}	26.29±0.05^{aB}	26.28±0.04^{aB}
	FSS	33.96±0.07^{aA}	34.00±0.03^{aA}	33.95±0.03^{aA}	33.92±0.02^{aA}	33.89±0.05^{aA}	33.85±0.04^{aA}
	RCS	21.75±0.07^{aC}	21.80±0.03^{aC}	21.76±0.03^{aC}	21.75±0.02^{aC}	21.70±0.05^{aC}	21.63±0.04^{aC}
Dietary fiber	CS	2.016±0.11^{aB}	2.028±0.10^{aB}	2.018±0.15^{aB}	2.010±0.12^{aB}	1.998±0.08^{aB}	1.990±0.10^{aB}
	FSS	16.58±0.11^{aA}	16.62±0.10^{aA}	16.53±0.15^{aA}	16.45±0.12^{aA}	16.46±0.08^{aA}	16.40±0.10^{aA}
	RCS	16.07±0.11^{aA}	16.13±0.10^{aA}	16.08±0.15^{aA}	16.00±0.12^{aA}	15.96±0.08^{aA}	16.00±0.10^{aA}

Parameters	Strawberry smoothie	Days					
		0	**3**	**6**	**9**	**12**	**15**
Viscosity (cp)	CS	960±25.43^{aC}	954.2±28.44^{aC}	930.2±17.17^{aC}	886.8±22.81^{abB}	819.6±22.04^{aC}	780±22.50^{aC}
	FSS	1470±25.43^{eA}	2099.4±28.44^{dA}	2679±17.17^{bA}	2962.8±22.81^{aA}	2640.6±22.04^{bA}	2595.2±22.50^{cA}
	RCS	1228±25.43^{dB}	1726.2±28.44^{cB}	2333.4±17.17^{aB}	2125.2±22.81^{bB}	1701.6±22.04^{dB}	1655±22.50^{cB}
L*	CS	65.19±0.02^{aA}	65.02±0.00^{bA}	64.80±0.00^{cA}	64.58±0.00^{dA}	64.44±0.01^{eA}	64.20±0.01^{fA}
	FSS	51.28±0.02^{aC}	51.13±0.00^{bC}	51.02±0.00^{cC}	50.93±0.00^{dC}	50.84±0.01^{eC}	50.58±0.01^{fC}
	RCS	55.09±0.02^{aB}	54.83±0.00^{bB}	54.82±0.00^{bB}	54.53±0.00^{cB}	54.44±0.01^{dB}	54.20±0.01^{eB}
a*	CS	16.99±0.02^{aB}	16.80±0.01^{bB}	16.82±0.02^{bB}	16.69±0.00^{cB}	16.65±0.01^{cB}	16.45±0.01^{dB}
	FSS	16.81±0.02^{aC}	16.68±0.01^{bC}	16.39±0.02^{cC}	16.25±0.00^{dC}	16.27±0.01^{dC}	16.15±0.01^{eC}
	RCS	17.55±0.02^{aA}	17.35±0.01^{bA}	17.26±0.02^{cA}	17.23±0.00^{cA}	17.20±0.01^{cdA}	17.08±0.01^{dA}
b*	CS	9.03±0.01^{aC}	8.92±0.01^{bC}	8.80±0.02^{cC}	8.78±0.01^{cdC}	8.74±0.01^{dC}	8.69±0.01^{eC}
	FSS	21.83±0.01^{fA}	21.94±0.01^{eA}	22.22±0.02^{dA}	22.28±0.01^{cA}	22.36±0.01^{bA}	22.45±0.01^{aA}
	RCS	20.08±0.01^{cB}	20.12±0.01^{cB}	20.28±0.02^{cB}	20.40±0.01^{bB}	20.44±0.01^{bB}	20.50±0.01^{aB}

Effect of storage on physicochemical attributes of strawberry smoothie.

*a_f Different letters in same row denote significant differences ($p<0.05$) for storage period among days.

A_C different letters in same column denote significant difference between control and fiber and reduced calorie smoothie ($p<0.05$)

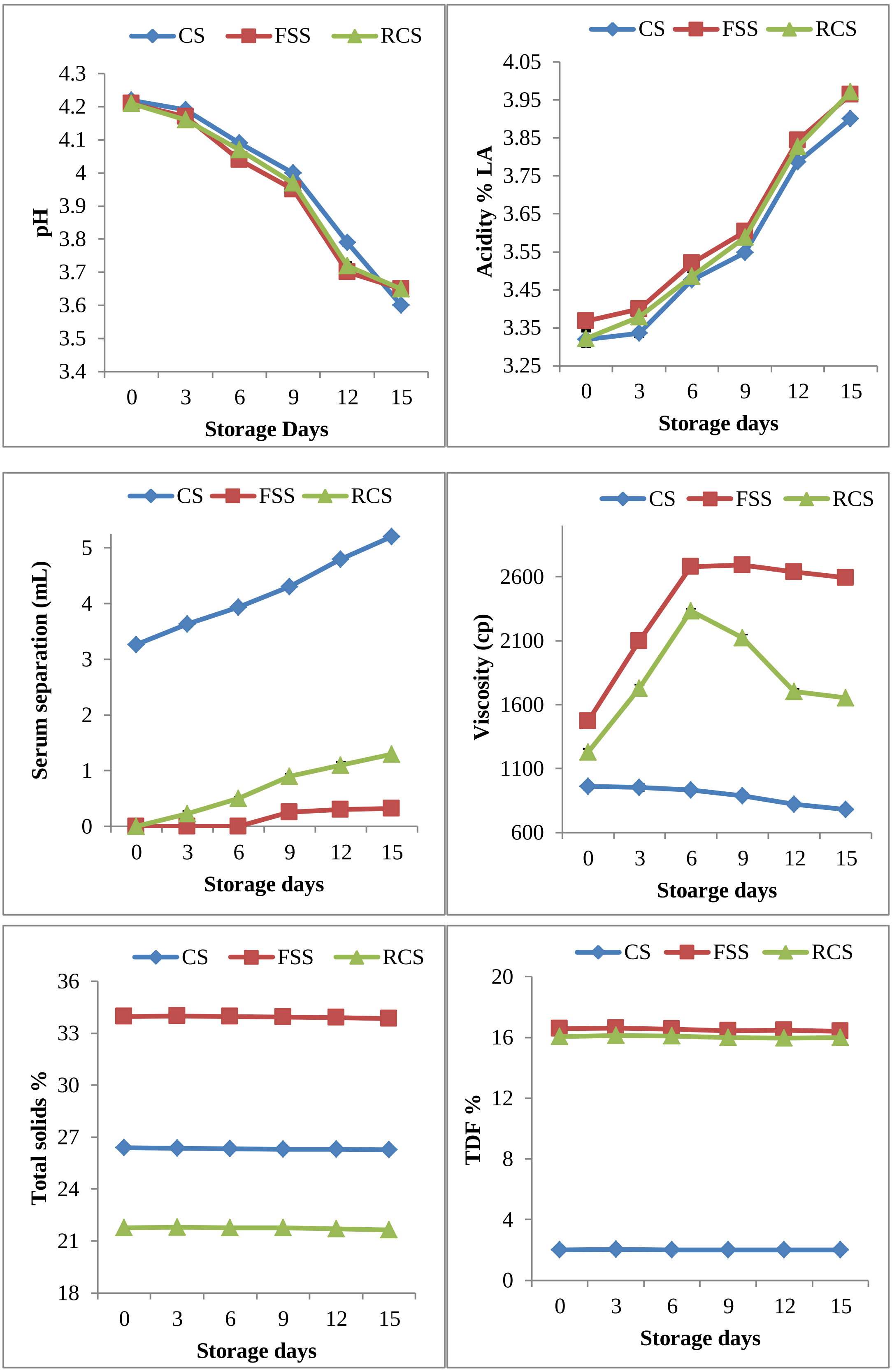


Fig. 4.3.3(a): **Effect of storage on physico-chemical properties of fiber fortified and reduced calorie fiber fortified strawberry probiotic smoothie**

