STUDIES ON USE OF PSYLLIUM HUSK AS A PREBIOTIC IN FOOD PRODUCTS

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B.Tech. (Food Science and Technology)

MASTER OF TECHNOLOGY

IN

FOOD TECHNOLOGY

DEPARTMENT OF FOOD MICROBIOLOGY AND SAFETY

COLLEGE OF FOOD TECHNOLOGY, PARBHANI

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PARBHANI - 431 402 (M.S.) INDIA

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STUDIES ON USE OF PSYLLIUM HUSK AS A PREBIOTIC IN FOOD PRODUCTS

By

HEMANTH M

B. Tech. (Food Science and Technology)

A thesis submitted to

Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani

in partial fulfillment of the requirements for the degree of

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IN

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DECLARATION BY THE CANDIDATE

I hereby declare that the thesis entitled **"STUDIES ON USE OF PSYLLIUM HUSK AS A PREBIOTIC IN FOOD PRODUCTS"** submitted by me is based on the actual work carried out by me under the guidance and supervision of **H. W. DESHPANDE** The extent of information derived from the exiting literature have been duly cited and referenced. The exiting research work or its any part is not submitted anywhere else for the award of any degree or diploma.

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ABBREVIATIONS USED

Abbreviation		Elaboration
Rpm	:	Rotations per minute
@	:	At the rate
%	:	per cent
CD	:	Critical Difference
Cm	:	Centimeter
SE	:	Standard Error
Mm	:	Milli meter
Mg	:	Milli gram
Gm	:	Gram
Kg	:	Kilogram
ºC	:	Degree Celsius
Psi	:	Pressure per square inch
et al.	:	et alibi (and associates)
Hr	:	Hour
i.e.	:	That is
Kcal	:	Kilo Calorie
No.	:	Number
Etc	:	Etcetera
CFU	:	Colony Forming Unit
SNF	:	Solids not fat
ND	:	Not detected
Min	:	Minute
Ml	:	Milliliter
Rs	:	Rupees
Sec	:	Second
cPs	:	Centipoise
N	:	Normality
W/W	:	Weight/weight
W/V	:	Weight/Volume

THESIS ABSTRACT

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Title of the thesis	:	STUDIES ON USE OF PSYLLIUM HUSK AS A PREBIOTIC IN FOOD PRODUCTS
Name of candidate	:	Hemanth M
Research Guide	:	H. W. Deshpande
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ABSTRACT

In the present study was carried out with Psyllium husk of native variety was treated with two different organic acids hydrochloric acid (HCL) and Sulphuric acid (H_2SO_4). Various concentrations (0.65%, 0.70% & 0.75%) of both HCL and H_2SO_4 was treated with ethanol as a solvent. Further these concentrations were applied to the psyllium husk-solvent ratios @ 1:2, 1:4 and 1:6 (W/V). The final results from the research showed that 0.75% HCL of concentration in ethanol solvent @ ratio 1:6 (W/V) was superior than the H_2SO_4 treated psyllium husk and the native psyllium husk with improved functional properties of psyllium husk was incorporated in the preparation of milkshake and smoothie. Organoleptic evaluation of the milkshake and smoothie with encapsulated psyllium husk beads with *L. acidophilus* and *L. bulgaricus* was performed and the results showed that encapsulated modified psyllium husk with 10 per cent probiotic culture having (10^9) cfu/gm had maximum score among all the samples as per the hedonic scale. The proximate composition of milkshake revealed that moisture content, total carbohydrate content, ash, total protein and crude fiber was 81.91percent, 8.21 percent, 0.92 percent, 3.89 percent, 2.73 percent and 2.25 percent respectively. Calculated energy value was found 79 Kcal/100ml. The viscosity of the selected milkshake sample M_2 was 0.56 Pa-S. The proximate composition of smoothie revealed that moisture content, total carbohydrate content, ash, total protein and crude fiber in was 77.2 percent,11.98 percent, 1.15 percent, 2.85 percent, 4.31 percent and 2.38 percent respectively. Calculated energy value was found 79 Kcal/100ml. The viscosity of the selected smoothie sample M_2 was 0.71 Pa-S. Microbial analysis of both the products were performed and the results show that that were stored under hygienic conditions and found to be safe for consumption.

Keywords: Psyllium husk, Acid modification, HCL, H_2SO_4, milkshake and smoothie

CHAPTER-I

INTRODUCTION

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INTRODUCTION

The name *Plantago* derived out of Latin, denotes sole of the foot and *Ovata* meaning the shape of leaves. Psyllium is a Greek term that means "flea" in reference to the shape, size and color of the seeds. Isabgol is taken from Persian and means "horse ear" because of the boat form of the *Plantago* seeds. The *Plantago* species are native to India and Pakistan, introduced as a medicinal herb for curing chronic dysentery and intestinal problems (Dhar *et al.,* 2005).

Isapgol is a rabi crop that requires well-drained loamy soil with a pH of 7.5 to 8.5 as well as chilly, dry weather. At maturation, heavy rains and overcast conditions have a negative impact on the yield and the crop is planted via disseminating, approximately 6 -12 kg seeds are required per hectare in november. A few of the species chosen for growth in Gujarat state are RI-87, RI-89, GI-1, GI-2, HI-1, HI-2, HI-5, and MIB-121. Irrigation occurs 7-8 times each year at 8-10 day intervals. At the moment, India produces 480,000 tonnes of seeds per year and global demand for psyllium seeds and husk is also expanding, with the main markets being the United States, France, Germany, and the United Kingdom. India maintains a monopoly in its manufacturing and trading on the global market (Anon, 2013). Given its high demand, this crop should be brought more into agricultural practise, and cultivation intensity should be increased. It has lately become a popular component among food product manufacturers, and as a result, it is now grown on more than 55,000 acres in Gujarat and Rajasthan and the exports are increasing at a rate of up to 14 percent per year, with the majority of growth occurring in India. India currently ranks top in terms of output and *Plantago Ovata* trades on the global market, earning a substantial foreign exchange profit (Anon, 2013).

The increase in demand for psyllium commodity has created urgency of need to develop high-yielding cultivars with superior husk quality and to investigate potential places for farming this crop in other Indian states. As a result, improvement in Isapgol for both quality and quantity of seeds and husks is critical for the country's economy. The Psyllium plants can grow up to 15 cm tall and are coated with fuzzy, white hair that grows at right angles to the stems and leaves. The leaves are basal, roughly linear, and green in color. The leaves produce spikes of miniature flowers, which develop into seedpods. Isapgol seeds are tiny (1.5-2 cm in diameter) and brown or reddish-brown in colour. This plant's seeds also contain a significant amount of mucilage as well as albuminous materials In order to process, the seed is crushed and dried inorder to perform winnowing (Sangan *et al.,* 1992).

The plant has been cultivated in India and Pakistan from century ago and traditional knowledge access is an asset easy to be explored. So, keeping in mind the wide industrial application as well as the increased market demands, appropriate method of cultivation and advance techniques are developed to enhance the yield and the quality of seeds and to get over the traditional process of milling the seeds. On the other hand, seeds may be used for preparation of high valued commercial products. It is desirable to explore the possibilities of intensify cultivation and yield of crop other than arable land which is not suitable for the cultivation of food crops. Moreover, it was concluded from the previous literature that isabgol seeds has the richest fiber contents consumed as natural source of fiber with various health benefits. This is the main cause of increasing interest of scientific community to do more research work in this direction in order to find newer ways to incorporate this fiber into dietary food products with health protective effects. The Psyllium addition in standard diet may support controlling those issues related to food deficient of soluble fiber and also *Plantago ovata* has potential role in industries for marketable product development and for benefit of a healthy society (Rehana *et al.*, 2015).

The Psyllium comes from the seeds of *Plantago* genus. The main component obtained from the seed is psyllium husk. The husk of the psyllium seed is made up of both soluble and insoluble fibres (Bijkerk *et al.*, 2004). The Psyllium has a molecular makeup of 75 percent xylose, 23 percent arabinose, traces of many other sugars, and about 35 percent minimal residues of non-reducing type. The polysaccharide is a highly branched acidic arabinoxylan with both (1→4) and – β (1→3) glycosidic linkages in the xylan backbone (Fischer *et al.,* 2004). And also, Psyllium can be used as a food additive to improve shelf life and consumer acceptance while also reducing stickiness due to its strong water-binding capability and stability at various PH levels and temperatures (Ibuki, 1989). Psyllium can be used as a fat and wheat flour alternative, as well as an emulsifier and stabiliser (Giuntini *et al.,* 2003 and Zandonadi *et al.,* 2010).

The *Plantago ovata* has been widely employed in the commercial manufacturing of seed husk, when soaked in water, a white thin membrane covering the seed has a higher mucilage content than husk. Because seeds are the most important portion of the plant, *Plantago ovata* has a long history of usage as a fibre supplement as it is a rich source of soluble fibre. Furthermore, psyllium husk has been used in Asia and Europe as an herbal treatment for chronic constipation since the 16th century (Abbas *et al.*, 1992).

Plantago ovata is currently in high demand on the market. So with rising market demand, cultivating this plant at the country level or globally is critical for a country's economic development. According to a scientific assessment, the United States is the leading importer of isabgol seeds and husk, consuming 8,000 metric tonnes per year, indicating the global market importance of this commodity. The natural dietary fibres appear to be the source of this growing interest and business and the crop also has a high export demand in the United States and Western Europe, with about 90% of production going to these nations. India is currently the world's largest producer and main supplier of psyllium husk and seed (Modi *et al.,* 1974).

Psyllium is found in a wide range of traditional foods. It has been incorporated water, usually mixed with sugar to make a refreshing drink. It is combined with fruit extracts such as coconut water, orange and prune juice and it is occasionally used in place of thin roots in a konjee, an Indian beverage. The Psyllium is used in the production of chocolate and jellies and its gum has the capacity to inhibit sugar crystallisation, hence used in various dairy products as a cream stabiliser. It also gives the ice cream a homogeneous, smooth, creamy structure that it keeps after the thawing process. Meer Corporation, which develops psyllium-based stabilisers marketed as Merecol IC, is one current application in this field. Psyllium is also utilised as dietary fibre in cereals (Thari *et al.,* 2019).

Microencapsulation is a process that involves packaging solid, liquid, or gaseous active substances within a second material in order to protect the active component from the surrounding environment. As a result, the active ingredient is referred to as the core material, while the surrounding material is referred to as the shell. This technology has been used in a wide range of industries, including chemicals and pharmaceuticals, as well as cosmetics and printing. As a result, there has been a surge in interest in microencapsulation technology (Dubey, 2009).

The beneficial colon microorganisms ferment non-digestible dietary compounds known as prebiotics and get their energy by breaking indigestible prebiotic bonds. The prebiotics are a type of nutrition that the gut bacteria degrades. In recent years, their association with human overall health has piqued people's interest. They have the ability to feed the gut bacteria, and their breakdown products are short-chain fatty acids that are delivered into the bloodstream, impacting not only the gastrointestinal tracts but also other distant organs (Bouhnik *et al.*, 2004).

Currently, the dairy business is involved in product development both actively and articulately. This includes new formulations and imitated products that are designed to compete with or replace existing products based on their superiority in terms of convenience, cost, and quality. The various sorts of links between milk products and the dairy processing industry characterise the dairy sector. The dairy processing business is dominated by a few large cooperatives that cater mostly to urban consumers, with unorganised small processors still capturing a large share of the market. According to India’s dairy report 46% of milk was sold in India in 2007. The structured sector processes about 13% of total milk produced in the country, whereas the unorganised sector processes 22% of total milk (MOFPI, 2006-2007).

The explosive growth of the dairy industry in many regions of the developing globe has been mostly driven by demand. The factors that have fuelled demand of growth in developing countries are rising incomes, urbanisation, and population increase, will continue to play a role in the future decades. The Population growth will continue, but at a slower pace and urbanization is thought to be unstoppable. The income growth is widely regarded as the most powerful driver of growing demand for milk products. The growing incomes will continue to underpin demand growth in the long run. The impact of economic expansion on dairy demand is determined by the pace of increase and the location of the growth. In low-income countries, demand responds more to income growth than in higher-income countries. Overall, the potential for increasing per capita consumption remains considerable in many regions of the developing world, as rising earnings translate into increased purchasing power, and growth in dairy consumption and production is projected to continue (FAO, 2006).

Various indigenous dairy products have traditionally been manufactured and consumed in significant amounts in the country. In current history, there has been a distinct trend in the intake of dairy-based goods. The scientific advancements have resulted in the exploitation of various benefits of milk and its products. With greater palatability and interesting forms with added fresh formulas, a variety of dairy products and milk-based products are entering the market. However, the manufacturing of various forms of milk beverages is rising rapidly in all regions of the world, and they are gaining in popularity by the day. Milk beverages can be flavoured with fruit flavour extracts, synthetic flavours that mimic natural fruit flavours, or by adding soft drinks, pulps, or pieces to increase their acceptance. To boost dairy intake and encourage the younger generation to drink milk, it has been regular practise to flavour the milk and then advertise it. The consumption of flavoured milk is constantly increasing since many young people dislike the taste of plain milk but readily accept it when it has been flavoured. (Taware, 2002).

Milk is a colloidal system made up of fat globules, lactose, proteins, vitamins, and minerals. Milk has been utilized as a primary source of nutrition throughout history and is a staple in Asian diets, providing a significant share of the RDA for nutrients such as protein, fat, vitamins, and minerals. As a result, milk is regarded as a vital source of calcium, fat, and protein. (Erzen *et al.,* 2014).

The milk-based beverages are becoming popular all across the world, particularly among indigenous who do not consume milk. Because of the variety of these beverages and their fortifying useful components, these new consumers prefer milk beverages. Milk continues to be a significant source of nutritional components as well as functional components with physiological effects. The future research on milk-based beverages will focus on understanding the mechanism of disease curative and preventative activity of functional milk components. These components will be commercially isolated, and other food uses such as integration or fortification in a variety of different food products and drinks will be investigated. Future milk beverage advances are likely to include brand extensions like flavoured milks with artificial sweeteners, milk beverages with natural fruit purees, and milk with bioactive components including phytosterols, peptides, and antioxidants. The milk ingredients can be mixed with a variety of traditional beverages as traditional beverage firms attempt to differentiate their products, allowing beverage companies to capitalise on the nutritional and functional benefits of milk (Mudgil and sheweta, 2019).

The milk shake, a cold beverage, prepared from milk and ice cream, is a palatable and perfect health–diet due to its low fat and sugar contents, and high milk solids-not-fat (MSNF) content than ice cream. It can be made more delicious and nutritious with addition of fruits as they are excellent source of phytochemicals which are essential for human health and relished by consumers in all season (Bakane *et al.,* 2016).

The milk shake that are commonly sold in the India subcontinent consist of sweetened cold milk added with colouring and flavouring agents without freezing but vigorously shaked. The most commonly used flavour blends are of rose, coffee and chocolate and they are sold by fruit juice centres in many parts of our country during the whole years and are liked mostly by consumers as they are palatable and nutritional. Several types of milk shakes are sold in the market i.e. mango milk shake, sapota milk shake, fig milk shake, banana milk shake, almond milk shake, custard apple milk shake and the method of manufacture of milk shake varies from region to region. The base for all this type of milk shake is however milk and cane sugar in different proportion and other specific ingredient are incorporated to cater and the different tastes of milk shakes occurs by wide variation in method of manufacture (Kuchekar *et al.,* 2019).

Apples are the world's second most consumed fruit after bananas, contain a variety of nutrients as well as non-nutrients such as dietary fibre, minerals, and vitamins. Furthermore, apples contain a high concentration of polyphenols, which are classified into several groups, including hydroxybenzoic acids, hydroxycinnamic acids and their derivatives, flavonols, dihydrochalcones, anthocyanids, monomeric flavanols, and oligomeric flavanols and also they have have shown therapeutic effects on the health against cancer, asthma and pulmonary dysfunction, cardiovascular illnesses, Alzheimer's disease, normal ageing decline, weight management, and diabetes due to their high nutraceutical qualities and different polyphenols (Boyer and liu, 2004).