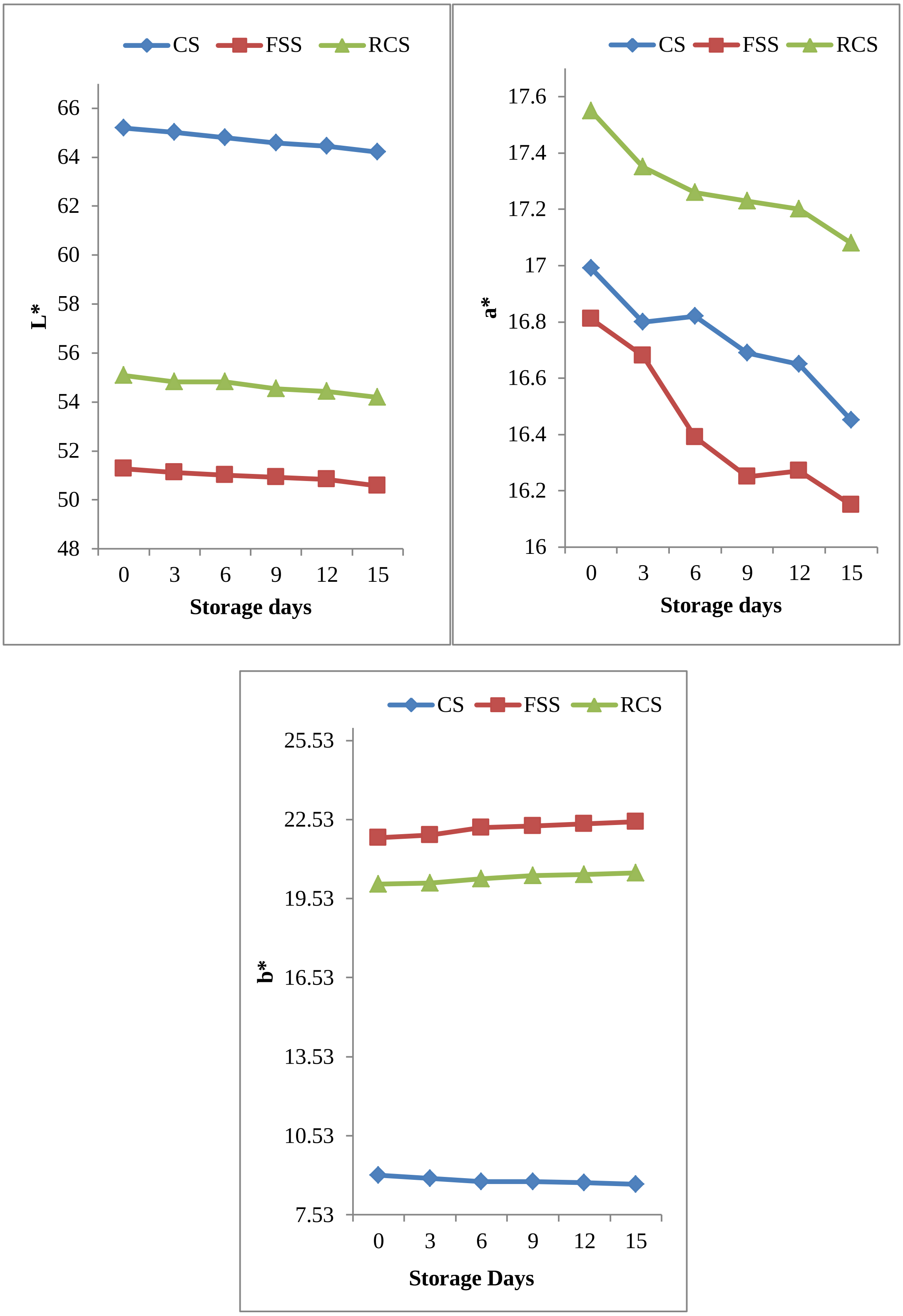


Fig. 4.3.3(b): Effect of storage on instrumental colour value of fiber fortified and reduced calorie fiber fortified strawberry probiotic smoothie

Table 4.3.4: Effect of storage on physico-chemical properties of fiber fortified and reduced calorie fiber fortified mango probiotic smoothie (n=3)

Parameters	Mango smoothie	Days					
		0	**3**	**6**	**9**	**12**	**15**
pH	Control	4.15±0.00^{aA}	4.07±0.01^{bA}	4.00±0.01^{cA}	3.88±0.01^{dA}	3.72±0.01^{eB}	3.60±0.01^{fB}
	Fiber + sugar	4.12±0.000^{aB}	4.05±0.01^{bA}	3.95±0.01^{cAB}	3.87±0.01^{dA}	3.78±0.01^{eA}	3.70±0.01^{fA}
	Reduced calorie	4.10±0.00^{aB}	4.05±0.01^{bA}	3.93±0.01^{cB}	3.86±0.01^{dA}	3.70±0.01^{eB}	3.65±0.01^{fB}
Acidity (%LA)	Control	3.483±0.01^{eA}	3.579±0.01^{dB}	3.664±0.01^{dA}	3.728±0.01^{cA}	3.967±0.02^{bA}	4.252±0.02^{aA}
	Fiber + sugar	3.485±0.01^{eA}	3.622±0.01^{dA}	3.695±0.01^{dA}	3.763±0.01^{cA}	3.927±0.02^{bA}	4.190±0.02^{aB}
	Reduced calorie	3.500±0.01^{fA}	3.608±0.01^{eAB}	3.690±0.01^{dA}	3.767±0.01^{cA}	3.927±0.02^{bA}	4.198±0.02^{aB}
Serum separation (ml)	Control	2.80±0.03^{eA}	2.80±0.03^{eA}	3.060±0.05^{dA}	3.530±0.01^{cA}	3.800±0.05^{bA}	4.125±0.05^{aA}
	Fiber + sugar	Nil	Nil	Nil	Nil	Nil	Nil
	Reduced calorie	Nil	Nil	Nil	Nil	Nil	Nil
Total solids	Control	25.12±0.05^{aB}	25.18±0.05^{aB}	25.15±0.04^{aB}	25.10±0.05^{aB}	25.10±0.05^{aB}	25.06±0.06^{aB}
	Fiber + sugar	29.22±0.06^{aA}	29.25±0.05^{aA}	29.25±0.04^{aA}	29.18±0.05^{aA}	29.14±0.05^{aA}	29.09±0.06^{aA}
	Reduced calorie	22.08±0.05^{aC}	22.14±0.05^{aC}	22.15±0.04^{aC}	22.14±0.05^{aC}	22.11±0.05^{aC}	22.12±0.06^{aB}
Total dietary fiber	Control	2.548±0.04^{aB}	2.550±0.05^{aB}	2.550±0.08^{aB}	2.545±0.06^{aB}	2.540±0.04^{aB}	2.540±0.05^{aB}
	Fiber + sugar	17.31±0.10^{aA}	17.30±0.10^{aA}	17.25±0.12^{aA}	17.25±0.12^{aA}	17.26±0.10^{aA}	17.25±0.12^{aA}
	Reduced calorie	17.14±0.14^{aA}	17.16±0.15^{aA}	17.15±0.14^{aA}	17.11±0.12^{aA}	17.08±0.14^{aA}	17.08±0.15^{aA}

Parameters	Mango smoothie	Days					
		0	**3**	**6**	**9**	**12**	**15**
Viscosity(cp)	Control	2249±85.08^{aC}	2124±129.70^{aB}	2109±102.59^{abC}	2025±63.13^{bC}	1879±85.86^{cC}	1512±91.42^{dC}
	Fiber + sugar	9113±85.08^{aA}	9120±129.70^{aA}	8448±102.59^{bA}	8085±63.13^{cA}	7793±85.86^{dA}	7585±91.42^{eA}
	Reduced calorie	8653±85.08^{aB}	8844±129.70^{aA}	8028±102.59^{bB}	7634±63.13^{cB}	7410±85.86^{cB}	7152±91.42^{dB}
L*	Control	77.08±0.01^{aA}	76.96±0.00^{bA}	76.81±0.01^{cA}	76.76±0.01^{cdA}	76.72±0.01^{dA}	76.60±0.01^{eA}
	Fiber + sugar	57.91±0.01^{aC}	57.81±0.00^{bC}	57.69±0.01^{cC}	57.67±0.01^{cB}	57.58±0.01^{dC}	57.49±0.01^{eC}
	Reduced calorie	61.49±0.01^{aB}	61.14±0.00^{bB}	61.00±0.01^{cB}	60.92±0.01^{dB}	60.84±0.01^{eB}	60.72±0.01^{fB}
a*	Control	5.62±0.00^{aC}	5.44±0.00^{bC}	5.43±0.00^{bC}	5.38±0.01^{cC}	5.21±0.02^{dC}	5.15±0.02^{eC}
	Fiber + sugar	9.33±0.00^{dA}	9.36±0.00^{cdA}	9.42±0.00^{cdA}	9.46±0.01^{cA}	9.53±0.02^{bA}	9.62±0.02^{aA}
	Reduced calorie	8.57±0.00^{eB}	8.64±0.00^{dB}	8.83±0.00^{cB}	8.90±0.01^{bB}	8.94±0.02^{bB}	8.99±0.02^{aB}
b*	Control	48.53±0.01^{aA}	48.11±0.00^{bA}	48.04±0.01^{cA}	47.98±0.01^{dA}	47.90±0.01^{eA}	47.79±0.01^{fA}
	Fiber + sugar	45.34±0.01^{aB}	45.30±0.00^{abB}	45.25±0.01^{bB}	45.20±0.01^{cB}	45.14±0.01^{dB}	45.00±0.01^{fB}
	Reduced calorie	41.98±0.01^{aC}	41.45±0.00^{bC}	41.17±0.00^{cC}	41.10±0.01^{dC}	40.99±0.01^{eC}	40.85±0.01^{fC}

Effect of storage on physico –chemical of mango smoothie.

*a_f Different letters in same row denote significant differences ($p<0.05$) for storage period among days.