For optimum health, one banana has 8.7 mg of vitamin C, 358 mg of potassium, and 2.6 mg of dietary fibre per 100 g. It also aids in protein metabolism, red blood cell production, and central nervous system activity. The bananas include vitamin C, which aids in healing and infection defence and they also have the most digestible carbs of any fruit. The benefit is that calories from carbohydrates are burned off more rapidly and easily than calories from protein or fat (Dhamsaniya and Varshney, 2013).

The smoothies are a great and practical way to include more fresh fruits and vegetables into your diet on a regular basis (Rodriguez-Verastegui *et al.,* 2015). They are non-alcoholic liquid refreshment drinks that are typically created from fresh or frozen fruits and vegetables. They are combined without straining and frequently served over crushed ice. The other components may include yoghurt, milk, ice cream, lemonade, tea, spices, and sauces in certain smoothies and they have a slightly thicker consistency than a slushie (Castillejo *et al.,* 2015).

The smoothies are split into fruit-only products, fruit-and-dairy products, and functional smoothies. The functional smoothies are a relatively new product on the market, usually incorporating probiotics among other ingredients. Fruit consumption is becoming more popular and vegetables is becoming more prominent than ever before.

This is due to consumers' thirst for new products that are less processed, healthy and nutritive and also smoothies are regarded as a healthy option to have one serving of fruit. Commercially, Smoothies on the market are high in calories and sugar and their fruit concentrations made from syrup are being varied. Instead of actual fruits, artificial fruits were used, resulting in a food with a high sugar content but minimal macronutrient content, Vitamin C, in particular. Smoothies aren't well-known among the general public, in fact they are nearly unknown and underrated (Banigo *et al.,* 2015).

Combining two or more fruits creates new flavours and tastes, which improves consumer acceptance. The blended beverages are currently available in a variety of flavours, including strawberry, chocolate, banana, vanilla, mango, raspberry, orange, and pineapple. The smoothies have a thick consistency and are typically consumed fresh or preserved for short periods of time (1-3 weeks) by storing in the refrigerator after pasteurisation or freezing. The pulsed electric field processing, when compared to mild thermal pasteurisation had low preservation effect of smoothie-type beverages (Walking *et al.,* 2010). The thermal and high hydrostatic pressure processing is done to preserve fruit smoothies containing apples, strawberries, bananas, and oranges in order to retain colour, polyphenols, and other quality attributes (Keenan and colleagues, 2011).

Therefore, the present investigation is undertaken with distinct and definite objects as follows.

1.1 OBJECTIVES

1.1.1 To study the physicochemical properties of psyllium husk

1.1.2 To standardize the process for the modification of psyllium husk

1.1.3 To study the physicochemical properties of prepared products i. e milkshake and smoothie

1.1.4 To study the microbial properties of prepared products

1.1.5 To study sensory characteristics of prepared products

1.1.6 To study textural (consistency) of prepared products

1.1.7 To access the techno-economical feasibility of prepared products

CHAPTER-II

REVIEW OF LITERATURE

CHAPTER-II

REVIEW OF LITERATURE

The proposed research work is investigated to develop two milk products milkshake and smoothie by using modified psyllium husk and have been made to review the pertinent literature in respect of technique standardization, sensory, chemical, nutritional and microbial quality, cost of production of milkshake and smoothie etc. and is presented in this chapter under the following heads.

2.1 Physico-chemical characteristics of psyllium husk

2.2 Modification of psyllium husk

2.3 Health benefits of psyllium husk

2.4 Microencapsulation of probiotics

2.5 Milk and dairy products

2.6 Chemical composition of milkshake and smoothie

2.7 Sensory properties of milkshake and smoothie

2.8 Textural properties of milkshake and smoothie

2.9 Health benefits of milkshake and smoothie

2.1 Physico-chemical characteristics of psyllium husk

Blackwood *et al.,* (2000) investigated the physiological responses of the heterogeneous mixture of psyllium substrates that were predictable on the premise of their physicochemical characteristics. The monosaccharide composition and regulations prescribed the influence rate and extent of fermentation. The Stool weight and gut transit-time were influenced by water holding capacity and the viscous polysaccharides caused delay in gastric emptying and slowed down transit through the small bowel, resulted in a slower rate of nutrient absorption. The polysaccharides with the large hydrophobic surface area played important roles in bile acid, carcinogen, and mutagen binding. Ispaghula had the capacity of binding bile acids over a higher number of less strengthened binding sites on polysaccharide structure and it also had good effect on the secondary bile acids that are harmful, lithocholic acid and deoxycholic acid.

Marlett and Fischer, (2005) studied extraction and fractionation of psyllium husk heteroxylan using alkaline and acid solutions. The highest yield was 57.5 percent for alkaline extracted gel and they also found differences in molecular composition.

Shin *et al.,* (2005) studied the bile acid binding capacity for the psyllium samples that was matched well with the order of swelling volume, indicating a potential link between swelling behavior and bile acid binding capability for hydroxypropylated psyllium samples. These findings also suggested that hydroxypropylation could alter its bile acid-binding capability and that the degree of substitution could be critical to its total bile acid-binding capacity. The bile acids binding to non-digestible polymers improved their elimination and promoted cholesterol conversion to bile acids, lowering plasma total and low density lipoprotein cholesterol levels and the risk of cardiovascular illness.

Jyothi *et al.,* (2007) the effects of hydroxypropylation on the physicochemical and biological properties of psyllium. It was reported that under alkaline conditions, hydroxypropylated psyllium derivatives were prepared using propylene oxide. FT-IR, hydroxypropyl content determination, and scanning electron microscopy were used to characterise the chemical structures of the hydroxypropylated psyllium derivatives and the results showed improved physic-chemical and biological properties.

Kshirsagar and Singhai, (2008) stated that hydroxypropylation is a widely used method for improving polysaccharide functionality. Many edible polysaccharides, including starch, chitin, and cellulose, have been hydroxypropylated to improve shelf life, freeze-thaw stability, cold water swelling, and reconstituting properties. Among starch ethers, hydroxypropyl starch with a low gelatinization property is particularly suitable for food applications.

Saghir *et al.,* (2009) studied a few psyllium derivatives that were prepared using chemical methods were investigated for their functionalities. Carboxymethylation was shown to improve the water soluble property of the arabinoxylan from psyllium and the ethylation was able to alter the intrinsic viscosity of the arabinoxylan derivatives.

Cheng *et al.,* (2009) demonstrated enzymatic and chemical approaches to be capable of altering the chemical and molecular structures of psyllium, potentially improving its physicochemical properties and biological activity. The availability of food grade enzymes and the overall cost limited enzymatic approaches, which are generally considered green because no organic solvents or chemicals were involved or generated. The chemical modification, on the other hand, was less expensive and may produce psyllium derivatives with more diverse chemical and molecular structures, making it an important approach for modifying the properties of natural biopolymers such as psyllium.

Yu *et al.,* (2008) studied the variety of physical, mechanical, enzymatic, and chemical processes to improve the physicochemical properties of psyllium and, as a result, to promote its use in food or other consumer products.

Van-Craeyveld and Courtin, (2009) collected more data on the extractability of psyllium husk polysaccharide, demonstrating that an extraction increase was more significant when the concentration was decreased rather than when the temperature was raised, with effects on gel structure.

Guo *et al.,* (2009) investigated the structure and rheology of gels made from alkaline extracted psyllium husk heteroxylans, as well as the effect of Ca2+. They discovered that adding Ca2+ changed the gel micro-structure from fibers to aggregate particles, increased elastic modulus and critical strain in a specific range of added levels and improved thermal stability.

Liu *et al.,* (2010) claimed that sulfation has reduced the gelling rate of psyllium husk while increasing its bile acid binding capacity. These studies have showed the chance of improving psyllium husk functional properties through chemical modifications.

Farahnaky *et al.,* (2010) reported the effects of temperature, concentration, and PH on gel properties. They discovered that freeze-dried psyllium gel has a lath sheet-like structure, had higher concentrations, resistance to heat treatments, and increased pH increased structure stability, had ordered structure and decreased pore size distribution respectively.

2.2 Modification of psyllium husk

Yu and Perret, (2003) studied the effects of a commercial food-grade xylanase on psyllium's physicochemical qualities. The enzymatic procedures were carried out in solid form at room temperature. The water-uptake properties, gelling capabilities, soluble and insoluble fibre contents, and surface structures of enzyme-treated psyllium preparations were studied and compared to the beginning psyllium. The solid-state xylanase application considerably reduced psyllium's water-uptake and gelling capacities (p 0.01), with a modest decrease in soluble fibre content but no effect on insoluble fibre content. The xylanase technique also resulted in smoother psyllium particle surface structure. Furthermore, no additional equipment or operation were necessary to carry out the enzymatic reaction, which produced no waste. These findings suggested that the employment of solid-state xylanase reactions may be used to improve the physicochemical properties of psyllium in order to promote the consumption of psyllium fibre in functional foodstuffs for the promotion of human health.

Yu *et al.,* (2003) investigated about psyllium husks that were treated with a commercial food-grade polysaccharidase combination to increase water-absorbing and gelling capabilities under solid-state reaction conditions. The water-absorbing ability, gelling capabilities, fibre contents, and surface structures of the changed psyllium preparations were compared to the original psyllium and the control, which was treated with no enzyme under the same reaction circumstances. The water-absorbing ability of the modified psyllium was tested using a gravimetric approach, and the gelling property was measured using a texture analyzer. The results demonstrated that the solid-state enzymatic alteration drastically reduced psyllium's water-absorbing and gelling capacities. When compared to the control, the unique psyllium preparation treated with the enzyme mixture at a concentration of 36 units/g psyllium showed reductions in water-absorbing ability, 71 percent in gel hardness, and 35% in gel adhesiveness. Scanning electron microscopy was used to analyse the surface structure of the transformed psyllium (SEM). The SEM data revealed that the enzymatic alteration reduced the total surface area. This may contribute to the mutated psyllium's decreased water-absorbing capabilities. This work proved the feasibility of producing innovative psyllium preparations for commercial food applications utilising a solid-state enzymatic technique.

Allen *et al.,* (2004) evaluated the hypolipidemic effects of two solid-state enzymatically modified psyllium preparations that were compared to the original psyllium husks. The libitum and hamsters were fed 0.2 weight percent cholesterol diets containing 12 percent cellulose or 5 percent cellulose + 7 percent raw or enzymatically processed psyllium preparations. The psyllium additions to the meal had no effect on food consumption or weekly mean hamster weight throughout the course of 5 weeks of feeding. However, the overall mass acquired during 35 days of feeding of altered psyllium Y-26-4, one of the modified psyllium preparations, was significantly lower, 48, 47, and 32 percent, respectively, than that of cellulose, raw psyllium, and modified psyllium Y-24-3 groups. Psyllium feeding substantially decreased plasma total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol by 50-100% compared to cellulose feeding at 35 days, with no major differences across the psyllium preparations. Dietary therapy had no effect on faecal dry weight. At day 2931, all three psyllium diets significantly increased faecal bile acid excretion by 30-70%, with no significant variations between psyllium preparations. These findings imply that enhancing the functional characteristics of psyllium using solid-state enzymatic methods, allowing for its absorption into food products, has no influence on the psyllium-mediated hypolipidemic effects.

Pei, (2008) stated psyllium, a form of dietary fibre has been found to provide a variety of health advantages, including cholesterol reduction, hypoglycemia prevention, cancer prevention, and laxative effects. However, due to its extraordinarily strong water-holding and gel-forming properties, it is difficult to incorporate psyllium into food products in the required amount per serving for health claims. The effect of acid treatment on the water uptake, swelling, gelling, and bile acid binding capabilities of psyllium samples was investigated in this study. The acid treatments were carried out at various reaction temperatures (25, 37.5, and 50 °C) and with various psyllium – solvent ratios (1:2.5, 1:5, 1:7.5, and 1:10 g/mL). The results showed that reaction temperature had a substantial influence on the effectiveness of acid treatment on the physical/chemical properties of psyllium samples, although the effects of varied psyllium-solvent ratios were not. This suggested that acid treatment at high temperatures could be a viable strategy for improving the physical/chemical properties of psyllium for integration into food products.

Cheng *et al.*, (2009) investigated about psyllium husks treated with varying doses of HCl in ethanol at room temperature to increase gelling and water uptake. The gelling characteristics, water uptake capacities, swelling volumes, and bile acid-binding capacities of acid-treated psyllium samples were investigated and compared to the control, which was exposed to the same method without acid treatment. The results demonstrated that 0.36–1.44 percent HCl treatment in ethanol can greatly reduce psyllium gelling, water absorption, and swelling capacities while not affecting bile acid binding abilities. SEM examination revealed that HCl treatment may lower the surface area of psyllium particles, which may contribute to their reduced water uptake capability. According to the findings of this investigation, a low concentration acid treatment in ethanol have improved psyllium functionality.

Bhatia and Ahuja, (2013) studied thiol modification in psyllium husk. Furthermore, to improve its mucoadhesive properties, psyllium was thiol functionalized. The psyllium was thiolated via esterification with thioglycolic acid. The thiolation was found to modify the morphology of psyllium's surface from fibrous to granular, resulting in a modest increase in crystallinity and swelling. The thiolated psyllium was discovered to have 3.282 m moles of thiol groups per gramme of polymer. The mucoadhesive uses of thiolated psylium were investigated by creating gels with metronidazole as the model medication. In a comparison study, thiolated psyllium gels demonstrated 3-fold stronger mucoadhesive strength than psyllium gels, as measured by modified physical balancing utilizing a chicken buccal pouch. The results of an in vitro release research demonstrated that thiolated psyllium gels offered sustained metronidazole release. Furthermore, the psyllium and thiolated psyllium gels were discovered to release the drug using first-order kinetics via a combination of polymer relaxation and diffusion through the matrix.

2.3 Health benefits of psyllium husk

McRorie *et al.,* **(**1998) claimed that psyllium is a laxative that forms bulk. It works by increasing the size of the stool and assisting with constipation relief. Initially, it works by binding to partially digested food as it passes from the stomach into the small intestine. It then aids in the absorption of water, increasing the size and moisture of stools and resulting in larger and easier-to-pass stools.

Wei *et al.,* (2008) reported psyllium clings to fat and bile acids, assisting the body in excreting them. To replace such lost bile acids, the liver uses cholesterol to make more. As a response, blood cholesterol levels fall and furthermore, psyllium can help boost HDL (“good”) cholesterol levels.

Warnberg *et al.,* (2009) stated that psyllium can help with diarrhea. It accomplishes this by acting as a water absorbent and by thickening the faeces and impeding its travel through the colon. Furthermore, Psyllium can assist to regular bowel motions by preventing constipation and reducing diarrhea.

Jovanovski *et al.,* (2018) stated that fiber of any kind can be beneficial to the heart and presence of it in the diet significantly improves cholesterol and reduce the risk of stroke disease, stroke, type 2 diabetes, and obesity. The psyllium and other water soluble fibres may help lower blood lipids, blood pressure, and the risk of heart attack. Furthermore, Psyllium has the potential to enhance heart health parameters such as LDL cholesterol. This may help to lower the risk of heart disease.

Mcrae, (2018) reported that fiber supplements can help manage the body's glycemic response to a meal by lowering insulin and blood sugar levels. This is especially true for water-soluble fibres like psyllium.

Noureddin *et al.,* (2018) has stated that Psyllium and other viscous fibres can help reduce hunger and aid in weight loss. Psyllium may help with appetite control by reducing stomach emptying and decreasing hunger and morover, reduced appetite and less calorie intake aids in weight loss.

2.4 Microencapsulation of probiotics

De vos, (2006) stated that polymers used in microcapsules must meet a variety of criteria, including the ability to be flexible, soft, mechanically stable, allow diffusion of molecules and be highly biocompatible to limit host immunological reactions. The polymers used are taken from either synthetic or natural sources. Polyethylene glycol, polyvinyl alcohol, polyurethane, polyethersulfone, polypropylene, sodium polystyrene sulphate, polyacrylate, agarose, chitosan, cellulose, collagen, xanthan, and alginate are the most often used sources, according to a recent research.

Chandramouli, (2004) reported that Calcium alginate microencapsulation is influenced by a number of parameters, including capsule size, alginate content, probiotic cell load, and hardening time in calcium chloride. Despite using the same dosage of alginate in the current investigation, microspheres containing quercetin required a larger microsphere size for coencapsulation of a probiotic and a prebiotic. Furthermore, different types of beads had different microsphere sizes because they contained a variable amount of probiotic.