A_C different letters in same column denote significant difference between control and fiber and reduced calorie smoothie ($p<0.05$)

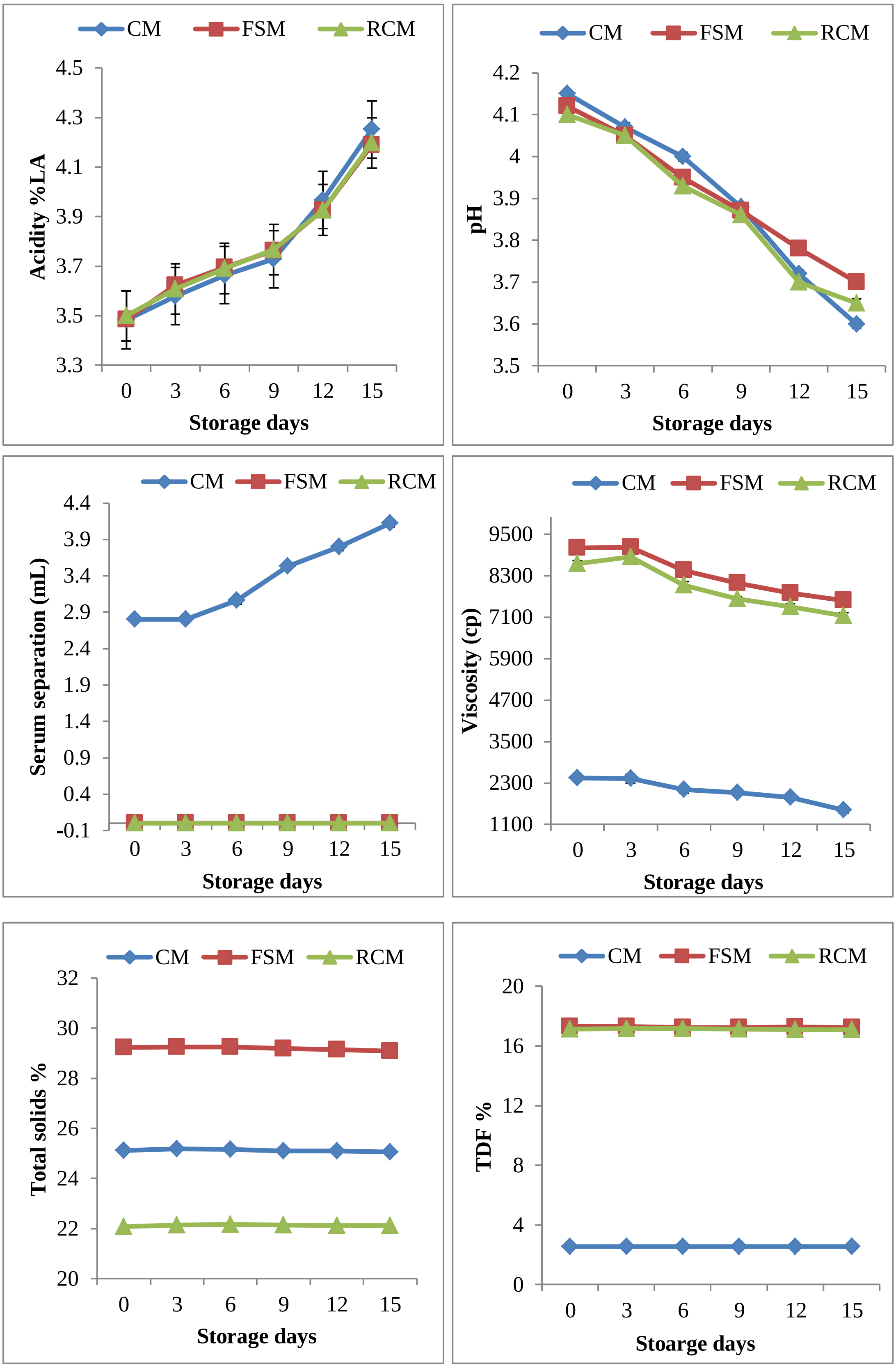


Fig. 4.3.4(a): Effect of storage on physico-chemical properties of fiber fortified and reduced calorie fiber fortified mango probiotic smoothie

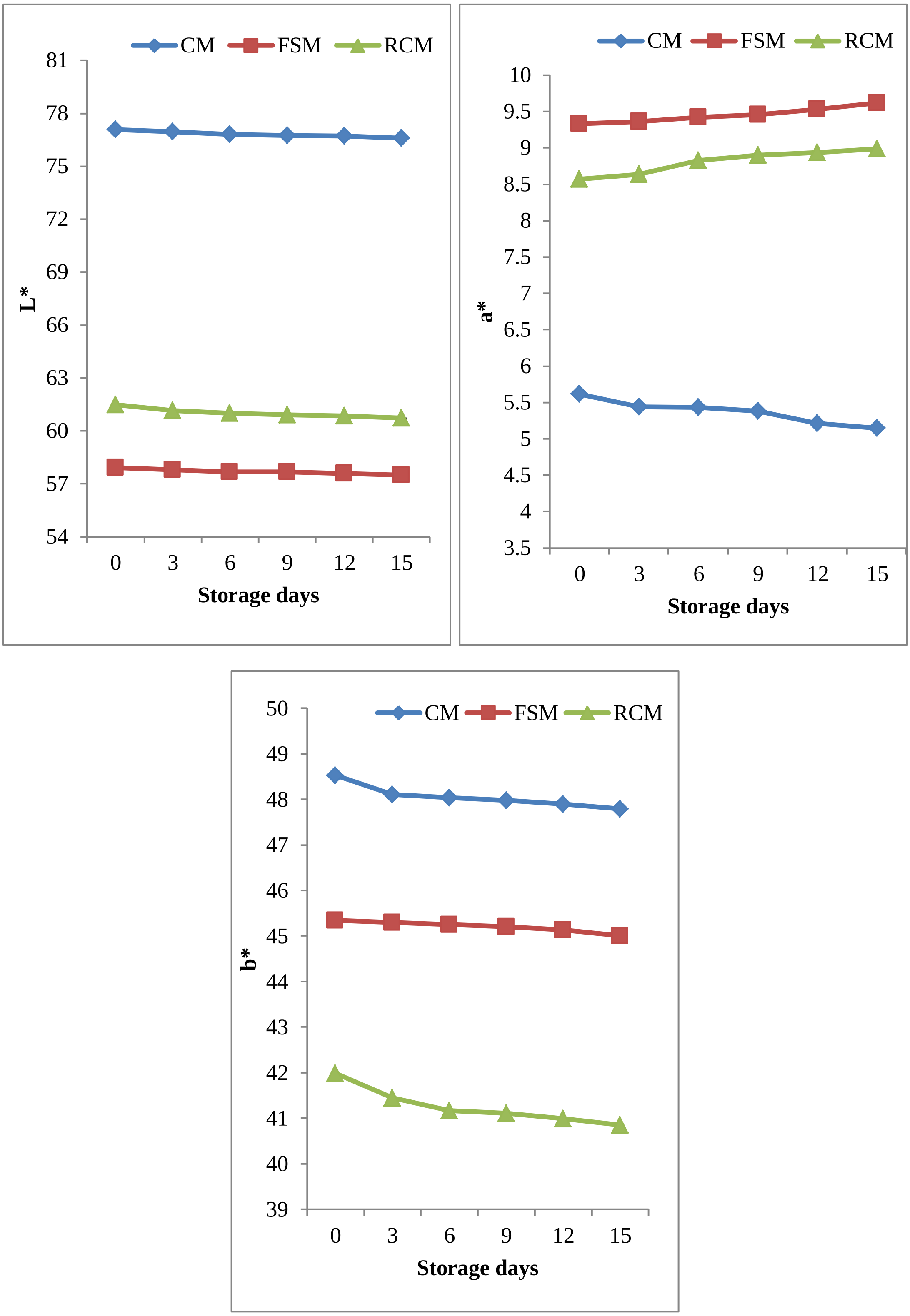


Fig. 4.3.4(b): Effect of storage on instrumental colour values of fiber fortified and reduced calorie fiber fortified mango probiotic smoothie

Instrumental colour

The change in colour of smoothie stored at refrigeration temperature in terms of lightness (L*), redness (a*) and yellowness (b*) were recorded and reported in Table 4.3.3 & 4.3.4.

In strawberry based smoothie, the colour value for FSS smoothie had significant difference ($p<0.05$) during storage. Lightness (L*) value for fiber fortified as well for sweetener sweetened smoothie was decreased significantly ($p<0.05$) with increasing the storage period as shown in Table 4.3.3. The mean score varied from 51.28-50.58 and 55.09-54.20 in fiber fortified and reduced calorie smoothie respectively that depicted darkening effect on the smoothie due to fiber incorporation. Similar results are reported by (Staffolo et al., 2004) that apple fiber imparted typical brownish and lower the L* value of yogurt.

The redness value (a*) decreased significantly ($p<0.05$) with increasing storage period of smoothie as shown in Table 4.3.3. The mean score value for redness (a*) varied from 16.81- 16.15 during storage period in case of fiber fortified smoothie. In reduced calorie smoothie (a*) value at zero day was 17.55 which decreased to 17.08 on 15th day of storage. Similar results were reported by (Raju & Pal, 2014) in oat fiber fortified yogurt.

The yellowness (b*) increased significantly ($p<0.05$) with increasing storage period of smoothie as shown in Table 4.3.3. The mean score value for yellowness (b*) varied from 21.83- 22.45 during storage period in case of fiber fortified smoothie. In sweetener sweetened smoothie (b*) value at zero day was 20.08 which increased to 20.50 on 15th day of storage. The results were in accordance with the study conducted by Manzoor et al., 2019 in preparation of stirred yogurt prepared by papaya peel powder. Karaca et al., 2019 reported significantly increasing b* values with increasing apricot fiber ratios.

In mango-based smoothie, the L*, a* and b* mean value scores in Fiber fortified smoothie was ranged 57.95- 57.49, 9.35- 9.62 and 45.34-45.00 during storage period respectively as shown in Table 4.3.4. In reduced calorie smoothie mean value for L*, a* and b* was ranged 61.49- 60.72, 8.51- 8.99 and 41.98-40.85 during storage. The lightness (L*) and yellowness (b*) value decreased significantly ($p<0.05$) while a* value increased significantly ($p<0.05$) with increasing storage period. This might be due to the distinctive brown colour of apple fiber which decreased the L* and b* value and increase a* value in both mango-based smoothie smoothies. Similar results were reported by (Staffolo et al., 2004).