Mandal *et al.,* (2006) Studied microencapsulated and free probiotic bacteria and tested their stability in simulated gastric fluid Encapsulation in chitosan-coated alginate microspheres increased bifidobacteria and lactobacilli survival considerably (pb0.05). Cell survival after 5 minutes of SGJ exposure was 95%, 94%, 78%, and 66% of the starting population in chitosan-coated alginate microspheres with *B. bifidum, L. gasseri, free L. gasseri*, and free *B. bifidum*, respectively. Microencapsulated *B. bifidum* and *L. gasseri* were both resistant to simulated stomach conditions After 120 minutes, more than 107 CFU/mL *B. bifidum* capsules and *L. gasseri* capsules survived, indicating that the *B. bifidum* capsule reduced by less than one logarithm unit and the *L. gasseri* capsule decreased by slightly more than one logarithm unit. It is believed that 10^7 CFU/mL of live probiotic cells are required to provide the consumer with health benefits.

2.5 Milk and dairy products

Wiley *et al.*, (2011) stated that milk and dairy products are the most common items in the diets of all demographics. The unique features and components of milk, as well as the ability of making a wide variety of dishes from this substance, are the reasons for their popularity. The milk products are functional foods because they include biologically active compounds with health-promoting qualities. Indeed, dairy has progressed from its traditional food/beverage position to that of a delivery method for health-promoting nutrients that appear to hold the key to a number of illnesses. Several factors, including feed source and breed, have been demonstrated to influence milk fat-soluble antioxidants. Among functional foods, dairy products, particularly ice cream and cheese, are excellent vehicles for delivering probiotics to the human digestive tract. Furthermore, milk products such as milkshakes, smoothies, and classic milk drinks serve to satisfy satiety while also giving necessary nutrients to the human body. Dairy products are medicinal and their health-care properties have been employed for many years and they also have a significant function in human health and are an essential component of the dietary pyramid.

Rozenberg *et al.,* (2016) claimed that dairy products provide a bundle of vital nutrients that are difficult to obtain in low-dairy or dairy-free diets, and for many people, a dairy-free diet makes it impossible to meet recommended daily calcium requirements. Despite the established benefits for bone health, some people avoid dairy in their diet because they believe it is harmful to their health, particularly those who have weight management issues, lactose intolerance, osteoarthritis, rheumatoid arthritis, or are attempting to avoid cardiovascular disease. This examined the information provided for health professionals so that they may assist their patients in making informed decisions about consuming dairy products as part of a balanced diet.

2.6 Chemical composition of milkshake and smoothie

Mule *et al.,* (2014) studied the proximate study of the fig milkshakes and showed that it contained considerably ($P \leq 0.05$) higher fat, protein, total sugar, and ash percent than the buffalo milkshake (control). The evaluation of the treated samples showed that the milkshake with 10 percent fig had considerably ($P \leq 0.05$) greater protein (4.52%) and sugar content (12.78%) than other two samples (5 percent fig and 7.5 percent fig). The overall acceptability score with 8.3 was the highest in the sample with 7.5% fig, but it didn’t differ ($P \geq 0.05$) from the other sample with 5 percent fig.

Usunobun and Egharebv, (2014) reported that increase in carbohydrate content of the smoothies may be attributed to an increasing percentage of banana pineapple addition. This fact could also explains that the energy content of the samples will be increased as the addition of banana and pineapple pulp increases. The high fibre content of the smoothies, ranging from 6.02 to 7.57 percent, indicates their ability to support good bowel movement. However, the crude fibre value obtained from this study is lower than the value mentioned in previous study. This, however, was unaffected by the incorporation of pumpkin leaves. The addition of pumpkin leaves accounted for the higher total ash content of pumpkin leaves supplemented smoothies.

Aderinola *et al.,* (2018) revealed the effect of *Moringa Oleifer*a leaf supplementation on the chemical composition of smoothies made from pineapple, apple and banana blends. The protein content of the control smoothie sample was compared to the protein content of the previous study, but the protein content of the supplemented smoothie samples was considerably higher. The fat content of sample varied from 0.72 percent to 1.86 percent, while its ash content varied from 1.01 percent to 9.71 percent. The apparent higher carbohydrate contents obtained for all supplemented samples when compared to the control smoothie sample was due to lower water content in these samples.

Shinde *et al.,* (2018) studied on the "physicochemical analysis of milkshake mixed with date pulp (khajur)". The goal of the study was to create milkshakes enhanced with date pulp in various concentrations using whole milk. On the basis of physicochemical analysis of milkshake, milkshake prepared with 7.5 percent date pulp was found to be the best treated beverage. The best tasting flavored milkshake was made by combining date pulp with 7.5 percent skim milk of buffalo, and it contained total solids, moisture content, protein, fat, ash, and titratable acidity with 29.27, 17.67, 3.67, 3.79, 1.70 and 0.30 percent, respectively.

Pandey *et al.,* (2020) investigated butter fruit milkshake formulation that was made by combining avocado pulp, maltodextrin, pasteurized milk and sugar in the following proportions: 84%, 10%, 28%, and 6%, respectively. The incorporation of milk and maltodextrin increased fat, protein, and ash contents while preserving the flavanoid, phenolic, and â-carotene contents of the avocado fruit pulp.

Uzodinma *et al.,* (2020) claimed smoothies made from various ratios of watermelon, pine apple and banana pulps blended with coconut milk constituted varying amounts of carbohydrate, fat, protein, ash, fiber, and moisture. Pasteurization, on the other hand, had no effect on the values of the proximate parameters ($p > 0.05$).

2.7 Sensory properties of milkshake and smoothie

Keenan *et al.,* (2012) claimed that the limonene content of smoothies was affected by processing, with thermally processed samples being found to be greater ($p<0.05$) than all of the other samples. The storage reduced the volatile content of all the smoothies, especially volatiles associated with ‘fresh/green/grassy' flavour notes, such as trans-2-hexanal. On day 1, principal component analysis (PCA) of descriptive sensory data revealed that pink color, apple aroma and flavour were much more related with both raw and processed smoothies. The physicochemical analysis revealed that both processing and storage had an effect on colour (p 0.05), but the changes were not consistent.

Morell *et al.,* (2014) investigated the sheds light on the factors influencing in mouth perspective of various hydrocolloids being used to design foods with improved satiety. Fortunately, sensory analysis revealed that the modified starch milkshakes achieved the highest expected satiety scores, with consumers finding them homogeneous, thick in the mouth, and very creamy. These findings suggested that consumers associated satiety with the creamy and thick characteristics at the start of intake rather than the full ingestion.

Hernandez *et al.,* (2015) evaluated the sensory attributes of persimmon milkshakes with enriched functional characteristics, as well as their liking, were investigated, and the results were related to consumer health and taste attitudes. According to the findings, customers perceived persimmon milkshakes to be a high anti-oxidant liquid beverage. Milkshakes made with HHP treated persimmon, irrespective of milk type, and even with untreated persimmon and whole milk received the highest overall rating. So, despite the seasonality of the fruit, treating persimmon with HHP allows for the creation of persimmon milkshakes with highly nutritious values and acceptability.

More *et al.,* (2017) evaluated the implementation of aloevera into custard apple milk shake and reported the impact of aloevera on sensory properties of prepared aloevera added custard apple milk shake. Furthermore, the (T2) milkshake sample prepared with 12% custard apple pulp, 4% aloevera pulp and 10% sugar received the highest score (7.96) and was deemed the most accepted of the treated samples.

Ribeiro *et al.,* (2018) investigated smoothie with strawberry, banana and jucara was prepared with a varied mixtural ratios. The banana and strawberry fruit pulps ratio was varied and banana pulp proportion was kept constant. From the five formulations, sample with highest percentage of strawberry pulp was rejected due to its higher acidity and lower sugar content. Moreover, the panelists decided to choose sweet formulations and went for the smoothie with highest percentage of banana. Related to consistency, same proportion of banana and strawberry pulp had better textural properties and provided better flow to the product.

Dilruksh *et al.,* (2021) evaluated the sensory attributes of Color, odour, appearance, texture, taste, and overall acceptability for the developed instant green smoothie. When considering the color, odor, appearance and overall acceptability, the actual likeness of the instant green smoothie sample was at the 75 percent. According to these findings, texture and taste was improved by the addition of pinch of salt with sugar or honey to taste while dissolving the instant powder in cold water and thickening agent was added to improve the score of texture.

2.8 Textural properties of milkshake and smoothie

Germain *et al.,* (2006) stated thickened milkshakes have long been utilised in the treatment of dysphagia to liquid in the clinic. The goal of this study was to look at the rheology of therapeutically effective cold thick beverages and see how they compared to the consistency groups. The consistency coefficient, flow behavior index, apparent viscosity and yield stress were calculated as rheological parameters. With yield stress, all of the beverages examined showed shear-thinning pseudoplastic flow behavior and were best suited to the Herschel–Bulkley method. The correlation values for apparent viscosity and bostwick consistency values were slightly higher (0.73–0.83). The yield stress, apparent viscosity and consistency coefficient values were found to be different at each Bostwick consistency level (p 0.05).

Camargo *et al.,* (2010) evaluated the project to determine the rheological properties of assai and passion fruit smoothies supplemented with unripe banana pulp. A Brookfield programmable rheometer, model DVIII, was used to determine rheological properties and viscosity. The viscosity evaluation as a function of temperature fits the Arrhenius model perfectly, and the parameters 0 and Ea was calculated. The rheological properties of an assai and passion fruit smoothie infused with banana pulp (unripe) followed the Herschel Bulkley model (HB), and it was a thixotropic fluid.

Keenan *et al.,* (2012) studied the use of power ultrasound as a non-thermal processing technique for fruit smoothies was investigated. The smoothies were sonicated at various amplitude levels (24.4–61.0 m) for different processing times (3–10 min). Total antioxidant capacity (TAC), particle size, and rheological properties were all evaluated. TAC (P0.001) and TP (P0.001) reductions were greatest at the maximum amplitude level (61.0 m). When compared to fresh and thermal smoothies, sonication reduced rheological parameters and shortened particle size.

Baiano *et al.,* (2012) evaluated the effects of cooking temperatures (45, 80, and 100 °C) and the inclusion of seed particles on the dynamic and mechanical properties of value-added Crimson seedless and Baresana grape-based smoothies. The increase in temperature caused an increase in the viscoelastic behaviour of Black Pearl and a slight decrease in the viscoelastic behaviour of Baresana grape-based smoothies. Smoothies made from Crimson grapes at an intermediate temperature (80 °C) had rheological properties comparable to those made at 45 °C and good than those made at 100 °C.

Hernandez-Carrion, *et al.,* (2015) stated milkshakes contain hydrocolloids, which give them viscosity and a high satiating capacity. The satiating capability of a large list of soluble gums that are viscous in solution has been studied extensively. Most of these types of compounds that impart viscosity to their solutions had an effect on the feeling of satiety that is caused by mechanisms that are related to slowing down enzyme action efficacy and gastric antrum distension (as they absorb large amounts of liquid) and/or delaying gastric emptying, which, in turn, may increase or prolong satiety signals from the stomach.

Dogaru *et al.,* (2014) classified two berry milk-based beverages in terms of rheological behaviour. The milk, 10% berry mixture (blueberry, raspberry and blackberry), and 2% sugar and honey, were used in the preparation of these beverages. Milk and combinations of sugar-milk and honey-milk were described rheologically in the temperature range of 5- 25°C. At all temperatures tested, these products exhibit non-newtonian behaviour patterns (pseudoplastic). The Oswald model was the best model for analysing experimental dependencies. The flow behaviour index (n) values for both berry milkshakes were less than one, indicating that all of these fluids are pseudoplastic. There were no changes in the rheological properties of the fluids studied at any temperature in the 5-25°C range.

Hemavathy and Anitha, (2014) studied bakery products and milk shakes that were chosen to be fortified with soy milk. These products are widely available and reasonably priced. The apple, grapes, and pomegranate were chosen as fruit varieties because they pair well with soymilk blends and promote good texture, taste, and flavour without curdling. The soymilk added to milkshakes improved their overall texture and consistency.

Milanovic *et al.,* (2014) investigated the purpose to look at the effect of fat content and kombucha inoculum concentration on the textural properties of milk beverages such as firmness, cohesiveness, consistency and viscosity index since production. The activity of the gel formed during milk fermentation was influenced by a variety of factors, including milk composition, starter culture, flavourings addition, and so on. The firmness, consistency, cohesiveness, and viscosity index of the beverage were all affected by its higher fat content.

Ribeiro *et al.,* (2018) evaluated the rheological characteristics of a juçara, banana and strawberry smoothie. The product was obtained by mixing the pulps of these fruits in previously defined proportions. The mixture was standardised in pilot disintegrator before being homogenised at 60 MPa in continuous mode and pasteurised for 35 seconds at 90 °C. Pasteurization also had an effect on the instrumental colour of the smoothie, as measured by the Hue angle ($p<0.05$). In terms of rheological behaviour, the smoothie demonstrated non-Newtonian fluid behaviour with pseudoplastic characteristics throughout all processing steps ($n<1$).

Rozhnov *et al.,* (2020) investigated the possibility of using amylolytic enzyme termamil 2XL to obtain the stable rheological properties of pumpkin-sea buckthorn smoothie, which together provided the drink with a stabilised texture. It was demonstrated that using coarse pumpkin pulp does not allow for the desired result in terms of stable rheological properties, because smoothing separation occurs almost immediately after preparation. The possibility of using enzymatic hydrolysis of pumpkin starch, which resulted in a stable framework due to starch granules in tissues of cells and better rheological behaviour of fruit smoothies has been established.

Mudgil *et al.,* (2020) investigated the response surface approach of variables such as sugar, corn flour, and almond paste on the viscosity of almond milkshake sample. To get the better levels of variables, the central composite design was adopted. The viscosity values recorded from various experiment runs ranged from 170 to 1085cps. The R2 (co-efficient determination) of the second-order polynomial model given by design software for viscosity of almond milkshake was 0.9871. The model F-values for almond shake viscosity was 84.9.

2.9 Health benefits of milkshake and smoothie

Baker *et al.,* (2009) claimed that milkshakes contain table sugar, which can readily give us with energy for our brains to function properly. The fruits, that are wonderful source of vitamins and dietary fibre for better digestion, can be added to milkshakes. Most fruits, such as strawberries, mangoes, and citrus fruits, are high in vitamin C, which aids in disease prevention. It also helps to strengthen our immune system.

Dreher, (2018) stated that fiber is an essential food that benefits our digestive, excretory, and even cardiovascular systems. The milkshakes include fibres that aid in digestion by acting as a cleanser for toxins and other undesirable lipids that clog our digestive tracts. With less fat in our bodies, our cardiovascular system will undoubtedly have a healthier blood flow and a more stable heart. The toxins and other leftovers will be washed away, allowing our excretory system to work properly.

Soares *et al.*, (2012) claimed an appropriate intake of milkshake enhances bone mineral density throughout skeletal growth and protects the elderly from bone loss and osteoporotic fractures. Dairy calcium was 50-100 percent more effective than supplementary calcium, according to clinical investigations. The calcium and other minerals in milkshake, whether full-fat or reduced-fat, lower body fat formation and speed up weight and fat loss during dieting.

Nowicka *et al.*, (2016) stated that smoothies are high in fibre since they contain a lot of fruits and vegetables. This can help bridge the gap between your regular fibre intake and the USDA's recommended fibre intake, lowering your risk of chronic illnesses and improving your general health.

Tkacz *et al.*, (2021) smoothies are reliable detoxifying drinks and the carotenoids present in them are highly beneficial for skin and complexion. The smoothies with certain fruits and vegetables also increase brain power and boost memory. They also help in weight loss and the beta-carotene in them boosts your immune system.

CHAPTER-III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODOLOGY

The present investigation entitled "Studies on exploration of modified psyllium husk in the formulation of milkshake and smoothie" was conducted in the Department of Food Microbiology and Safety, College of Food Technology, VNMKV, Parbhani.

During the present research, different physicochemical, nutritional, sensory characteristics of the psyllium husk, prepared probiotic milkshake and probiotic smoothie were studied. The materials used and methods adopted in analysis are summarized as following.