4.3.3 Effect of storage period on the microbiological analysis of smoothie

Probiotic viability

The changes in probiotic viability of smoothie during storage have been illustrated in Table 4.3.5. The mean score of probiotic viability was affected by progressive storage days. In strawberry based smoothie, the mean of probiotic viability for fiber fortified smoothie was 8.56 at zero days which decreased significantly ($p<0.05$) after 6th day of storage and reached viability 7.95 at 15th day. In reduced calorie smoothie the mean value for the probiotic viability was ranged 8.38-7.71 during storage.

In mango-based smoothie, the probiotic viability for fiber fortified smoothie significantly ($p<0.05$) decreased with increasing storage period (8.56 -7.88) period. In reduced calorie smoothie, initial viability was 8.50 which decreased to 7.67 on 15th day.

The progressive decline in probiotic count during storage could be due to continuous increase in acidity and low pH of smoothie samples. Similar results were reported by (Gallina et al., 2019). in probiotic fermented smoothie during storage. Karaca et al. (2019) reported the number of *L. acidophilus* increased with increasing apricot fiber ratios but significantly decreased with storage duration

Yeast and mould count not detectable till 6th day of storage. However, with advancing storage period, both mango as well as strawberry-based smoothie showed an increase in yeast and mould count after 9th day. Yeast and mold count varied from 1.53-2.18 and 1.67-2.23 in fiber fortified and reduced calorie strawberry smoothie respectively. In mango smoothie, yeast and mold count varied from 1.45-2.20 and 1.52-2.25 in fiber fortified and reduced calorie smoothie respectively. A significant ($p<0.05$) change observed in yeast and mold count during storage period of smoothie as shown in Table 4.3.5. Coliforms in any dairy product represent the extent of hygienic conditions maintained during production, packaging and storage. In present study, coliforms were found to be absent in all the samples, indicating that proper hygienic precautions were taken during production and packaging of smoothie. After 15 days storage study for smoothie was ended as microbial spoilage occurred.

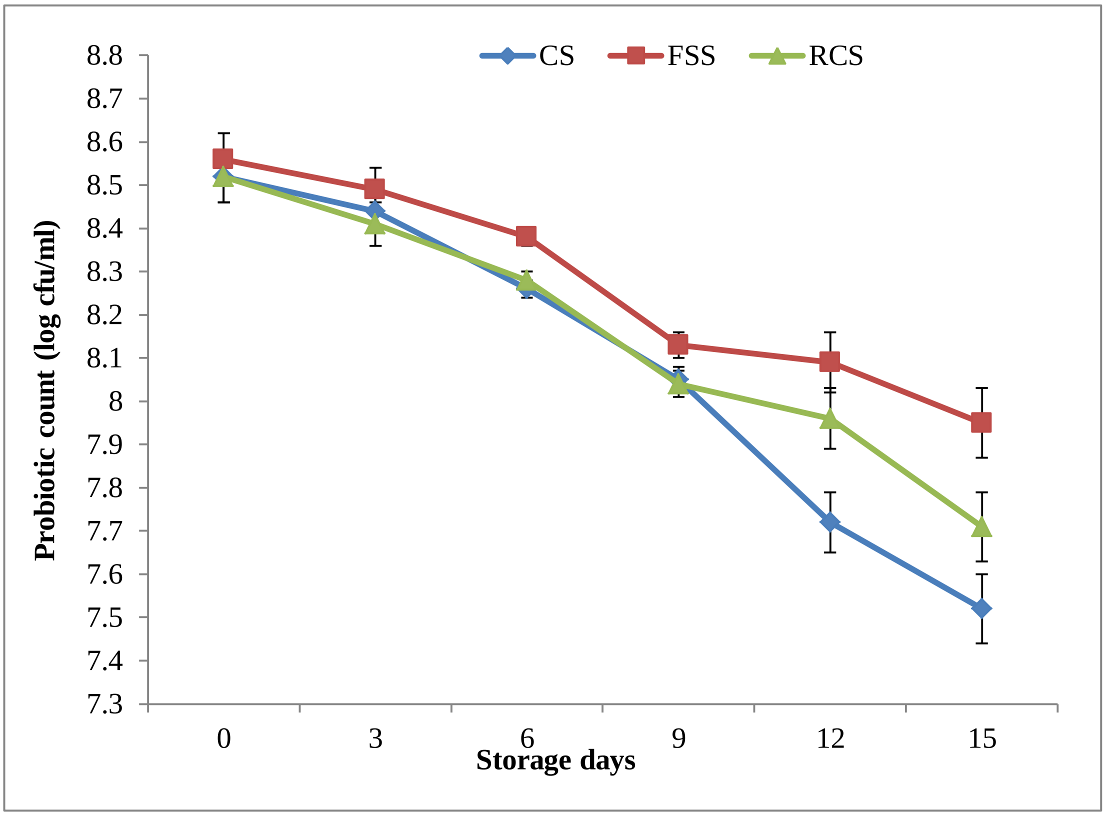
Table 4.3.5: Microbial changes during storage study of strawberry-based and mango-based smoothie (n=3)

Parameter	Product	Days					
		0	**3**	**6**	**9**	**12**	**15**
Probiotic viability	CS	8.52± 0.06^{aA}	8.44± 0.05^{aA}	8.26± 0.02^{bB}	8.05± 0.03^{cA}	7.72± 0.07^{dB}	7.52±0.08^{eC}
	FSS	8.56± 0.06^{aA}	8.49± 0.05^{aA}	8.38± 0.02^{aA}	8.13± 0.03^{bA}	8.09± 0.07^{bA}	7.95±0.08^{cA}
	RCS	8.52± 0.06^{aA}	8.41± 0.05^{abA}	8.28± 0.02^{bB}	8.04± 0.03^{cA}	7.96± 0.07^{cAB}	7.71±0.08^{dA}
Probiotic viability	CM	8.58± 0.03^{aA}	8.50± 0.03^{aA}	8.30± 0.02^{bA}	8.03± 0.03^{cB}	7.85± 0.04^{dB}	7.59±0.06^{eC}
	FSM	8.56± 0.03^{aA}	8.45± 0.03^{bA}	8.32± 0.02^{cA}	8.24± 0.03^{cA}	8.04± 0.04^{dA}	7.88±0.06^{eA}
	RCM	8.50± 0.03^{aA}	8.47± 0.03^{aA}	8.25± 0.02^{bA}	8.09± 0.03^{cB}	7.93± 0.04^{dAB}	7.67±0.06^{eB}
Yeast and mold	CS	Nil	Nil	Nil	1.74± 0.19^{bA}	2.14± 0.05^{aA}	-
	FSS	Nil	Nil	Nil	Nil	1.53± 0.11^{bB}	2.18±0.06^{aA}
	RCS	Nil	Nil	Nil	Nil	1.67± 0.10^{bB}	2.23±0.10^{aA}
Yeast and mold	CM	Nil	Nil	Nil	1.65± 0.09^{bA}	2.10± 0.10^{aA}	-
	FSM	Nil	Nil	Nil	Nil	1.45± 0.12^{bB}	2.20±0.13^{aA}
	RCM	Nil	Nil	Nil	Nil	1.52± 0.16^{bB}	2.25±0.10^{aA}
Coliform	Control	Nil	Nil	Nil	Nil	Nil	Nil
	FSS	Nil	Nil	Nil	Nil	Nil	Nil
	RCS	Nil	Nil	Nil	Nil	Nil	Nil
Coliform	CM	Nil	Nil	Nil	Nil	Nil	Nil
	FSM	Nil	Nil	Nil	Nil	Nil	Nil
	RCM	Nil	Nil	Nil	Nil	Nil	Nil

Effect of storage on physico –chemical of mango smoothie.

*a_c Different letters in same row denote significant differences ($p<0.05$) for storage period among days.

A_C different letters in same column denote significant difference between control and fiber and reduced calorie smoothie ($p<0.05$)