3.1 Materials

3.1.1 Psyllium husk

Psyllium husk is procured from the local market.

3.1.2 Milk

Fresh cow milk was procured from the local market.

3.1.3. Sugar

Good quality white crystalline food grade cane sugar was procured from super store of local market, for the production of milkshake and smoothie.

3.1.4 Honey

Honey was purchased from the local market.

3.1.5 Bananas and apples

Bananas for the preparation of smoothie and apples for the preparation of milkshake were purchased from local fruit vendors.

3.1.6 Packaging material

The glass bottles for storage of milkshake and smoothie will be purchased from the local market.

3.1.7 Chemical

All the chemicals used in this investigation will be of analytical grade were procured from Department of Food Microbiology and Safety and Department of Food Chemistry and Nutrition, College of Food Technology, V.N.M.K.V, Parbhani.

3.1.8 Processing and analytical equipments

The processing and analytical equipments included an electronic weighing balance with the accuracy of 0.0001g for weight measurements, autoclave, BOD incubator, pH meter, hot air oven, muffle furnace, soxhlet apparatus, micro kjeldhal assembly, glass wares. All these were obtained from College of Food Technology, V.N.M.K.V. Parbhani.

3.1.9 Milko testometer (milky lab)

The parameter of milk like lactose, protein, fat, freezing point, PH and SNF of buffalo milk were determined by using milko testometer (milky lab).

3.2 Methodology

3.2.1 Starter culture

The probiotic organisms viz. *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* were individually grown in MRS broth at 37°C for 48 hrs. The cultivated MRS broth was then centrifuged at 4,000 rpm for 10 min to harvest the cells. The harvested cells were washed twice with sterile water. The biomass was taken as starter culture.

Flow Sheet 1: Preparation of starter culture

Stock culture

↓

Activation of bacterial strains in MRS broth separately at 37°C for 48 hrs

↓

MRS broth containing desired strains

↓

Centrifugation at 4000rpm/10min

↓

Biomass (Starter culture)

3.2.2 Encapsulation of probiotics

The microencapsulation of probiotic bacteria was performed using the extrusion technique. Extrusion method is the oldest and most common procedure of producing hydrocolloid capsules (King, 1995). It is a simple and cheap method with gentle operations which makes cell injuries minimal and causes relatively high viability of probiotic cells. Biocompatibility and flexibility are some of the other specifications of this method (Klien *et al.,* 1983; Tanaka *et al.,* 1984).

Hydrocolloid solution was prepared by using sodium alginate at 1 per cent (w/v) for preparation of 100ml probiotic milkshake and probiotic smoothie, 10 ml of inoculum (5ml each of *L. acidophilus* and *L. bulgaricus*) was mixed in 2 gm of modified

Plate 1: psyllium plant

Plate 2: Raw native psyllium husk

Plate 3: Acid modified psyllium husk

psyllium husk powder. Probiotic culture and modified psyllium husk powder normal mixed properly and passed through a syringe in the form of droplets into 0. 3M calcium chloride solution. Interaction between the two solutions led to formations of beads (2-5mm) and the resulting beads were then stored in 0.1 per cent peptone (Karthikeyan *et al.,* 2014).

Flow sheet 2: Microencapsulation of Strains

Preparation of polymer solution

↓

Addition of probiotic culture and modified psyllium husk powder

↓

Extrusion of the cell- polymer solution into calcium chloride solution

↓

Capsule formation by cross linking

↓

Recovery of capsule and storage in 0.1 percent peptone solution at 4°C

3.2.3 Quality characteristics of psyllium husk

Determination of Quality parameters and technical specification of psyllium husk was carried out standard for isabgol husk given by Bureau of Indian Standards.

3.2.3.1 Proximate composition

Proximate composition such as moisture, fat, crude protein, ash and crude fibre were determined as per AOAC 2005 and carbohydrate by difference method.

1. Determination of moisture

The moisture was estimated by weighing accurately 5 g of sample which was, ground to pass through 60 mesh size and subjected to oven drying at 105°C for 4 hrs. It was again weighed after cooling in desiccators and the procedure was repeated until get constant weight. The resultant loss in weight was calculated as moisture content.

$$\text{Moisture\%} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight of sample}} \times 100$$

2. Determination of Fat

The 5g ground de-moisturized sample weighed accurately in thimble and defatted with petroleum ether in Soxhlet apparatus for 6-8 hrs at 600C. The resultant ether extract was evaporated and lipid content was calculated (AOAC, 2005).

$$\text{Fat (\%)} = \frac{\text{Final weight of flask-Empty weight of flask}}{\text{Weight of sample}} \times 100$$

3. Determination of protein

The Protein content was determined by Micro-Kjeldhal method with 0.2g of ground defatted sample by digesting the sample in digestion tube with H_2SO_4 in presence of digestion mixture for 3-4 hrs till the contents of digestion flask get transparent colour. The samples were then diluted with distilled water up to 50 ml in a volumetric flask. The ammonia from the samples was liberated through distillation after adding 40% NaOH solution and collected in flask containing 4 % boric acid solution using methyl red as an indicator. The nitrogen content in the samples was determined by titrating against standard 0.1 N H_2SO_4 solution and the crude protein percentage was calculated by using formula.

$$\text{Nitrogen (\%)} = \frac{\text{(Sample- blank) x N of } H_2SO_4 \text{ x 0.014 x DF}}{\text{Aliquote x Weight of sample taken}} \times 100$$

$$\text{\% Protein} = \text{Nitrogen \%} \times 6.25$$

4. Total carbohydrate

Total carbohydrates were calculated by difference method as follows. Cabohydrate = 100 − % (Moisture + Fat + Protein + Ash + Crude fibre).

5. Total ash

Total ash was determined according to A.O.A.C. (2005). Sample (5g) was weighed into a crucible and ignited at low flame till all the material was completely become smokeless. Then it was kept in muffle furnace for 5 hrs at 550◦C and further cooled in desiccators and weighed. This was repeated till two consecutive weights were constant and percentage ash was calculated. The per cent ash was calculated by knowing the difference between the initial and final weight.

$$\text{Ash (\%)} = \frac{\text{Weight before heating – Weight after heating}}{\text{Weight of sample}} \times 100$$

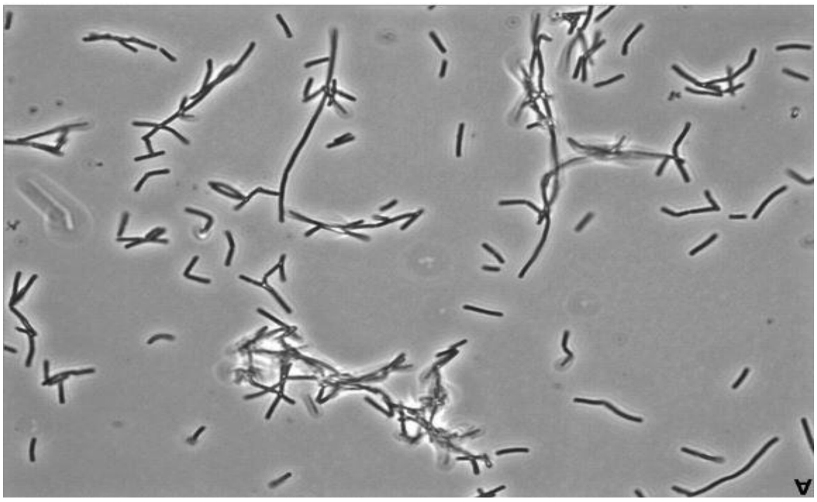

Plate 4: ***Lactobacillus acidophilus*** **under fluorescent contrast microscope**

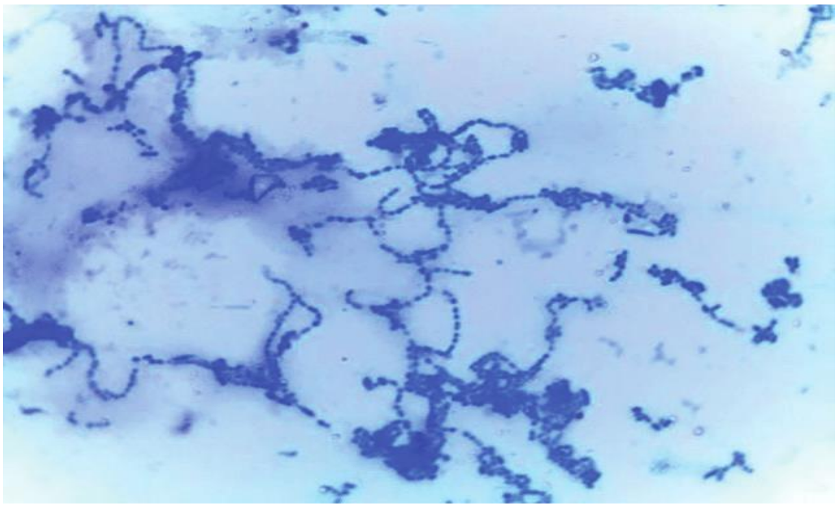

Plate 5: ***Lactobacillus bulgaricus*** **under fluorescent Contrast microscope**

Plate 6: Prepared Encapsulated modified psyllium husk beads with ***Lactobacillus acidophilus and Lactobacillus bulgaricus***

6. Estimation of crude fibre

About 2 to 5 g of moisture and fat free samples were weighed into 500 ml beaker and 200 ml boiling 0.255 N H2SO4 was added. The mixture was boiled for 30 minutes keeping the volume constant by addition of water at frequent intervals. At the end of this period, the mixture was filtered through a filter paper and the residue washed with hot water till free from acid. The material then transferred to the same beaker and 200 ml of boiling 0.313 N NaOH solution added. After boiling for 30 minutes the mixture was filtered through filter paper. The residue was washed with hot water till free from alkali followed with some alcohol. It was then transferred to crucible. The crucible was heated in a muffle furnace at 550-6000C for 4 hours cooled and weighed again. The difference in the weights represented the weight of the crude fibre.

$$\text{Crude fiber (\%)} = \frac{\text{Weight of residue} - \text{Weight of ash}}{\text{Weight of sample}} \times 100$$

7. Mineral analysis

Mineral contents like calcium, magnesium, iron and zinc etc. of raw ingredients were measured by using titration and spectrophotometric method (Atomic absorption spectrophotometer) (Ranganna, 1986).

7.1 Mineral solution preparation

The obtained ash moistened with a small amount of glass distilled water (0.5-1.0 ml) and 5 ml of distilled hydrochloric acid was added to it. The mixture was evaporated to dryness on a boiling water bath. Another 5 ml of hydrochloric acid was added again and the solution evaporated to dryness as before. 4 ml of hydrochloric acid 27 and a few ml of water added in this solution, warmed over boiling water bath and filtered into 100 ml volumetric flask using watman no 40 filter paper. After cooling, the volume was made up to 100 ml and suitable aliquots were used for the estimation of different minerals.

Minerals such as calcium, magnesium, phosphorus, zinc, iron and copper were determined as per the procedures given by Ranganna, (1986).

Estimation of Calcium

The 25 ml mineral solution was diluted to 150 ml with distilled water and neutralized with ammonia solution using methyl red as indicator till pink color changes to yellow. Further the solution was boiled and 10 ml of 6 per cent ammonium oxalate was added. This mixture was boiled for few minutes and added with conc. glacial acetic acid (99.5%) till the color was distinctly pink. The mixtures was kept aside in warm place (overnight) and when precipitate settle down, the supernatant was tested with a drop of ammonium oxalate to ensure the completion of precipitation. The contents were filtered through Whatman no.4 filter paper and give washings of warm distilled water. The precipitate was transferred to a beaker by making a hole in the center of filter paper and by giving washings of sulphuric acid (2N, 5 ml) twice. Then the solution was heated to 700ºC and titrates against N/100 $KMnO_4$. Simultaneously a blank was also run. The results were expressed as mg calcium/100g sample (Ranganna, 1986).

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

7.2 Determination of Iron

Iron content was determined by a-a, depyridyl method described in (AOAC, 2005) exactly 10 ml of wet digested sample solution was pipette into volumetric flask of 25 ml capacity in triplicates. 1ml of hydroxylamine hydrochloride solution, 5 ml of acetate buffer solution and 2ml of a-a, depyridly solution were added into each volumetric flask. The volume was made up to 25 ml with glass distilled water and the content was mixed well. The intensity of the colour developed was read in spetronic 20 at 510 nm. Iron content of the digested sample solution was read from the standard curve of known concentration of iron.

Preparation of standard curve

Pipette 0.0, 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 ml of Fe standard solution into a series of 25ml volumetric flasks and add to each of them exactly 0.2 ml of conc. HCl. Dilute each of them to exactly 10 ml with water, and then add reagents in the same way as for the sample, plot the quantity of Fe (in mg) against the absorbance (ICMR, 1990).

Iron content of sample

$$\text{Mg FE/100g sample} = \frac{\begin{array}{c}\text{Quantify of Fe in} \\ \text{aliquot of ash solution} \\ \text{(from calibration curve)}\end{array}}{\begin{array}{c}\text{Aliquot of ash solution} \\ \text{taken for determination}\end{array}} \times \frac{\begin{array}{c}\text{Total vol.} \\ \text{of ash solution}\end{array}}{\begin{array}{c}\text{Weight of sample} \\ \text{taken for ashing}\end{array}} \times 100$$

7.3 Determination of Magnesium

Magnesium was estimated by colorimetric method. Measure 10 ml of ash solution into a 15 ml graduated centrifuge tube. Add 1 drop of methyl red indicator. Neutralize solution with NH_4OH and ammonium oxalate and make the solution to a volume of 13 ml. Mix and allow to stand overnight. Centrifuge for 10 minutes and discard precipitate. Measure 1 ml of the supernatant liquid from above into a 15 ml centrifuge tube. Add 3 ml of water, 1 ml of ammonium phosphate and 2 ml of NH_4OH. Mix and allow standing overnight. Centrifuge for 7 min, discard the supernatant liquid, mix with 5 ml of dilute NH_4OH, centrifuge for 7 minutes and discard supernatant liquid. Dry the precipitate by placing the tube to container of hot water. Add 1 ml of dilute HCl and 5 ml hydroquinone and 0.5 ml sodium sulphite solution. Mix and allow to stand for 30 min. Transfer the solution to colorimeter tube and read the absorbance in a colorimeter using a No. 66 red filter. Set the instrument scale at zero with scale (Ranganna, 1986).

3.2.3.2 Acid modification of psyllium husk with hydrochloric acid (HCL) and sulphuric acid (H2SO4)

The acid treatment levels for psyllium husk with HCL and H2S04 are depicted in table 1 and 2.

Table 1: Acid treatment levels for psyllium husk with hydrochloric acid (HCL)

Concentration of HCL in ethanol solvent	Psyllium husk: Solvent ratio
0.75%	1:2, 1:4 and 1:6 (w/v)
0.00% for control	1:2, 1:4 and 1:6 (w/v)

Table 2: Acid treatment levels for psyllium husk with sulphuric acid (H_2SO_4)

Concentration of H_2SO_4 in ethanol solvent	Psyllium husk: Solvent ratio
0.75%	1:2, 1:4 and 1:6 (w/v)
0.00% for control	1:2, 1:4 and 1:6 (w/v)

Acid modification of psyllium husk was carried out as per the method described by Pei Xiaoyin (2008) with certain changes in concentration of HCl in ethanol solvent as per the results of the research study conducted by the (Syed *et al.,* 2018) on the standardization of acid concentration and solvent ratio for modification of psyllium husk (*Plantago Ovata*). Hence, acid modification with concentration of 0.65 per cent HCl in the ethanol solvent for solvent ratio of 1:6 (w/v) as PSH: Solvent ratio was carried out to improve functional properties of psyllium husk as required for exploration in the value addition of processed food products. The solvent used for psyllium husks treatment was vacuum filtered, rinsed with 95 per cent ethanol and 100 per cent for 2 times each, then dried and stored. Control group was treated with 100 per cent ethanol and followed the steps of preparation mentioned above (Table 1). The same above steps were followed for the H_2SO_4 modified psyllium husk and followed in (Table 2).

3.2.3.3 Functional Properties of Native and Acid Modified Psyllium Husk

Functional properties of native and modified psyllium husk were determined in terms of hydration capacity, oil absorption capacity and water up-take rate.