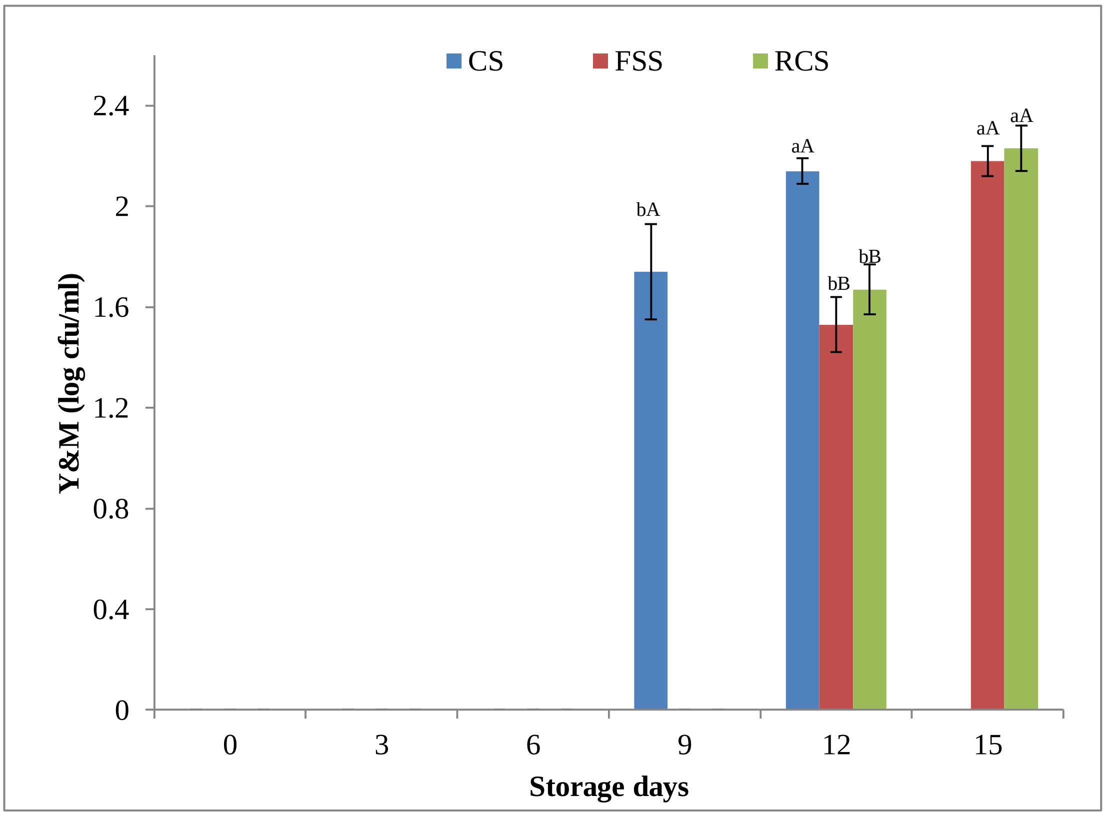
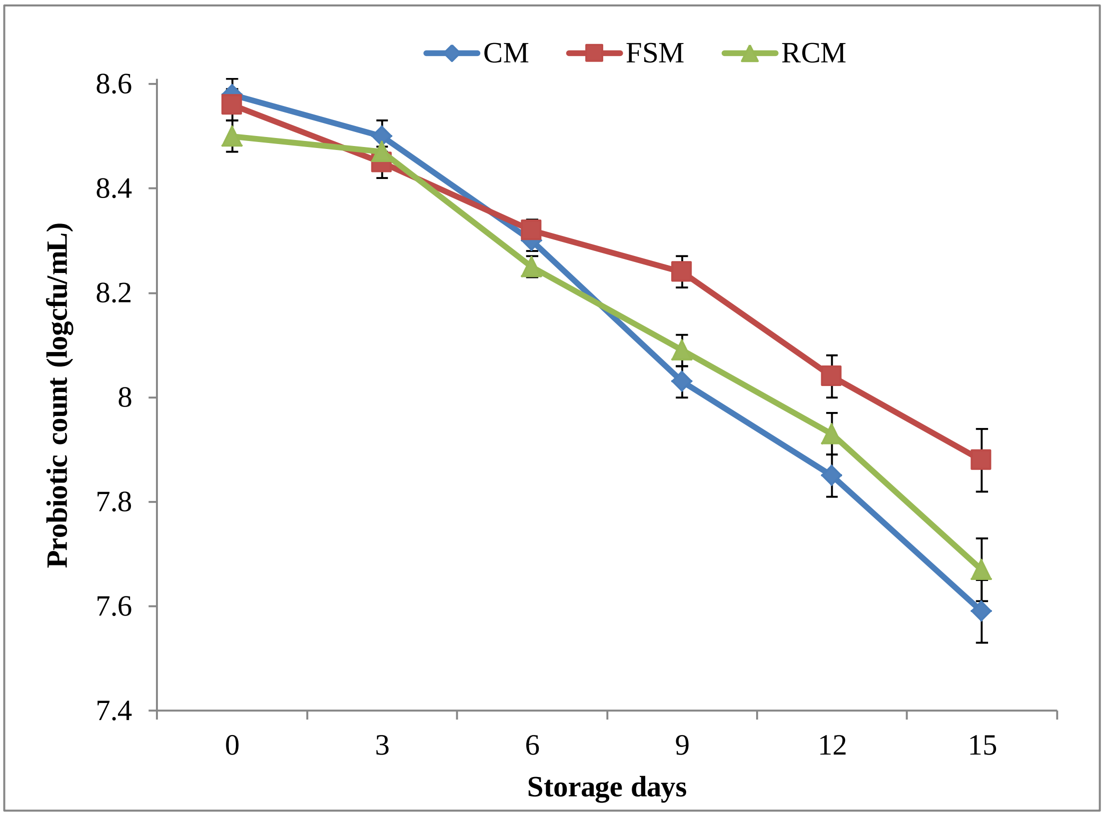


Fig. 4.3.5(a): Microbial changes during storage study of strawberry-based smoothie



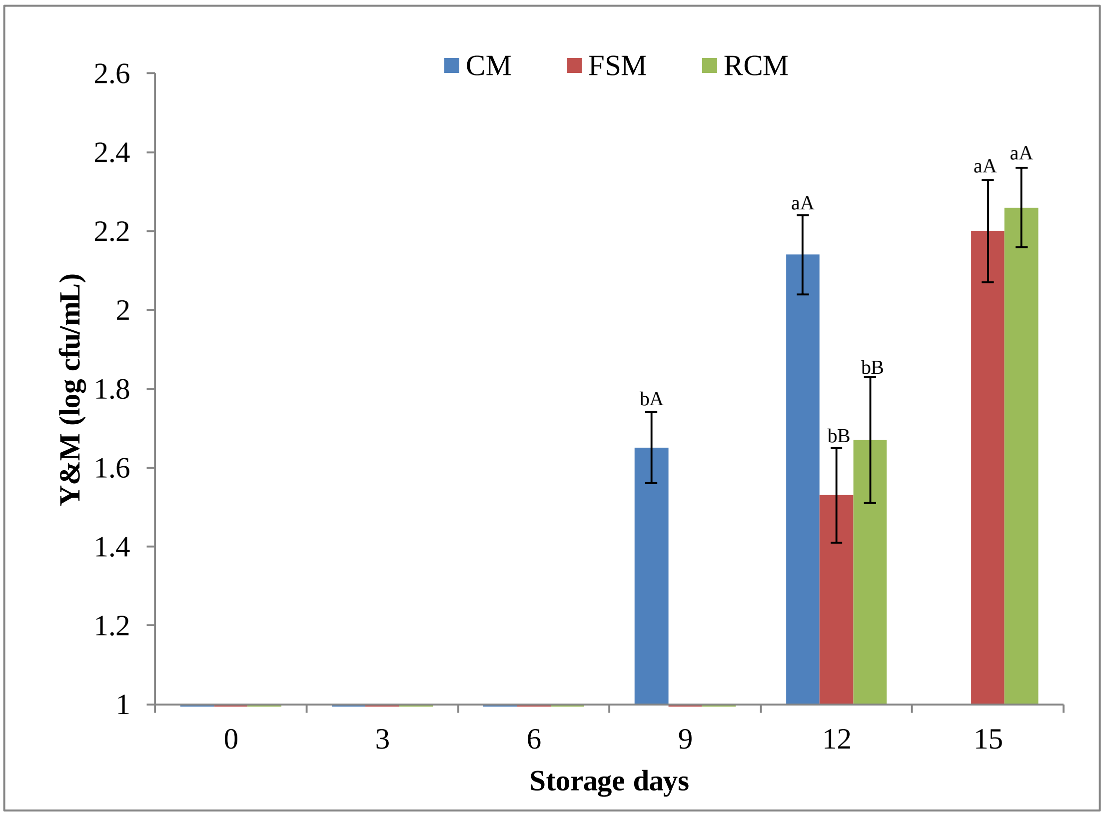


Fig. 4.3.5(b): Microbial changes during storage study of mango-based smoothie

4.3.4 Study of neotame stability in smoothie during storage employing HPLC

Table 4.3.6: Stability of neotame during storage

Storage period (days) (5° C)	Stability %	
	Reduced calorie strawberry probiotic smoothie	Reduced calorie mango probiotic smoothie
0	96.02±0.66[a]	96.53±0.49[a]
12[th]	96.24±0.58[a]	96.42±0.66[a]

Values presented are Mean score ±SE (n=3)
Values with same superscripts within the same column differ non significantly (p>0.05)

It was apparent from the Table 4.3.6 that there was a non significant (p>0.05) change in neotame content during storage of smoothie as pH of sample varied from 4.21- 3.70 which fall within the pH range of maximal neotame stability. Similar results were reported by Nofre and Tinti (2000) in neotame added yoghurt at 5°C for 6 weeks. Prakash et al. (2002) also reported that about 98 per cent of the initial neotame remained in yoghurt at the end of 6 week. Kumari et al., 2016 also observed maximal stability during storage of neotame sweetened yogurt.