1. Oil absorption and hydration capacity

Oil absorption and hydration capacities were determined by the method of Rosario and Flores (1981). One-gram sample was mixed with 10 ml. distilled water or 10 ml. oil (refined groundnut oil) for 30 second in a mixer. The samples were then allowed to stand for 30 min at 30oC in a water bath and centrifuged at 3000 rpm for 20 min. The volume of supernatant was recorded to calculate the amount of hydration or oil absorption capacity.

2. Water Up-Taking rate

According to the method described by Yu *et al*., (2003). 1.0 g psyllium sample was equilibrated in 10% relative humidity (RH) desiccator for 48 h. After accurately weighing, all the samples were transferred into a 90% RH desiccator and exposed to moisture for 30 min. The moisture-absorbed samples were then accurately weighed and the weights were recorded. The amount of the absorbed water was presented as weight change of the dry matter after exposure to high RH environment. The data were reported as the average amount of water taken up by each gram of the psyllium samples per minute (mg/ (g×min)).

3. Gelation

Least gelation concentration of samples was determined by the method of Iyen and Singh (1997). Sample suspension containing 8-15 per cent (w/v) sample in 0.5 per cent increments were prepared in 15ml of distilled water. The test tubes were heated for 1 h in boiling water, rapidly cooled under running tap water and refrigerated for 3 hrs at 5°C.The least concentration was determined as that concentration at which the sample did not fall down or slip from inverted test tubes.

3.2.4 Milkshake with modified psyllium husk

3.2.4.1 Standardization of milkshake preparation

The production of milkshake was carried out in the Department of Food Microbiology and Safety, College of Food Technology, V.N.M.K.V, Parbhani by using modified psyllium husk with several ingredients in formulation of milkshake. The processing methodology standardized by using organoleptic evaluation. The recipe used for preparation of milkshake is mentioned below in table 3. The standardization of recipe for preparation of milkshake has been done as follows.

Table 3: Standard recipe for preparation of milkshake

Ingredients	Standardization
Milk (ml)	630
Apple pulp (gm)	270
Sugar (gm)	100

3.2.4.2 Preparation of milkshake

Milk with fat 6 percent and SNF 9% percent was taken. And pan heating was done up to 38°c by addition of sugar 10 percent and was allowed to dissolve. The apple fruits that were sorted and washed were made crushed into pulp. The crushed fruit pulp was added and further heating of mix done at 71°C for 30 min. And the whole mix of ingredients were stirred at the time of boiling. Ageing of mix was done at 6-10°C for 2-3 hours followed by addition of encapsulated modified psyllium husk with probiotic culture deep and freezing of mix was done for -2 to -6 °C. And the above steps lead to the final product. Then the milkshake was refrigerated to 18 °C for 24 hours for storage. The flowchart for the preparation of milkshake is mentioned below in flowsheet 3.

Flow sheet 3: Preparation of probiotic milkshake

Apples | Milk

↓ | ↓

Sorting and washing | Standardization milk (Fat-6% SNF-9%)

↓ | ↓

Pulping of fruits | Pan heating to 38°c

↓

Addition of sugar (10%)

↓

Addition of crushed fruit pulp

↓

Heating of mix (71°C, 30 min)

↓

Ageing of mix (6-10°C, 2-3 h)

↓

Addition of encapsulated modified psyllium husk with probiotic culture

↓

Deep Freezing of mix (-2 to -6 °C)

↓

Final product

3.2.5.1 Physical properties of milkshake

3.2.5.1. Viscosity

The viscosity of the milkshake samples was determined using a Brookfield viscometer (spindle-type). The viscometer was fixed with UL adaptor and spindle no. 63. Before viscosity measurement of the samples, the viscometer was subjected for auto zeroing in air. After this, the type of spindle and speed of rotation (rpm) were mentioned in the viscometer as per instructions.63 spindle was selected for viscosity measurement of milkshake samples at 30 rpm. Viscosity measurement of almond milkshake samples was carried out at 20°C (Briggs and Steffe 1997).

3.2.5.2 Proximate composition of milkshake

Proximate composition such as moisture, fat, crude protein, ash and crude fibre were determined as per A.O.A.C 2005 and carbohydrate by difference method.

3.2.6 Smoothie with modified psyllium husk

3.2.6.1 Standardization of Smoothie preparation

The production of smoothie was carried out in the Department of Food Microbiology and Safety, College of Food Technology, V.N.M.K.V., Parbhani by using modified psyllium husk with several ingredients in formulation of smoothie. The processing methodology standardized by using organoleptic evaluation. The recipe used for preparation of smoothie is mentioned in table 4. The standardization of recipe for preparation of smoothie has been done as follows.

Table 4: Standard recipe for preparation of smoothie

Ingredients	Standardization
Milk (ml)	400
Banana pulp (gm)	400
Sugar (gm)	80
Water (ml)	70
Honey (gm)	50

Milk with fat 4 percent and SNF 7% percent was taken and pasteurized to 63°c for 30 minutesfollowed by cooling of milk up to 12°c. The banana fruits that were sorted and washed were made crushed into pulp. Blending of ingredients until obtaining fine thin paste of mixture. Then followed the addition of encapsulated modified psyllium husk with probiotic culture to the mixture. The smoothie was refrigerated to 4-10°C for 36 hours and stored. The flowsheet for the preparation of smoothie is mentioned in flowsheet 4.

Flow sheet 4: Preparation of probiotic smoothie

Bananas | Milk
↓ | ↓
Sorting and washing | Standardization milk (Fat 4% and SNF 7%)
↓ | ↓
Pulping of fruits | Pasteurization of milk (63°C for 30 minutes)
↓ | ↓
↘ ↙
↓
Addition of Sugar (10%)
↓
Addition of Banana Pulp
↓
Heating of mix (71°C for 30 min)
↓
Fine Mixture and Cooling
↓
Addition of encapsulated modified psyllium husk with probiotic culture
↓
Refrigeration (4 to 8 °C)
↓
Final Product

3.2.7.1 Physical properties of smoothie

1. Viscosity

The viscosity of the smoothie samples was determined using a Brookfield viscometer (spindle-type). The viscometer was fixed with UL adaptor and spindle no. 63. Before viscosity measurement of the samples, the viscometer was subjected for auto zeroing in air. After this, the type of spindle and speed of rotation (rpm) were mentioned in the viscometer as per instructions.63 spindle was selected for viscosity measurement of smoothie samples at 30 rpm. Viscosity measurement of almond milkshake samples was carried out at 20°C (Briggs and Steffe 1997).

3.2.7.2. Proximate composition

Proximate composition such as moisture, fat, crude protein, ash and crude fibre were determined as per A.0.A.C 2005 and carbohydrate by difference method.

3.2.7.3 Microbial examination of prepared probiotic milkshake and smoothie.

Microbial examination is the perfect quality assessment protocol performed in quality analysis of food products. However, in a dairy product it is a mandatory one. Total Plate count (TPC), Yeast and mold count and coliform count were analysed. The milkshake and smoothie samples were analysed for yeast and mould count and coliform count.

3.2.8.1 Dilution blanks

The dilution blanks consisted of 0.85 to 0.90 per cent sodium chloride in test tubes (9 ml) and citrate phosphate buffer in flasks (99 ml). These were autoclaved at 121°C for 20 min. The dilution blanks (citrate phosphate buffer) were warmed to 45°C before the use of sample preparations.

3.2.8.2 Sampling

3.2.8.2.1 Sampling of milkshake

Samples were collected from selected glass bottles of milkshake. The sample was taken with a sterile pipette and remaining product was mixed thoroughly to make the contents homogenous. Then representative samples (11 ml) of milkshake was mixed thoroughly with citrate phosphate buffer (99 ml) to get first dilution of the sample. This dilution was serially diluted by transferring 1ml dilution to 9 ml of dilution blank

3.2.8.2.2 Sampling of smoothie

Samples were collected from selected glass bottles of smoothie. The sample was taken with a sterile pipette and remaining product was mixed thoroughly to make the contents homogenous. Then representative samples (11 ml) of smoothie was mixed thoroughly with citrate phosphate buffer (99 ml) to get first dilution of the sample. This dilution was serially diluted by transferring 1ml dilution to 9 ml of dilution blank.

3.2.8.3 Total plate count

Microbial analysis was done to determine Total Plate Count (TPC) of the samples on the nutrient agar media for bacterial count by the method recommended by Harrigan and Mc-Cance (1966).The Total Plate Count of sample was determined by use of nutrient agar medium. The prepared media sterilized at 15 psi for 15 min. The nine serial dilution i.e.10-1, 10-2, 10-3, 10-4, 10-5, 10-6, 10-7, 10-8 and 10-9 was pour in petri plates containing nutrient agar medium, when it will have solidified. The plates were incubated at 37°C for 48 hr and colonies were counted on colony counter. Total bacterial Count (CFU/g) = no. of colonies × dilution factor/ ml of aliquot.

3.2.8.4 Yeast and mold

Microbial analysis was done to determine total yeast and mold count of the samples on the potato dextrose agar media for yeast and mold count by the method recommended by Harrigan and Mc-Cance (1966). The yeast and mold count of sample was determined by using potato dextrose agar (PDA) and the pour plate technique was used for the Isolation. The media was sterilized and poured into plates. The dilutions of sample were made up to 10-8 and then the 1 ml of aliquot was used for streaking. Plates were incubated at 37°C for 48-72 hrs, and results noted in cfu/ml. The Yeast and mold count of the milkshake and smoothie was checked at weekly intervals for 3 weeks.

3.2.8.5 Coliform

The Coliform and basically E.Coli are the indicator microbes of water contamination by faeces and therefore it is mandatory to examine the contamination. The Coliform gives red pink colonies on VRB agar so it was used for examination. Using the pour-plate technique, appropriately 0.1 ml aliquots was taken in duplicate plates and tempered VRB agar was added. The agar was allowed to solidify and then overlay of about 5 ml of Violet Red Bile Agar (VRB) was added. Allow agar to solidify. Plates were inverted and incubated at 37°C for 24 hrs. Red colonies surrounded by a zone of precipitate and report as “presumptive coliforms cfu/g”.

3.2.9 Organoleptic evaluation of probiotic milkshake and smoothie

The probiotic milkshake and smoothie was evaluated for sensory characteristics like appearance, color, taste, flavor and overall acceptability post incubation period and also during storage at refrigerated conditions (4°C). Sensory evaluation was conducted in the laboratory by a panel of semi trained judges which comprised of postgraduate students and academic staff members of College of Food Technology, V.N.M.K.V., Parbhani. Samples were scored based on a nine point hedonic scale. Judges were asked to rate the product on 9 point Hedonic scale with corresponding descriptive terms ranging from 9 “like extremely” to “dislike extremely”

3.2.10 Techno-economic feasibility of the product

The cost of production of most acceptable milkshake and smoothie was calculated by considering the raw materials cost, processing cost and miscellaneous cost. Processing cost was considered 10 per cent of total raw material cost. Packaging cost is considered 5 per cent of total raw material cost. Further yield of the milkshake and smoothie to be considered as 90-98 per cent.

CHAPTER-IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

The present investigation entitled "Studies on use of psyllium husk as a prebiotic in food products" had been carried out and the results obtained are summarized as follows.

The physico-chemical properties of psyllium husk were studied. Efforts were made to select the acid with improved functional properties among HCL and H_2SO_4 and the acid with better functional properties was used in the preparation of probiotic milkshake and probiotic smoothie. The prepared dairy product were further analysed for physico-chemical and sensorial quality parameters. The results obtained during the present investigation have been discussed in the light of relevant information available in scientific literature under suitable headings and subheadings.

A. Psyllium husk

4.1 Psyllium husk

4.1.1 Physico-chemical characteristics of psyllium husk

4.1.1.1Proximate composition of psyllium husk

4.1.1.2 Mineral composition of native psyllium husk

4.1.1.3 Functional properties of native psyllium husk

4.2.2 Acid modification of native psyllium husk

4.2.2.1 Effect of acid modification on functional properties of psyllium husk

4.2.2.2 Effect of acid modification on textural profile of acid modified psyllium husk

4.2.2.3 Effect of acid modification on proximate composition of acid modified psyllium husk

B. Milkshake

4.3 Standardization of the processing technology of preparation of milkshake

4.4 Sensory evaluation of milkshake

4.5 Proximate Composition of milkshake

4.6 Mineral composition of milkshake

4.7: Textural properties of milkshake

4.8 Storage and microbial characteristic of milkshake

4.8.1 Viable counts (LAB) present in milkshake during storage.

4.9 Microbial quality of milkshake during storage

4.10 Techno-economical feasibility of milkshake

C. Smoothie

4.11 Standardization of the processing technology of preparation of smoothie

4.12 Sensory evaluation of the prepared encapsulated probiotic basundi

4.13 Proximate Composition of smoothie

4.14 Mineral composition of Smoothie

4.15 Textural properties of Smoothie

4.16 Storage and microbial characteristic of smoothie

4.16.1 Viable counts (LAB) present in smoothie during storage.

4.17 Microbial quality of Smoothie during storage

4.18 Techno-economical feasibility of smoothie

4.1 Psyllium husk

4.1.1 Physico-chemical characteristics of psyllium husk

4.1.1.1 Proximate composition of psyllium husk

The proximate analysis of the psyllium husk is crucial because inclusion of husk in the products may have a significant role in the modulation of physical, chemical and ultimately sensory features. Proximate analysis is also crucial to measure the efficiency of the psyllium husk. Thus, husk was investigated for its contents such as moisture, ash, protein, crude fat, and crude fibre, as well as dietary fibre. The results for the proximate composition of native psyllium husk, such as moisture, fat, protein, ash, carbohydrate, crude fibre and dietary fibre are depicted in table 5.

Table 5: Proximate composition of psyllium husk

Chemical parameters	Native psyllium husk
Moisture (%)	6.91 ± 0.07
Ash (%)	2.64 ± 0.03
Total fat (%)	1.91 ±0.03
Total protein (%)	2.31 ±0.01
Total carbohydrate (%)	86.82 ±0.7
Crude fiber (%)	3.2 ± 0.02
Dietary fiber (%)	76.59 ±0.4
Energy value (Kcal/100g)	375

* Each value is average of three determinations

The results in Table 5 showed that the moisture content of native psyllium husk was 6.91 ± 0.07 per cent on whole weight basis, fat content per cent 1.91 ±0.03 and protein content was 2.31 ±0.01 per cent. It is clear from the Table 5 that crude fibre, ash and total carbohydrate content of native psyllium husk were found to be 3.2 ± 0.02per cent, 2.64 ± 0.03 per cent and 86.82 ±0.7per cent respectively. The dietary fiber was found to be76.59 ±0.4and and calculated energy value was 375 Kcal/100g. The results were in similar trend to the values obtained by (Liangh *et al.,* 2003) and (Guo *et al.,* 2008), they found moisture, ash, protein, fat and NFE values in psyllium husk as 6.83 ± 0.04, 4.07 ± 0.02, 0.94 ± 0.00,0.04 ± 0.11 and 84.98 ± 4.26 per cent, respectively.

4.1.1.2 Mineral composition of native psyllium husk

The mineral composition of the native psyllium husk was inspected in terms of iron, copper, manganese and zinc content and average values are outlined in Table 6.

Table 6: Mineral composition of psyllium husk

Parameters	Results (mg/100 g)
Iron (Fe)	7.88±0.04
Copper (Cu)	0.713±0.002
Manganese (Mn)	0.650±0.003
Zinc (Zn)	0.356±0.001

* Each value is average of three determinations.

It is revealed from Table 6 that iron and copper content of native psyllium husk was found to be 7.88±0.04 mg/100 g and 0.713±0.002 mg/100 g respectively while manganese and zinc content was found to be 0.650±0.003 mg/100 g and 0.356±0.001 mg/100 g. The results are in good agreement with the results reported by (Hashmi *et al.,* 2018).

4.1.1.3 Functional properties of native psyllium husk

The functional properties of psyllium husk play a crucial role in assessing the efficiency of the modified psyllium husk. The improved functional properties also helps in easy incorporation of psyllium husk in food products without affecting the textural properties. The techniques used for analysing functional properties are mentioned in materials and methods chapter 3.2.1.3. The hydration capacity, oil hydration capacity and water up-taking rate of native psyllium husk were studied and the results are depicted in Table 7.

Table 7: Functional properties of native psyllium husk

Parameters	Results
Hydration capacity (ml/g)	3.2 ±0.03
Oil absorption capacity (ml/g)	1.19 ±0.02
Water up-taking rate (mg/(g×min)	2.21 ±0.02

* Each value is average of three determinations.

The depicted results are presented in Table 7 reveals the different functional properties of native psyllium husk. The hydration capacity of native psyllium husk was found to be 3.2 ±0.03 ml/g while the oil absorption capacity and water up-taking rate was 1.19 ±0.02 ml/g and 2.21 ±0.02 mg/g x min respectively. Kamaljit *et al.*, (2011) and Hashmi *et al.*, (2018) reported similar findings for native psyllium husk functional properties as an ingredient for high fibre bread and probiotic basundi respectively.

4.2.2 Acid modification of native psyllium husk

Acid modification of psyllium husk was conducted out according to the procedure reported by Pei Xiaoyin (2008), with changes in HCl and H_2SO_4 concentration in ethanol solvent based on the findings of a study performed by (Syed *et al.*, 2018) on the standardisation of acid concentration and solvent ratio for modification of psyllium husk (*Plantago ovata F*.), i.e. The solvent used for psyllium husk modification was ethanol containing 34 to 37% hydrochloric acid (HCl) at a concentration of 0.75 percent (w/v).

The acid modification is less expensive than enzymatic procedures, and hence it has the potential to be used in the food business. As a result, an attempt was made to improve the physical and chemical properties of psyllium husk for incorporating in foods by acid treatment of raw psyllium husk, so that it can serve as a source of dietary fibre while not interfering with the nutritional and sensorial properties of psyllium husk incorporated food products. An acidic aqueous solution comprising a solvent and an acid was used to treat raw psyllium husk. The solvent must be able to dissolve the acid and can be organic, inorganic, or a mixture of the two. Based on the investigations conducted by the Pei (2008) ethanol was a chosen organic solvent. The acid is preferably hydrochloric acid, a moderate to strong acid with a pka of not more than 5, ideally between 1 and 5, and most preferably between 3 and 1. The treatment was carried out for an adequate period of time and under suitable processing circumstances so that the resulting psyllium husk has the necessary specified qualities, which will vary depending on the intended application of the modified psyllium husk product.

As a result, additional research was carried out to explore the effects of various acid solvent ratios at a reaction temperature of 37.5°C on the physical, chemical and functional features of both the acid-treated psyllium husk samples. Psyllium husk – solvent ratios (PSH: Solvent @ 1:6 (w/v)) were evaluated at 37.5°C, and the entire technique used in the experiment is explained in the materials and methods chapter 3.2.1.2**.**

Table 8: Acid treatment for psyllium husk with hydrochloric acid (HCL)

Concentration of HCl in Ethanol (Solvent)	**Psyllium husk (PSH): Solvent Ratio**
0.75%	1:2, 1:4 and 1:6 (w/v)
0.00% for Control	1:2, 1:4 and 1:6 (w/v)

Table 9: Acid treatment for psyllium husk with sulphuric acid (H_2S0_4)

Concentration of H_2SO_4 in Ethanol (Solvent)	**Psyllium husk (PSH): Solvent Ratio**
0.75%	1:2, 1:4 and 1:6 (w/v)
0.00% for Control	1:2, 1:4 and 1:6 (w/v)

After treating the psyllium husk with the acids HCL and H_2SO_4, the psyllium husk was kept at 48 hrs at room temperature. Further the product was recovered by vacuum filteration. The vacuum filteration was done to remove uncertain fractions along with solvent from the final product. After recovery, the resulting acid modified PSH was air dried at room temperature until the requisite water content was obtained.

4.2.2.1 Effect of acid modification on functional properties of psyllium husk

The effect of acid treatment on functional qualities such as hydration capacity, oil absorption capacity, and water uptake rate of control sample, acid treated samples of HCL and H_2SO_4 and native psyllium husk was tested, and the findings are shown in table 10.

Table 10: Effect of acid modification on functional properties of psyllium husk

Acids	Concentration of acids in Ethanol (Solvent)	Psyllium husk (PSH) : Solvent Ratio	Hydration capacity (ml/g)	Oil absorption capacity (ml/g)	Water up-taking rate (mg/(g×min)
HCL	Control	1:6	2.6±0.06	0.9±0.03	1.8±0.02
	0.75%	1:6	0.8±0.04	0.3±0.02	1.54±0.05
H_2SO_4	control	1:6	3.2±0.09	1.1±0.05	2.45±0.06
	0.75%	1:6	1.5±0.03	0.4±0.04	1.62±0.07
	Native psyllium husk	1:6	3.2 ±0.03	1.19 ±0.02	2.21 ±0.02

* Each value is average of three determinations

Hydration capacity

The results depicted in table 10 showed that hydration capacity for native psyllium husk was 3.2 ±0.03 ml/g followed by control samples of H_2SO_4 and HCL with 3.2±0.09 ml/g and 2.6±0.06 ml/g respectively. There was substantial decrease with improved hydration capacity of psyllium husk after the acid treatment. The HCL treated sample had lowest hydration capacity with 0.8±0.04 ml/g compared to the H_2SO_4 sample with 1.5±0.03 ml/g. In terms of physiological functionality, the water-binding capacity of fiber can be used to predict its ability to increase stool weight. Milk proteins are examples of compounds that can aid in water binding and are be considered by food technologists. Understanding these characteristics is critical in developing efficient food processes capable of producing consumer-acceptable food products. DFs with high water holding capacity, for example, can be used not only as DF enrichment, but also as functional ingredients to decrease calorific value, avoid syneresis, and modify the viscosity and texture of the finished product (Holtekjølen *et al.*, 2008). The nature of water holding capacity's response to impending spoilage suggests that it could be used to estimate the microbial properties of products (Jay, 1965).

Oil absorption capacity

The Oil absorption capacity (OAC), is the binding of fat by the nonpolar side chain of proteins. Oil absorption capacity is an important functional property that contributes to enhancing mouth feel while retaining the flavor of food products.

Protein's ability to bind oil and water in food is determined by intrinsic factors such as protein conformation, amino acid composition, and surface polarity or hydrophobicity (Iwe *et al.,* 2016). Acid treated psyllium husk samples with 0.75% HCL and H_2SO_4 for ratio 1:6 had a better oil absorption capacity values of 0.3±0.02 ml/g and 0.4±0.04 ml/g respectively. The HCL and H_2SO_4 control samples showed higher oil absorption capacity with 0.9±0.03 ml/g and1.1±0.05 ml/g respectively and for the native psyllium husk it was found to be 1.19 ±0.02 ml/g. The oil absorption capacity improved with the addition of acid treatment in psyllium husk. The presence of nonpolar side chains in foods may bind the oil hydrocarbon side chains in foods which may explain the increase in OAC. High OAC ingredient may be beneficial in structural interactions in foods, particularly in terms of palatability, shelf-life extension, and flavor retention (Jitngarmkusol *et al.,* 2008). Protein, which is made up of both hydrophobic and hydrophilic parts, is the most important chemical component influencing oil absorption capacity. The non-polar amino acid side chains can form hydrophobic interactions with lipid hydrocarbon chains. The oil absorbing mechanism frequently involves capillary interactions in the food matrix, which allows the absorbed oil to be retained (Suresh *et al.,* 2013).

Water up-taking rate

The occurrence of swelling of biological products, which occurs concurrently with water diffusion and affects the water absorption rate, should not be overlooked. It is critical to investigate the swelling phenomenon in order to gain a better understanding of the water up-taking rate process of food products. The water up taking rate of acid modified psyllium husk was also investigated and the revealed results are presented in table 10. The water up-taking rate was lowest for the 0.75%HCL psyllium husk- solvent ratio (1:6) with 1.54±0.05 mg/ (g×min) followed by 0.75% H_2SO_4 psyllium husk-solvent ratio (1:6) with 1.62±0.07 mg/ (g×min). Furthermore, the data showed that the water uptaking rate of native psyllium was 2.21 ±0.02 mg/ (g×min) followed by the control samples of H_2SO_4 and HCL with 1.62±0.07 mg/ (g×min) and 1.54±0.05 mg/ (g×min) respectively. The comparision among these shows that HCL treated psyllium husk sample had the improved water up taking rate when compared to the native psyllium husk and the H_2SO_4 treated psyllium husk.

Water uptake rate is an important factor in determining the physicochemical and sensorial properties of processed food products fortified with psyllium husk as a source of dietary fiber, particularly dairy-based, baked, and fried products. (Bello *et al.,* 2004) used a similar approach for the process of water absorption during hydration, including considerations for volume change as well as an expression for variable diffusivity for solving the Fick's law-based diffusion equation. The magnitude of the volume change was proportional to the amount of water taken in. The results for the water up-taking rate were similar to the findings Pei Xiaoyin, (2008) for water up-taking rate for acid treated PSH.

After analysing the results, it showed that HCL treated psyllium husk with a concentration of 0.75% of ratio 1:6 psyllium husk- solvent ratio was superior with better functional properties when compared to native psyllium husk and H_2SO_4 treated psyllium husk and was considered to be incorporated in food products and for further studies on its proximate composition and textural profile analysis.

4.2.2.2 Effect of acid modification on textural profile of acid modified psyllium husk.

Table 11: Effect of acid modification on textural profile of acid modified psyllium husk

Samples	Hardness (gm)	Adhesiveness (gm)
Native PSH	85.5 ± 1.1	10.8 ± 0.75
Acid modified PSH	48.3 ± 3.1	8.4 ± 0.27

*Each value is average of three determinations

The effect of acid modification on textural profile of acid modified psyllium husk is depicted in table 11. The two particulars, native PSH and selected acid modified PSH were taken for analysis. Acid modified psyllium had decreased hardness and adhesiveness compared to native PSH when observed results from the table 11.The depicted results shows that hardness was reduced from 85.5 ± 1.1 to 48.3 ± 3.1 gm. And the adhesiveness was reduced from 10.8 ± 0.75 to 8.4 ± 0.27 gm. The reduction in hardness and adhesiveness was due to the swelling power of psyllium husk that was obtained by acid modification which resulted in reduced hydration capacity and water up-taking rate. This indicates that addition of acid to the husk results in decreased hardness and adhesiveness.

4.2.2.3 Effect of acid modification on proximate composition of acid modified psyllium husk.

Table 12: Effect of acid modification on proximate composition of acid modified psyllium husk

Chemical parameters	Native psyllium husk	Modified psyllium husk
Moisture (%)	6.91±0.07	7.11±0.09
Ash (%)	2.64±0.03	2.24±0.02
Total fat (%)	1.91±0.03	0.65±0.02
Total protein (%)	2.31±0.01	1.11±0.01
Total carbohydrate (%)	86.82±0.7	89.01±0.9
Crude fiber (%)	3.2±0.02	2.75±0.02
Dietary fiber (%)	76.59±0.4	78.65±0.5
Energy value (Kcal/100g)	375	369

*Each value is average of three determinations

The proximate composition of modified psyllium husk was depicted in table 12 and it revealed that moisture content in psyllium husk was 6.91±0.04 percent and was increased to 7.11±0.03 percent in modified psyllium husk. Carbohydrate and dietary fiber content was found to be increased from 86.82±0.7 to 89.01±0.9 percent and 76.59±0.4 to 78.65 ±0.5 respectively. Crude fiber and Ash content decreased from 3.2±0.02 to 2.75±0.02 percent and 2.64±0.03 to 2.24±0.02 percent respectively. Total fat content decreased from 1.9±0.031 to 0.65±0.02 percent. Total protein in native PSH was found to be 2.31±0.01 percent and decreased to 1.11±0.01 percent in modified PSH. Present results are also in corroborated with the finding of Guo *et al.,* (2008), they were found to be similar. There was decrease in fat, protein, ash and crude fiber content due to gel hardness of psyllium formed by partial degradation after acid modification. The carbohydrate content was increased due to acid hydrolysis of psyllium husk caused by HCL and the results are similar with results shown by (Syed *et al.,* 2018).

4.3 Standardization of the processing technology of preparation of milkshake

Table 13: Standardization of the processing technology of preparation of milkshake

Ingredients	Standardization
Milk (ml)	630
Apple pulp(gm)	270
Sugar(gm)	100

The ingredients that are necessary to standardize the preparation of milkshake are depicted in table 13. The standard receipe contained milk of 630 ml followed by apple pulp 270 gm and sugar 100gm. The encapsulated probiotic beads with modified psyllium husk were added to the final product of milkshake aseptically. Detailed process for the preparation of milkshake is mentioned in materials and methods chapter 3.2.3.2.

4.4 Sensory evaluation of milkshake

Table 14: Organoleptic evaluation of prepared milkshake

Sample	Appearance	Color	Taste	Flavour	Texture	Overall acceptability
M_0	8.1	6.9	6.5	6.1	7.5	7.1
M_1	7.2	7.3	8.5	6.5	7.6	7.8
M_2	7.4	7.4	8.3	6.9	8.2	**8.1**
M_3	7.2	7.5	8.3	6.8	8.1	8.0
SE ±	0.036	0.029	0.065	0.047	0.089	0.081
CD@5%	0.065	0.049	0.092	0.145	0.197	0.247

* Each value is average of three determinations

M_0- Milkshake with native psyllium husk

M_1- Milkshake + encapsulated modified psyllium husk with 10 per cent probiotic culture having (10^8) cfu/gm

M_2- Milkshake + encapsulated modified psyllium husk with 10 per cent probiotic culture having (10^9) cfu/gm

M_3- Milkshake + encapsulated modified psyllium husk with 10 per cent probiotic culture having (10^{10}) cfu/gm

Color is used as a preliminary criterion for food approval. Color and appearance are a significant sensory components in determining customer acceptance of milkshake. The results from the table showed that color and appearance of milkshake with encapsulated modified psyllium husk with 10 per cent probiotic culture having (10^9) cfu/gm had maximum hedonic score of 7.4.

Flavor means an overall integrated perception of taste and aroma associated with the product. The results showed that milkshake with encapsulated modified psyllium husk with 10 per cent probiotic culture having (10^9) cfu/gm had the highest score with 6.9 and the control sample showed the least score with 6.1 among all the samples.

Taste is one of the major parameters when it comes to the acceptance of food products. The product should have the satisfactory taste before it benefits with the health benefits. The sample M_1 had the maximum score followed by M_2, M_1 and M_0 with 8.3, 8.3 and 6.5 respectively.

Texture (hardness) is a set of physical properties that can be detected with a mouth bite. The results revealed that milkshake with encapsulated modified psyllium husk with 10 per cent probiotic culture having (10^9) cfu/gm had the highest score with 8.2 and the control sample showed the least score with 7.5 among all the samples.

Overall acceptability is based on several organoleptic quality indicators, such as colour, flavour, taste, texture, and so on, and represents the panellists' collective perception and acceptance. The sample M_2 had the best score of overall acceptability and control sample M_0 had the lowest score.

4.5 Proximate Composition of milkshake

Table 15: Proximate composition of milkshake

Chemical parameters	Mean value
Moisture (%)	81.91±0.7
Ash (%)	0.92±0.01
Total fat (%)	3.89±0.04
Total protein (%)	2.73±0.03
Total carbohydrate (%)	8.21±0.1
Crude fiber (%)	2.25±0.01
Energy value (Kcal/100g)	79

* Each value is average of three determinations

The proximate composition of milkshake was depicted in table 15 and it revealed that moisture content in psyllium husk was 81.91±0.7 percent and total carbohydrate content found to be 8.21±0.1 percent. It is clear from the table 15 that Ash, Total fat, Total protein and Crude fiber content were found to be 0.92±0.01 percent, 3.89±0.04 percent, 2.73±0.03 percent and 2.25±0.01 percent respectively. Calculated energy value was found 79 Kcal/100ml. The following results are good confirmatory with (Dantas *et al.*, 2017).

4.6 Mineral composition of milkshake

Mineral composition of psyllium husk with the parameters like iron, copper, manganese and zinc are revealed in table 16.