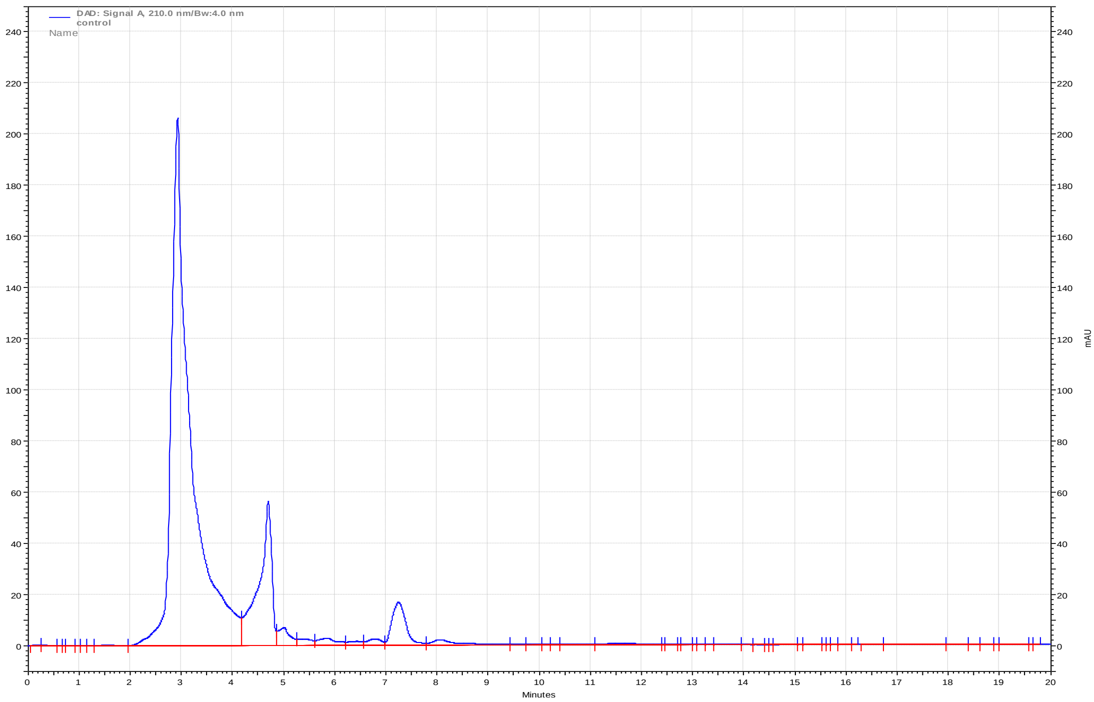


Fig. 4.3.6: Chromatogram of control sample

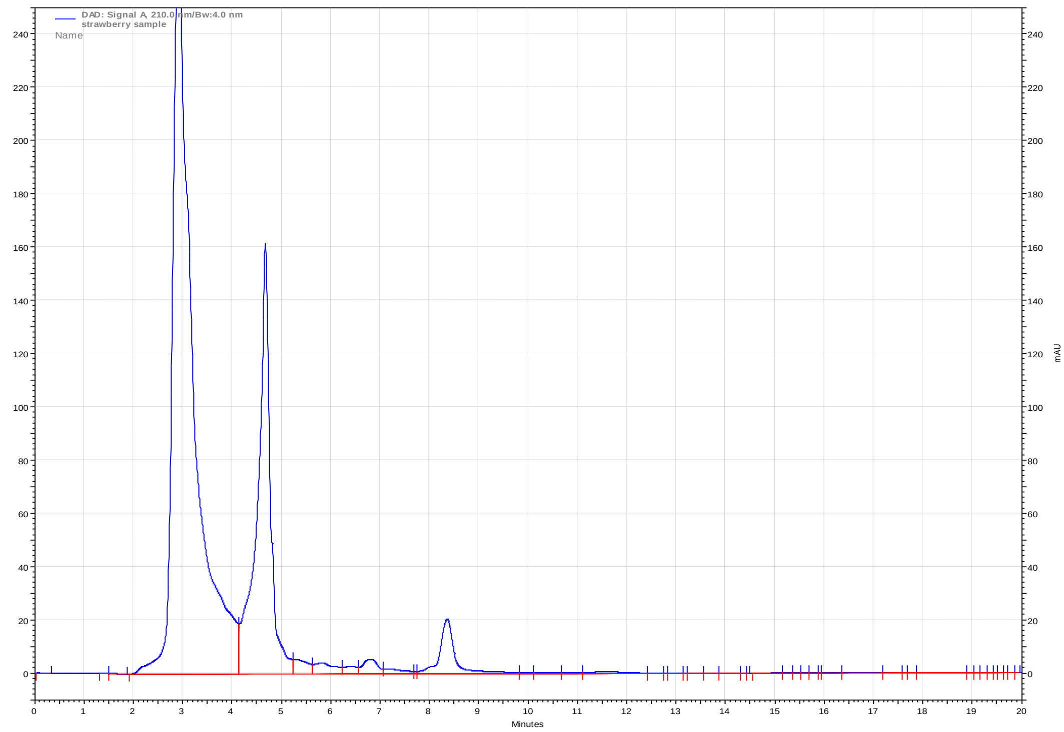


Fig. 4.3.7(a): Chromatogram of fiber fortified reduced calorie strawberry probiotic smoothie at 0 day

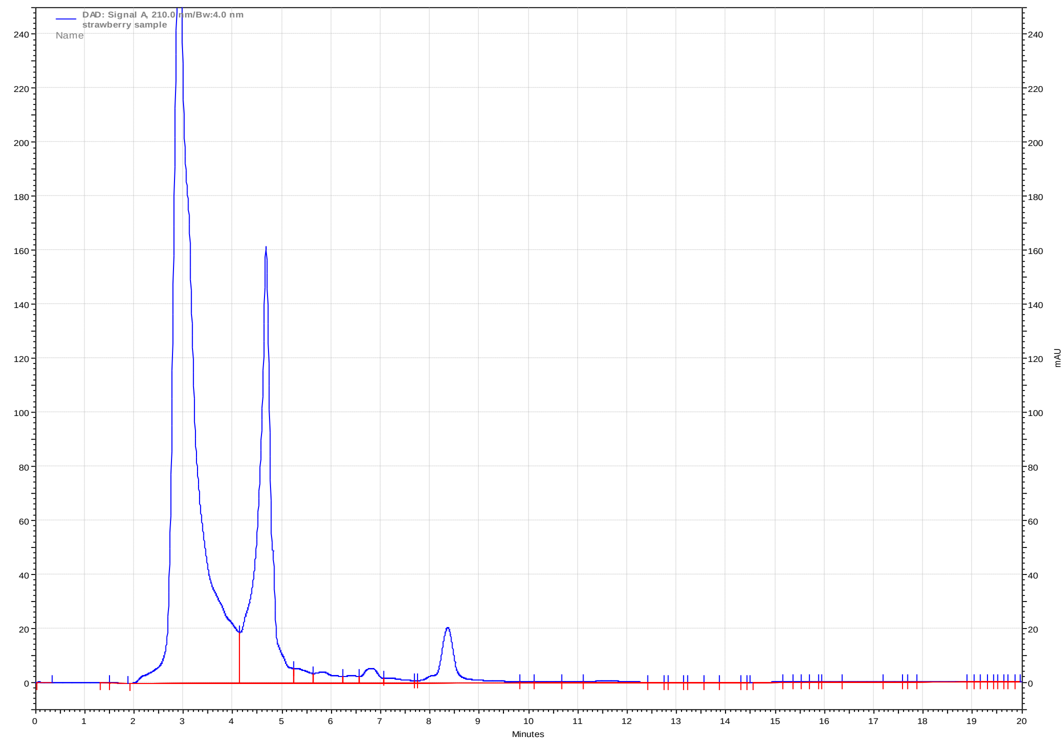


Fig. 4.3.7(b): Chromatogram of fiber fortified reduced calorie strawberry smoothie at 12th day

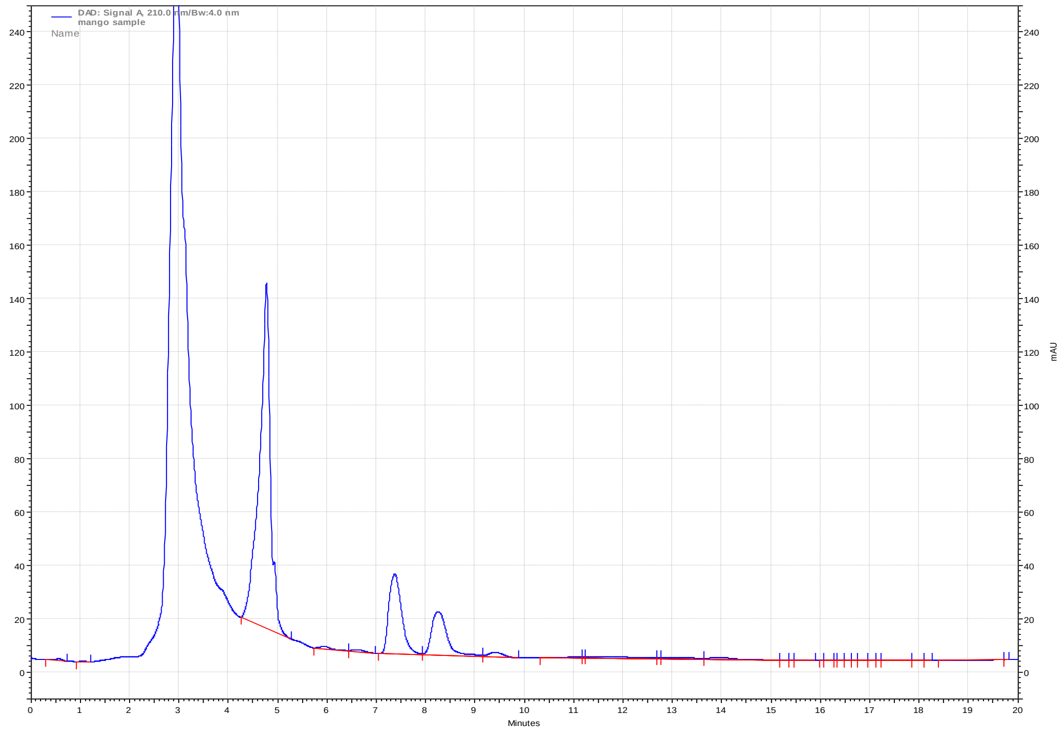


Fig. 4.3.8(a): Chromatogram of fiber fortified reduced calorie mango smoothie at 0 day

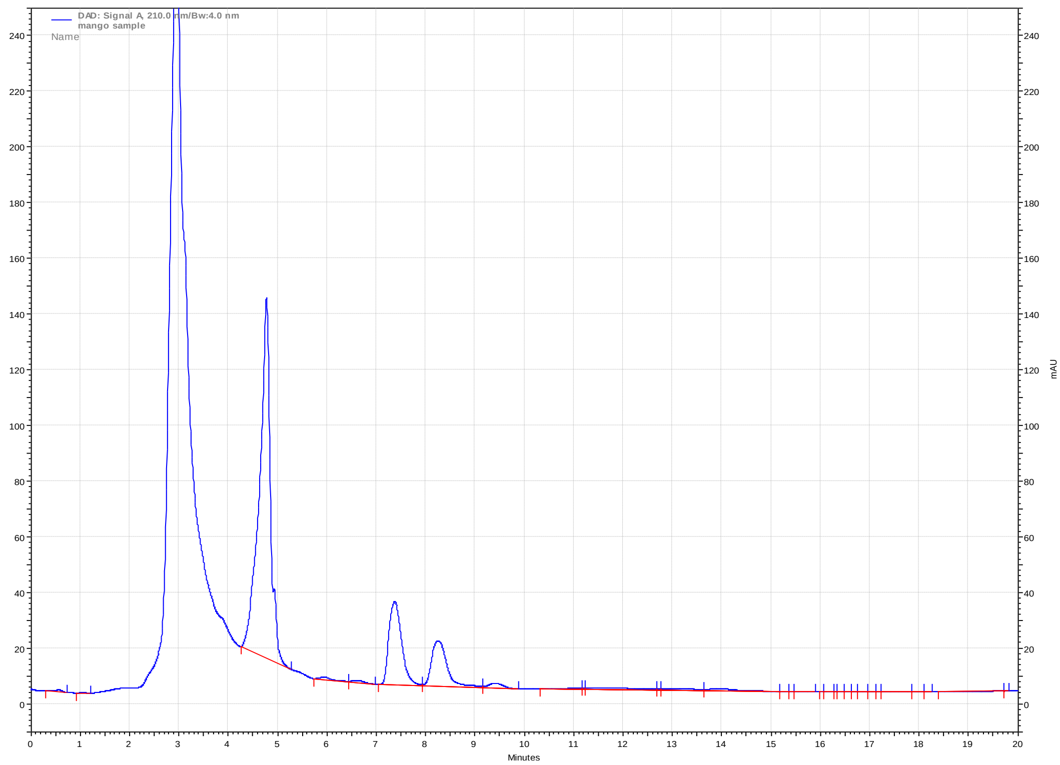


Fig. 4.3.8(b): Chromatogram of fiber fortified reduced calorie mango smoothie at 12th day

4.3.4 Cost Analysis of Mango smoothie-

Every market product needs to be commercially viable to become attractive for entrepreneurs and industrialists so that its mass production can be taken up. Further, the product should also be palatable and price should be within reach of common people for commercial sustainability of the product in a competitive market. Cost of production is one of the most important deciding factors for the commercial viability of any industry. Therefore, through present investigation, an attempt was made to estimate the cost of production of control smoothie and experimental smoothie (mango) taking into consideration certain set of assumptions.

1. **Land and building:** The working space required for Smoothie unit was 375 sq. feet (25×15 sq. feet) and it was taken on rent in a peri urban area (@ ₹5500/- per month). Building includes processing section, store and utilities section.
2. **Machinery and Equipment:** Capital investment (₹5,23,500)/-) includes the cost of all equipments viz. refrigerator, diesel stove, display cabinet, cream separator, food processor, packaging machine, stainless steel vat and weighing balance. Depreciation on machinery and equipments was taken according to their expected life. Interest on capital investment and variable cost was taken @12 percent per annum.
3. **Raw material:** Raw materials such as milk (₹35/kg), sugar (₹40/kg), mango (₹250/kg) (present rate ₹100/500gm price vary between season to season), sunfiber (₹500/kg), apple fiber (₹300/kg) were required for preparation of smoothie manufacturing.
4. **Capacity:** One batch of mango smoothie was made from total of 55.65 kg of curd, and yield of the final product was 500 bottles including losses.
5. **Manpower:** Four unskilled person was required for manufacturing smoothie (as per Punjab government new rate DC approved unskilled worker ₹390/person)
6. **Packaging Material:** Glass bottle (₹4/ bottles) were used as packaging material.

Table 4.3.7: Cost analysis of Mango smoothie

Sr. No.	Total Capital investment required	523500	
	Particulars	**Cost (in ₹)**	
A.	Fixed cost		
	Depreciation on Equipments p.a.	52350	
	Interest on Capital Investment @12% p.a.	62820	
	Building rent per annum	66000	
	Fixed cost per annum	181170	
	Total fixed cost per batch (500 bottles) (A)	**496.36**	
B.	Variable cost	**Quantity required per batch**	**Cost (in Rs.)**
	Raw Materials		
1.	Milk (Rs35/lt)	55.65 Kg	1947.75
2.	Sugar (Rs 40/kg)	10.43 Kg	417.20
3.	Pasteurized water	13.04 Kg	0
4.	Apple fiber (Rs. 300 Kg)	3.48 Kg	1044
5.	Sunfiber (Rs. 500/Kg)	8.70 Kg	4350
6.	Mango pulp (Rs. 250/ Kg)	8.70 Kg	2175
	Total	100 Kg	9933.95
	Labour		
	Labour @ 390/ Day for unskilled (1 man day= 8 Hr) Half Shift	Unskilled= 4 man	1560
	Packaging Material		
1.	Glass bottles (Rs. 4/bottle)	500	2000
	Fuel/ Electricity		
1	Electricity @ 5.85/ unit	21	122.85
2	Fuel charges (Diesel @ 82)	7	574
	Losses @ 1%		20
	Total		
	Total variable cost per day (per batch) (B)		14210.80

C.	**Total cost of the batch = A+B**		**14707.16**
D	Total number of bottles manufactured after losses		500
E	Total cost per bottle		29.41
F	Sale price per bottle		35
G	Gross return		17500
H	Net return		2792.84
I	Net return per bottle (before taxes)		5.58
J	Net returns per month		83758.20
K	BC ratio		1.18
L	Break even point		75.45
	Double shift		
A.	Total fixed cost per day		496.36
B.	Total variable cost per day		28421.60
C.	Total cost per day		28917.96
D	Number of bottles		1000.00
E	Gross return		35000.00
F	Net returns		6082.04
G	Net return per bottle (before taxes)		6.08
H	Net return per month		182461.20
J	BC ratio		1.21
I	Break even point		75.45

4.3.4.1 Component wise cost classification

The component wise cost classification of fiber fortified mango smoothie is given in Table 4.3.7. The total fixed cost per day was estimated as 496.36 and the total variable cost per batch was 14210.80 (contributing major share in total cost because of raw material), both of these resulted into total cost of per day as 14707.16, respectively. The total numbers of bottles produced after losses @ 1% were 500 and if the sale price is set as 35 per bottle, net returns of 5.58 can be obtained per bottle. The net profit per month in a single shift was estimated as 837855.20 which increased to

182461.2 during double shift. The profit margin is quite good and can be easily increased further by increasing the sale price of the product once the market has been established.

4.3.4.2 Break-even analysis

The break-even output is the minimum number of units of finished product produced at which the total revenue equals total cost. A firm will continue its production process or will remain solvent as long as the marginal revenue is greater than or equal to the marginal cost. Break even output provides us with an estimate of the output produced at that level. The break-even output was calculated at 35 per bottle of product. The BC ratio (beneficial cost ratio) of the product was above 1 that signifies that business is running in safe direction with enhanced profits.

4.3.5 Cost analysis of strawberry smoothie

Every market product needs to be commercially viable to become attractive for entrepreneurs and industrialists so that its mass production can be taken up. Further, the product should also be palatable and price should be within reach of common people for commercial sustainability of the product in a competitive market. Cost of production is one of the most important deciding factors for the commercial viability of any industry. Therefore, through present investigation, an attempt was made to estimate the cost of production of control smoothie and experimental smoothie (strawberry) taking into consideration certain set of assumptions.

1 **Land and building:** The working space required for Smoothie unit was 375 sq. feet (25×15 sq. feet) and it was taken on rent in a peri urban area (@₹5500/- per month). Building includes processing section, store and utilities section.

2 **Machinery and Equipment:** Capital investment (₹5,23,500)/-) includes the cost of all equipment’s viz. refrigerator, diesel stove, display cabinet, cream separator, homogeniser, food processor, packaging machine, stainless steel vat and weighing balance. Depreciation on machinery and equipment’s was taken according to their expected life. Interest on capital investment and variable cost was taken @12 percent per annum.

3 **Raw material:** Raw materials such as milk (₹35/kg), sugar (₹40/kg), Strawberry pulp (₹300/kg) (present rate ₹100/500gm price vary between season to season),

apple fiber (₹300/kg) and sunfiber (₹500/kg) were required for preparation of smoothie manufacturing.

4 **Capacity:** One batch of Smoothie was made from total of 51.30 Kg of curd and yield of the final product was 500 bottles including handling losses.

5 **Manpower:** Four unskilled person was required for manufacturing Smoothie (as per Punjab government new rate DC approved unskillled worker ₹390/person)

6 **Packaging Material:** Glass bottle (₹4/ bottles) were used as packaging material.