Table 16: Mineral composition of milkshake

Parameters	Results (mg/100 g)
Iron (Fe)	0.453
Copper (Cu)	0.279
Manganese (Mn)	0.153
Zinc (Zn)	0.595

Plate 7: M_2 sample with encapsulated psyllium husk having 10^9 cfu/gm

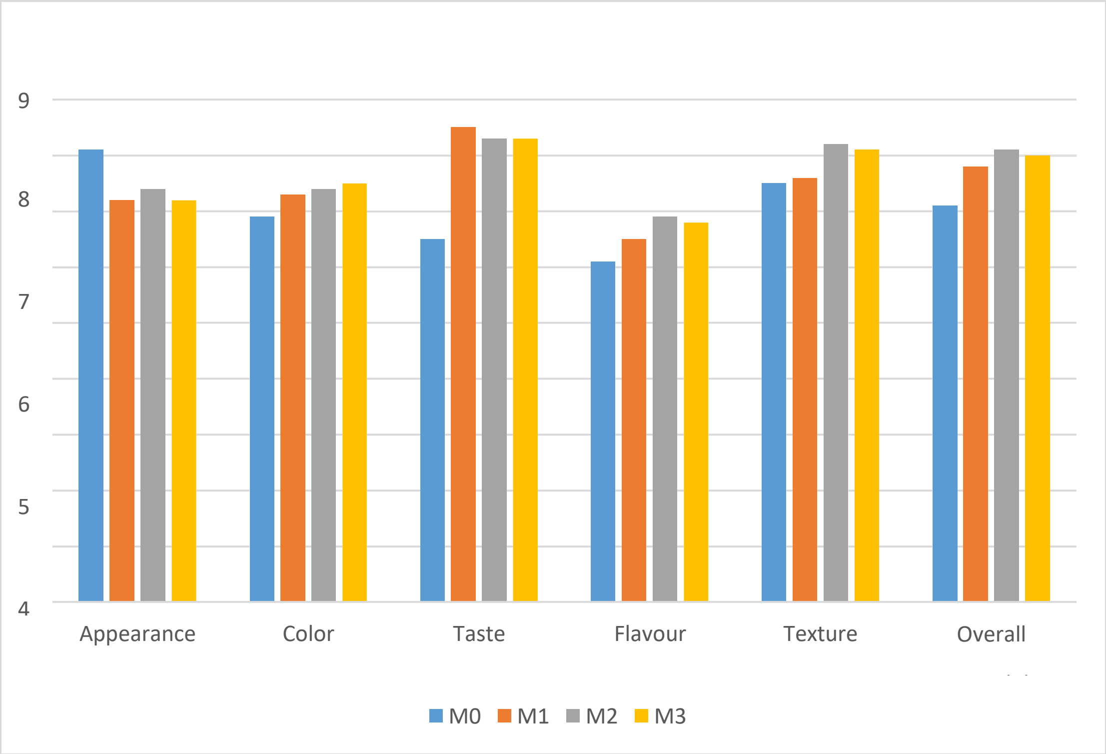

Figure 1: Sensory Evaluation of prepared milkshake using hedonic scale

The results from the table 16 show that the zinc content was the highest with 0.595 mg followed by iron and copper with the values of 0.453 mg and 0.279 mg respectively. The manganese content was the lowest with the value 0.153 mg.

4.7: Textural properties of milkshake

Table 17: Textural properties of milkshake

Sample	Viscosity (Pa-s)
Control	0.72
M_2	0.56

From the above table 17 results showed that viscosity of control sample with native psyllium husk had more viscosity 0.72 Pa-s when compared to milkshakes with modified psyllium husk. The viscosity of the samples M_2 was 0.56. The milkshake sample with highest hedonic score had viscosity value within the accepted range. There was a decrease in viscosity due to reduced swelling of the psyllium particles caused by the acid for the modified psyllium husk. The results for the viscosity of milkshake were supported by the findings of (Mudgil *et al.,* 2020) in literature. The viscosity of milkshake was more for the control sample that had native psyllium husk and the viscosity steadily decreased after the addition of acid modified to the milkshake samples and also corroborated with the findings of (Dogaru *et al.,* 2014) and the values were found to be similar.

4.8 Storage and microbial characteristic of milkshake

4.8.1 Viable counts (LAB) present in milkshake during storage.

Table 18: Viable counts (LAB) of milkshake during storage

Time in (days)	Viable count		
	(cfu/ml)x10^8	(cfu/ml)x10^9	(cfu/ml)x10^{10}
0	2.3	1.9	1.0
2	2.5	2.1	1.3
4	2.9	2.5	1.5
6	3.3	3.0	1.8

The total viable counts of encapsulation beads added accepted milkshake sample C was observed at different storage period on MRS agar media. The results are presented in table 18. It is evident that viable counts are observed at dilutions rate of

10^8, 10^9 and 10^{10}. Cell viability on on MRS agar plates of 1st day and 6th day are showed in plates 8 and 9 respectively.

4.9 Microbial quality of milkshake during storage

The acceptable milkshake sample sample was then subjected to microbiological investigations for total plate count, yeast and mould count, and coliform growth during storage according to Cappuccino and Sherman's method (1996). The table 19 depicts the recorded results. Plates 10, 11, and 12 show photographs of TPC, yeast and mould counts, and coliform counts, respectively.

Table 19: Microbial quality of milkshake during storage

Time in weeks	Total Plate Count (cfu/g)x10^4	Yeast and Mold (cfu/g)x10^4	Coliform count (cfu/g) x10^3
1	1.6	ND	ND
2	3.5	2.1	ND
3	4.2	2.9	ND

The results from the table 19 show that TPC growth was found to be 1.6 x10^4 , 3.5 x10^4 and 4.2 x10^4 (cfu/g) for the first, second and third weeks respectively. There was no yeast and mold growth in the first week but in the second and third week growth of 2.1 x 10^4 and 2.9 x10^4 (cfu/g) can be seen. Coliform count was not found in the first two weeks under good hygienic and refrigeration of milkshake. The above results for TPC, yeast and mold were similar to the results reported by (More *et al.,* 2014).

4.10 Techno-economical feasibility of milkshake

The techno-economical feasibility is a important parameter in calculating the total cost of a product and for the commercial selling of the food produce. The techno-economic feasibility of milkshake was determined by calculating the total cost of production for one litre of milkshake prepared with acid modified psyllium husk. The cost of production was estimated using a standard method of calculation based on raw material cost, processing cost (10% of raw material cost), and packing cost (5% of raw material cost), and the unit net cost were evaluated and are shown in table 20.

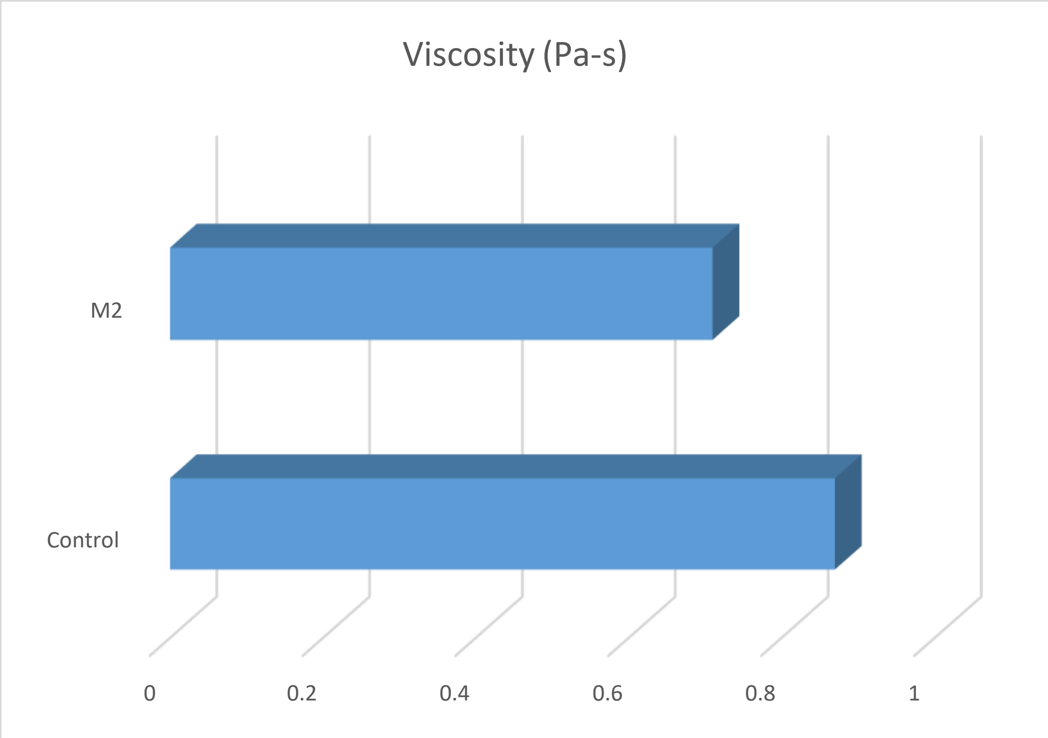

Figure 2: Textural properties of milkshake

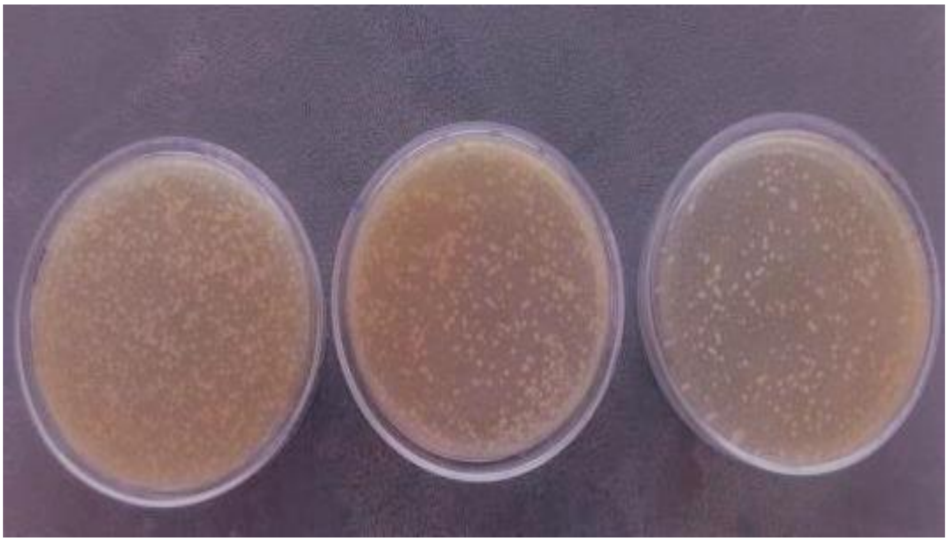

Plate 8: Cell viability on MRS agar plates of probiotic milkshake on 1st day

Plate 9: Cell viability on MRS agar plates of probiotic milkshake on 6th day

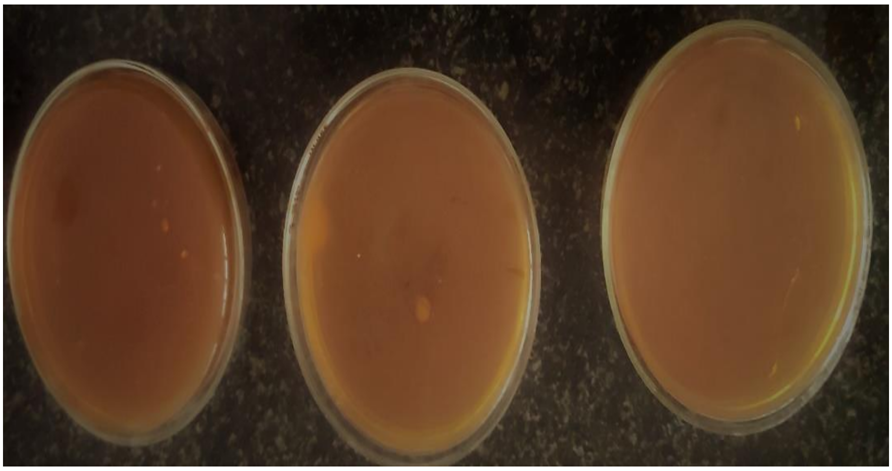

Plate 10: TPC count of milkshake

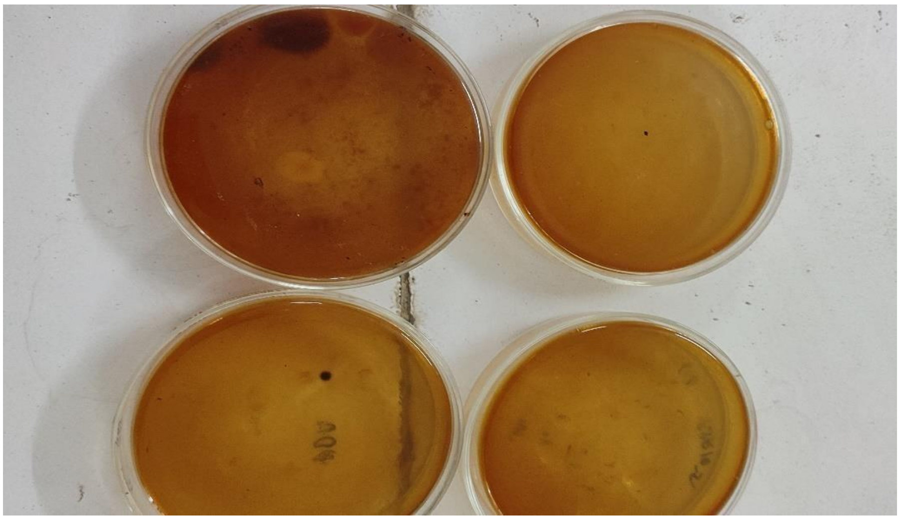

Plate 11: Yeast and mould count of milkshake

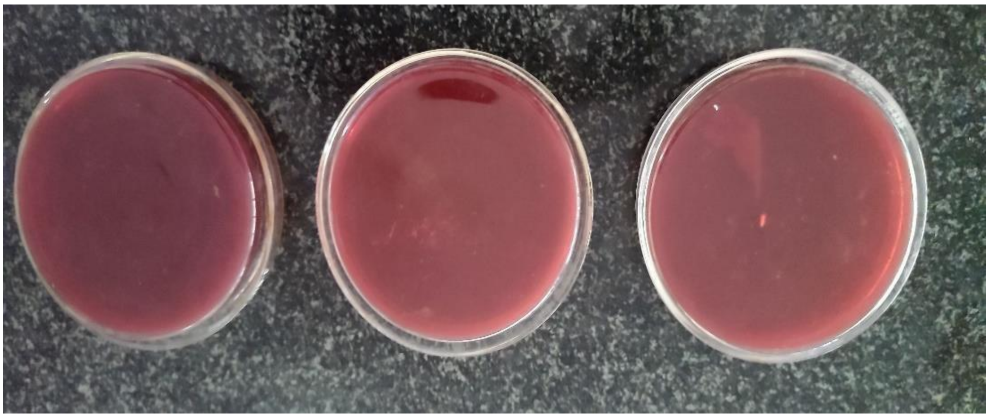

Plate 12: Coliform count of milkshake

Table 20: Techno-economic feasibility of milkshake

Particulars	Price for 1Lit / Kg(Rs)	Quantity (in ml /g)	Cost (rs)
Milk	40/lit	630 ml	25.2
Psyllium husk	100/kg	6.0 gm	0.60
Apple	100/kg	270 gm	27
Sugar	40/kg	100gm	4.0
Total raw material cost			56.8
Processing cost (10% of Raw Material Cost)			5.68
Packaging cost (5% of Raw Material Cost)			2.84
Production cost of 100 ml of milkshake			6.53
Production cost of 1 lit of milkshake			**65.3**
Production cost of 10 lit of milkshake			653

The Table 20 depicts the price of 1 kg/ml of raw material and the cost of producing 1 lit of milkshake from sample M2. The cost of production for 1ooml of milkshake is Rs.6.53, 1 lit of milkshake is Rs.65.3 and the cost of production for 10 kg of sample is Rs.653, assuming a 97 percent yield of product. Considering the health benefits, the milkshake with psyllium husk provides like improving the gut health and improving blood cholesterol levels, the cost was not higher and was affordable.