Table 4.3.8: Cost analysis of Strawberry smoothie

Sr No.	**Total Capital investment required**	**523500**	
	Particulars	**Cost (in Rs.)**	
A.	Fixed cost		
	Depreciation on Equipments p.a.	52350	
	Interest on Capital Investment @12% p.a.	62820	
	Building rent per annum	66000	
	Fixed cost per annum	181170	
	Total fixed cost per batch (500 bottle) (A)	**496.36**	
B.	Variable cost	**Quantity required per batch**	**Cost (in Rs.)**
	Raw Materials		
1.	Milk (Rs35/kg)	51.30 Kg	1795.50
2.	Sugar (Rs 40/kg)	13.05 Kg	522.0
3.	Pasteurized water	13.04 Kg	0
4.	Apple fiber (Rs. 300/ Kg)	3.48 Kg	1044
5.	Sunfiber (Rs. 500/Kg)	8.70 Kg	4350
6.	Strawberry pulp (Rs. 300/ Kg)	10.43 Kg	3129
	Total	100 Kg	10840.50
	Labour		
	Labour @ 390/ Day for unskilled (1 man day= 8 Hr) Half Shift	Unskilled= 4 man	1560

	Packaging Material		
1.	Glass bottles (Rs. 4/bottle)	500	2000
	Fuel/ Electricity		
1	Electricity @ 5.85/ unit	21	122.85
2	Fuel charges (Diesel @ 82)	7	574
	Losses @ 1%		20
	Total		
	Total variable cost per day (per batch) (B)		15117.35
C.	**Total cost of the batch = A+B**		**15613.71**
D	Total number of bottles manufactured after losses		500
E	Total cost per bottle		31.22
F	Sale price per bottle		35
G	Gross return		17500
H	Net return		1886.29
I	Net return per bottle (before taxes)		3.77
J	Net returns per month		56588.70
K	BC ratio		1.12
L	Break even point		104.16
	Double shift		
A	Total fixed cost per day		496.36
B	Total variable cost per day		30234.70
C	Total cost per day		30731.06
D	Number of bottles		1000.00
E	Gross return		35000.00
F	Net returns		4268.94
G	Net return per bottle (before taxes)		4.27
H	Net return per month		128068.20
I	BC ratio		1.13
J	Break even point		104.16

4.3.5.1 Component wise cost classification

The component wise cost classification of fiber fortified strawberry smoothie is given in Table 4.3.8. The total fixed cost per day was estimated as 496.36 and the total variable cost per batch was 15117.35 (contributing major share in total cost because of raw material), both of these resulted into total cost of per day as 15613.71, respectively. The total number of bottles produced after losses @ 1% was 500 and if the sale price is set as 35 per bottle, net returns of 3.77 can be obtained per bottle. The net profit per month in a single shift was estimated as 56588.7 which increased to 128068.20 during double shift. The profit margin is quite good and can be easily increased further by increasing the sale price of the product once the market has been established.

4.3.5.2 Break-even analysis

The break-even output is the minimum number of units of finished product produced at which the total revenue equals total cost. A firm will continue its production process or will remain solvent as long as the marginal revenue is greater than or equal to the marginal cost. Break even output provides us with an estimate of the output produced at that level. The break-even output was calculated at □35 per bottle of product. The BC ratio (beneficial cost ratio) of the product was above 1 that signifies that business is running in safe direction with enhanced profits.

CHAPTER V

SUMMARY AND CONCLUSIONS

Fruits and vegetables generally are considered good sources of fiber, out of which few sources were chosen for the present investigation based on the criteria of cost, seasonal availability and being abundant source of nutritional reservoirs. Based on the maximum preferences, two major fruits were chosen. The study was focused on the fiber fortification with the help of easily adaptable product, which has a place in the diet of the consumers from a long time, i.e., smoothie. Therefore, the natural sources rich in fibers like apple fiber, sunfiber, diesol which are also a rich source of minerals, antioxidants too, were chosen to provide nutritional and functional benefits. To start with the study, various preliminary trials were taken with varying concentrations of fiber, ranging from 4-10 per cent, pulp of two different fruits in varying concentration i.e., 10-12 percent along with sugar for the preparation of smoothie. To select the optimum level, sensory evaluation was performed to find out the acceptability of consumers in terms of various parameters. Results indicated that, 4 per cent Apple fiber, 10 per cent Sunfiber®, 15 per cent sugar and 12 per cent pulp were found optimized for the preparation of Strawberry smoothie where, 4 per cent Apple fiber, 10 per cent Sunfiber®, 12 per cent sugar and 10 per cent pulp was selected during sensory evaluation for mango smoothie. Also, Diesol™ was tried as fiber source but could not give desirable sensory attributes.

The second objective was to prepare the reduced calorie probiotic smoothie. In this objective sugar was replaced with artificial or natural sweeteners. The prime reason to prepare reduced calorie counterpart was to prepare beverage for calorie conscious segment. Various trials were taken with sweeteners singly or in combination. To select the optimum level of sweeteners, sensory evaluation was performed to find out the acceptability of consumers in terms of various parameters. Sweeteners added singly could not fetch remarkable sensory acceptability by panellists. This might be due to the bitter taste of stevia and intense sweetness after taste in neotame. Results indicated that blend of sweeteners were liked by the panellist at the range of (stevia and neotame) (0.03 and 0.002 per cent) in strawberry smoothie and (0.03 and 0.001 per cent) in mango smoothie. The physico-chemical properties were also analyzed in fiber fortified smoothie and reduced calorie smoothed in both variants of smoothie (strawberry and mango smoothie). Better sensory acceptability and proximate behaviour was found for fiber fortified smoothie sample.

To evaluate life of product during storage, the control (smoothie without fiber) and fortified product were kept under refrigeration temperature to visualize the changes in sensory, physico-chemical and microbiological parameters. Fiber fortified smoothie lasted upto 12 days whereas control smoothie gave a shelf life of 9 days. Control smoothie in both the variants gave a life of 9 days at similar temperature conditions. Probiotic viability in smoothie decreased significantly ($p<0.05$) with increasing storage period in both variants of smoothie. However, remained on the upper side where it can be termed as the probiotic till its last day. Yeast and mold count were detectable on 9^{th} day of storage in fiber fortified and reduced calorie, which increased significantly ($p<0.05$) with storage days. However, in case of reduced calorie smoothie the sweetness was the main reason for its unacceptability. However, coliforms were absent during the entire study. Various other proximate parameters of fiber fortified product were also found superior in the above-mentioned treatments.

There was no serum separation and sediment seen in fiber fortified smoothie during the storage where in control sample it increased with progressive days and was maximum as compared to other variants. This was due to the addition of fiber which bound the free water and decreased the serum separation in product. Also, the high solid content in fiber fortified smoothie decreased syneresis. The L*value of control sample was less as compared to fiber fortified smoothie. This was due to the addition of apple fiber which imparted the typical brown colour which reduced the L* value in fiber fortified smoothie. The probiotic viability was also good in fiber fortified smoothie. Microbiologically, the probiotic count sustained a good count over the storage period. However, yeast and mold count reached to a value of 2.06, 2.10 on 15^{th} and 12^{th} day in case of prepared smoothie and control smoothie. Therefore, life of product was counted an interval earlier than this, wherein it was safe to consume.

Neotame degradation was analyzed using HPLC wherein the added amount of 1 and 2 mg in fiber fortified mango smoothie and fiber fortified strawberry smoothie was analyzed. Recovery rate of 96.53 per cent and 96.02 per cent was found in mango and strawberry smoothie respectively, which was quite appreciable. This indicates that sweeteners were available for its perceivable intensity and even after 12 days of storage sweetener was not much degraded.

Cost analysis revealed that finalized product can be manufactured with a cost of 31.22 per 200 ml of strawberry smoothie 29.41 ml for mango smoothie, which then can further be sold for Rs. 35 harvest better returns.

From the results obtained, it can be concluded that mango and strawberry pulp-based smoothies, mango was best acceptable. This smoothie can be safely consumed till 12 days. Also, in coming years, smoothie market will grow owing to the changing food habits, busy schedule and shifting trend towards healthy grab and go meal alternatives.

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APPENDIX 1

Score Card for sensory evaluation of Smoothie

Kindly evaluate the given samples of Smoothie for sensory attributes, colour & appearance, consistency, sweetness, flavour, overall acceptability using the following 9-point hedonic scale and enter the score for each sample in the space provided.

Hedonic ratings	**Score**
Like Extremely	9
Like Very Much	8
Like Moderately	7
Like Slightly	6
Neither Like nor Dislike	5
Dislike Slightly	4
Dislike Moderately	3
Dislike Very Much	2
Dislike Extremely	1

Sensory attributes				
Colour & Appearance				
Consistency				
Sweetness				
Flavour				
Overall acceptability				

Remarks (If any) : ________________

Signature ___________________

VITA

Name of the Student	:	Kusum Lata
Father's Name	:	Jagdish Kumar
Mother's Name	:	Banti Devi
Nationality	:	Indian
Date of Birth	:	15.11.1992
Permanent Home Address	:	Vill. Sosan, Tehsil Sadar, Distt. Bilaspur, Himachal Pradesh, 174 032

EDUCATIONAL QUALIFICATION

Bachelor's Degree	:	B. Tech (Food Technology)
University	:	Shoolini University, Solan
Year of Award	:	2016
OCPA	:	7.40/10.00
Master's Degree	:	M. Tech.
OCPA	:	7.688/10.00