B. Smoothie

4.11 Standardization of the processing technology of preparation of smoothie

Table 21: Standardization for the preparation of smoothie

Ingredients	Standardization
Milk (ml)	400
Banana pulp(gm)	400
Sugar(gm)	80
Water (ml)	70
Honey (gm)	50

The ingredients used to standardize the preparation of smoothie are reported in table 21. The standard receipe contained milk of 400 ml followed by banana pulp 400 gm and water 70ml. And the sugar and honey was added at 80 gm and 50 ml respectively to impart sweetness to the product. The encapsulated probiotic beads with psyllium husk was added to the smoothie samples aseptically. The complete process for the preparation of smoothie is mentioned in materials and method 3.2.5.1.

4.12 Sensory evaluation of the prepared smoothie

The sensory evaluation of smoothie is one of the important parameter in regarding acceptance of the developed product. It was done by using hedonic scale by the panellist and the scores of appearance, color, taste, flavour, texture and overall acceptability was given by the analyst. The results of the organoleptic evaluation of the smoothie is table 22.

Table 22: Organoleptic evaluation of prepared smoothie

Sample	Appearance	Color	Taste	Flavour	Texture	Overall acceptability
M_0	7.8	7.0	6.7	6.8	7.8	7.2
M_1	7.4	7.4	8.2	6.6	8.1	7.7
M_2	7.3	7.6	8.1	6.7	8.3	**8.0**
M_3	7.1	7.8	7.8	6.5	8.0	7.9
SE ±	0.029	0.018	0.053	0.042	0.076	0.075
CD@5%	0.072	0.052	0.086	0.126	0.183	0.212

* Each value is average of three determinations

M_0- Smoothie with native psyllium husk

M_1- Smoothie + encapsulated modified psyllium husk with 10 per cent probiotic culture having (10^8) cfu/gm

M_2- Smoothie + encapsulated modified psyllium husk with 10 per cent probiotic culture having (10^9) cfu/gm

M_3- Smoothie+ encapsulated modified psyllium husk with 10 per cent probiotic culture having (10^{10}) cfu/gm

Colour and appearance are potentially the most important sensory property in the food and beverages industry. Food colour gives consumers an almost immediate impression about the freshness, flavour and quality of a product. This affects the consumer decision to purchase that product or select something that looks more appealing. The Sample M_3 had the best hedonic score of color with 7.8 and sample M_0 had the better appearance with 7.8.

Flavor is the parameter that affects the sense of a product and it also taste. The results showed that the lowest value was found to be 6.5 for the M_3 sample and the higher value with better taste was for sample M_2.

Taste is one of the major parameters that is necessary for consumer acceptance of the product. The sample M_2 had the maximum score with 8.1 and M_0 had the lowest score.

Responsible for triggering different sensations and reactions, texture is truly the charm card, the major contributor to consumer seduction. The results revealed that smoothie with encapsulated modified psyllium husk with 10 per cent probiotic culture having (10^9) cfu/gm had the highest score with 8.3 and the control sample showed the least score with 7.8 among all the samples.

Overall acceptability is based on several organoleptic quality indicators, such as colour, flavour, taste, texture, and so on, and represents the panellists' collective perception and acceptance. The sample M_2 had the best score of overall acceptability with 8.0 and control sample M_0 had the lowest score followed by M_3 and M

4.13 Proximate Composition of smoothie

The proximate composition of smoothie was studied with respect to moisture, ash, crude fat, crude protein, crude fiber and carbohydrate .The results are presented in table 23.

Table 23: Proximate composition of smoothie

Chemical parameters	Mean value
Moisture (%)	77.2±0.6
Ash (%)	1.15±0.01
Total fat (%)	2.85±0.02
Total protein (%)	4.31±0.06
Total carbohydrate (%)	11.98±0.2
Crude fiber (%)	2.38±0.02
Energy value (Kcal/100g)	90

* Each value is average of three determinations

The proximate composition of smoothie was depicted in Table 23 and it revealed that moisture content in psyllium husk was 77.2±0.6 percent and total carbohydrate content found to be 11.98±0.2 percent. It is clear from the Table that Ash, Total fat, Total protein and Crude fiber content were found to be 1.15±0.01 percent, 2.85±0.02 percent, 4.31±0.06 percent, 2.38±0.02 percent respectively. Calculated energyvalue was found 90 Kcal/100ml. The following results are in` good confirmatory with (Odiye *et al.*,2021).

The results were also similar in trends to the findings of (Nwaoha *et al.*, 2020) as they reported values for protein, ash, and total carbohydrates as 4.92 per cent, 1.25 per cent and 12.31 percent, respectively in husk

4.14 Mineral composition of Smoothie

The mineral analysis of smoothie was evaluated and the minerals that were assessed are Iron, copper, Manganese and zinc and the measured mineral composition is depicted in table 24.

Table 24: Mineral composition of Smoothie

Parameters	Results (mg/100 g)
Iron (Fe)	0.527
Copper (Cu)	0.215
Manganese (Mn)	0.196
Zinc (Zn)	0.768

The results from the Table 24 show that the zinc content was the highest with 0.768 mgfollowed by iron, copper and manganese with the values of 0.527 mg, 0.215 mg and 0.196 mg respectively.

4.15 Textural properties of Smoothie

The viscosity of smoothie was studied and revealed in table 25. This parameter is one of the primary component in providing consistency and acceptance to the product.

Table 25: Textural properties of smoothie

Sample	Viscosity (Pa-s)
Control	0.87
M_2	0.71

Plate 13: M_2 sample with encapsulated psyllium husk having 10^9 cfu/gm

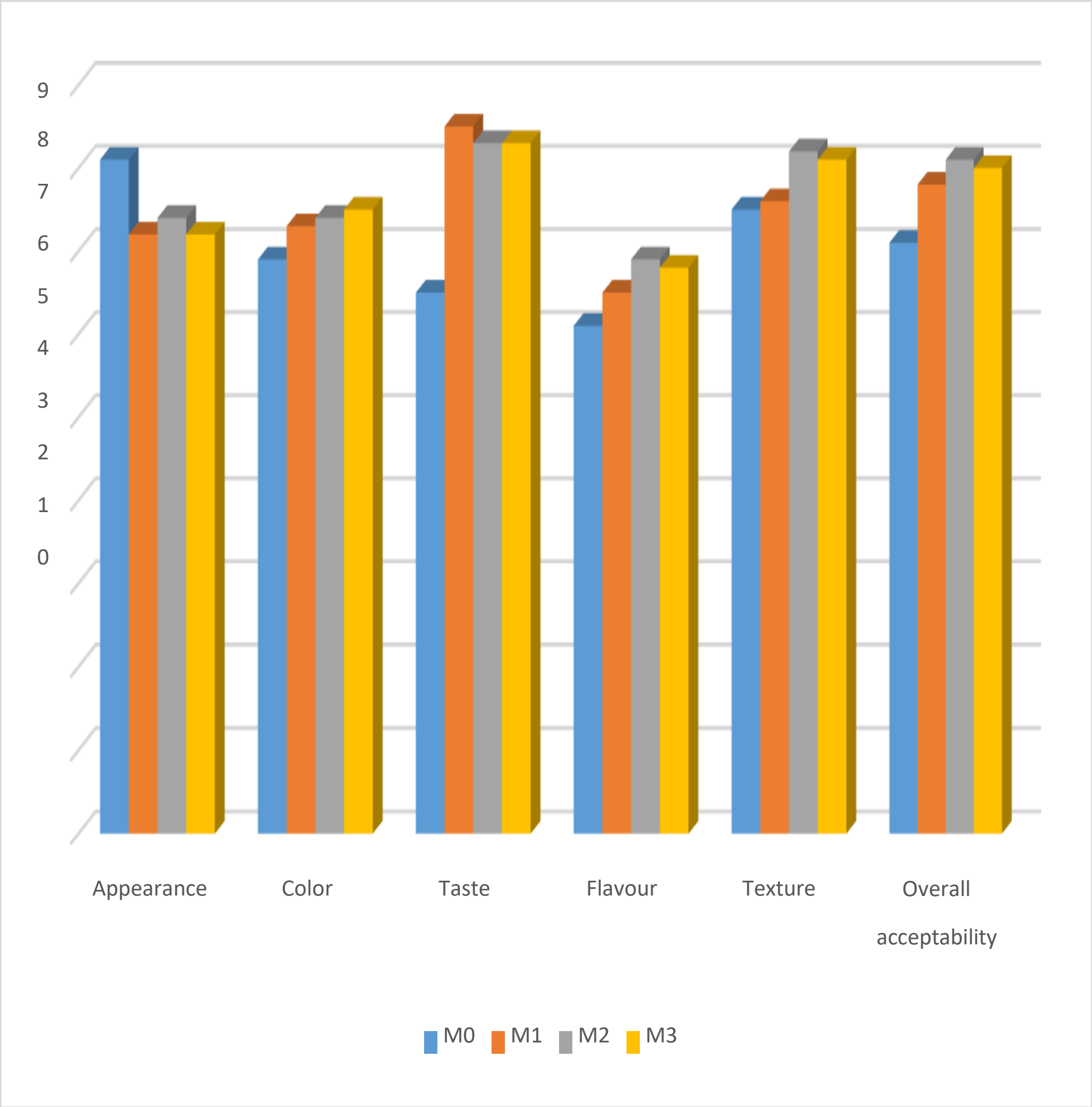

Figure 3: Sensory Evaluation of prepared smoothie using hedonic scale

From the above table 25, results showed that viscosity of control sample with native psyllium husk had more viscosity 0.87 Pa-s when compared to smoothie prepared with modified psyllium husk. Viscosity of the sample M_2 was found to be 0.71 Pa-S. The fruit smoothie viscosity depends upon the blenders and the total blending time taken for the preparation. The particle size decreases upon on more blending time and the necessary viscosity varies according to the addition of different ingredients such as gums, carrageenan, dietary fibres etc.. (Duffy *et al.*, 2015). There was a decrease in viscosity due to reduced swelling of the psyllium particles caused by the acid for the modified psyllium husk. The results for the viscosity of smoothie were supported by the findings of (Sun-Waterhouse *et al.*, 2014) in literature.

4.16 Storage and microbial characteristic of smoothie

4.16.1 Viable counts (LAB) present in smoothie during storage.

The total viable counts of encapsulation beads added accepted smoothie sample C was observed at different storage period on MRS agar media. The results are presented in table 26. It is evident that viable counts are observed at dilutions rate of 10^8, 10^9 and 10^{10}. The graphical representation of the below table is presented in figure 14. Cell viability on MRS agar plates of 1st day and 6th day are shown in plates 14 and 15 respectively.

Table 26. Viable counts (LAB) of smoothie during storage

Time in (days)	Viable count		
	(cfu/ml)x10^8	(cfu/ml)x10^9	(cfu/ml)x10^{10}
0	1.8	1.5	0.7
2	2.1	1.8	1.0
4	2.3	2.1	1.3
6	2.7	2.4	1.5

To achieve the requirements of a probiotic food, a food product should contain at least 10^7–10^8 plate bacteria per gramme or millilitre, according to the Japanese Fermented Milk and Lactic Acid Bacteria Drinks Association (Ishibashi and Shimanura 1993).

4.17 Microbial quality of Smoothie during storage

The final accepted smoothie sample was tested for microbiological characteristics for a period of three weeks. According to (Cappuccino & Sherman, 1996) approach, the accepted drink sample was subjected to microbiological investigations for total plate count, yeast and mould count, and coliform growth over the storage period. The table 27 shows the results of the current inquiry, as well as images of petri plates indicating total plate count, yeast and mould count, and coliforms count, which are displayed in plates 16, 17, and 18 accordingly.

Table 27: Microbial quality of smoothie during storage

Time in weeks	Total Plate Count (cfu/g)x10^4	Yeast and Mold (cfu/g)x10^4	Coliform count (cfu/g) x10^3
1	1.2	ND	ND
2	2.9	1.7	ND
3	3.5	2.6	ND

It is apparent from the table that the highest TPC count was after 3 weeks of storage of the product 3.5 x10^4 cfu/g and the lowest TPC growth was on first week 1.2 x10^4 cfu/g. And the final data revealed that TPC growth growth on first, second and third week was 1.2 x10^4, 2.9 x10^4 and 3.5 x10^4 (cfu/g) respectively. The microbial investigation showed that the TPC growth was increase with increase in duration or storage period. There was no yeast and mold growth in the first week but in the second and third week growth of 1.7 x 10^4 and 2.6 x10^4 (cfu/g) can be seen. This showed that even yeast and mold growth also increased with increase in storage period. Coliform count was not found in the first two weeks under good hygienic and refrigeration of smoothie. It also proves that smoothie is safe to consume. And the above results for TPC, yeast and mold were similar to theresults reported by (Krahulcová, *et al.,* 2021).

4.18 Techno-economical feasibility of smoothie

The total cost of production for 1 kg of smoothie was used to determine the techno-economical feasibility of smoothie. The production costs was estimated using a standard calculation approach, based on raw material cost, processing cost (10% of raw material cost), and packing cost (5% of raw material cost), and the unit cost of production and net cost were evaluated and shown in table 28.

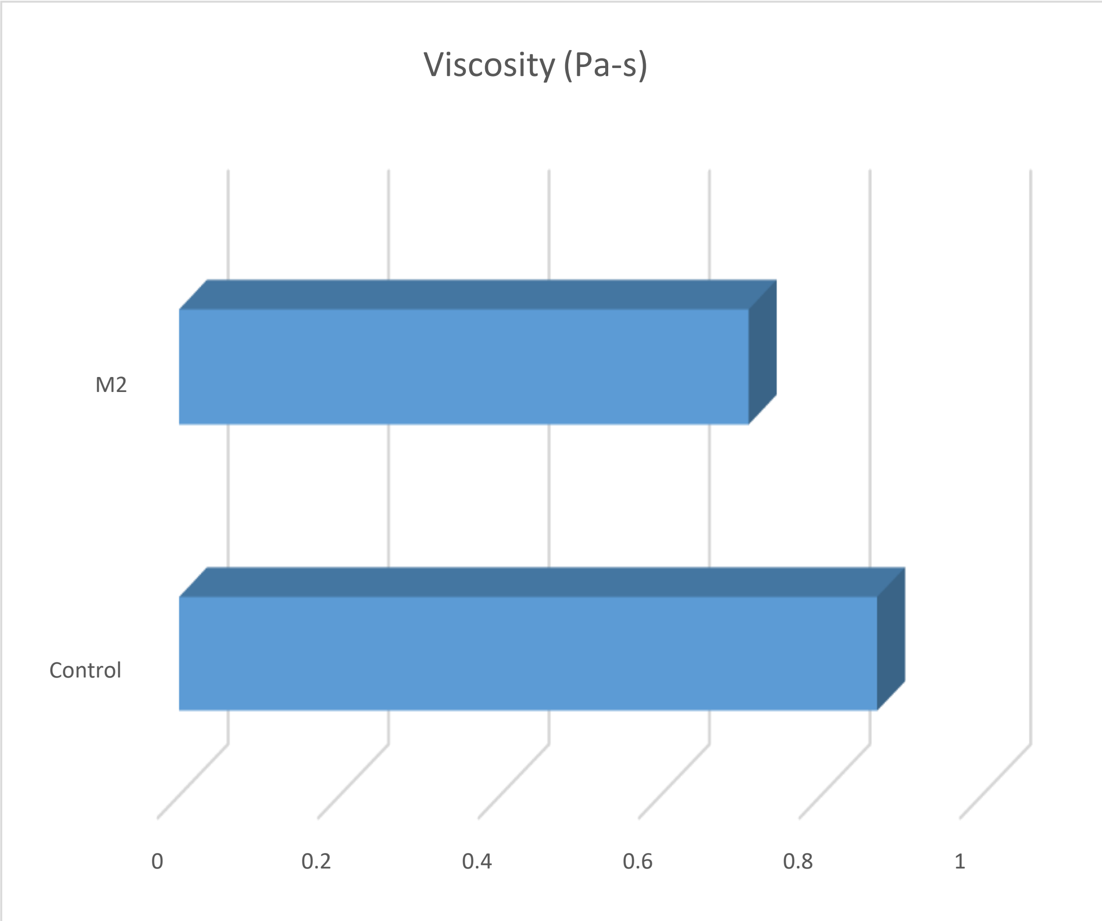

Figure 4: Textural properties of smoothie

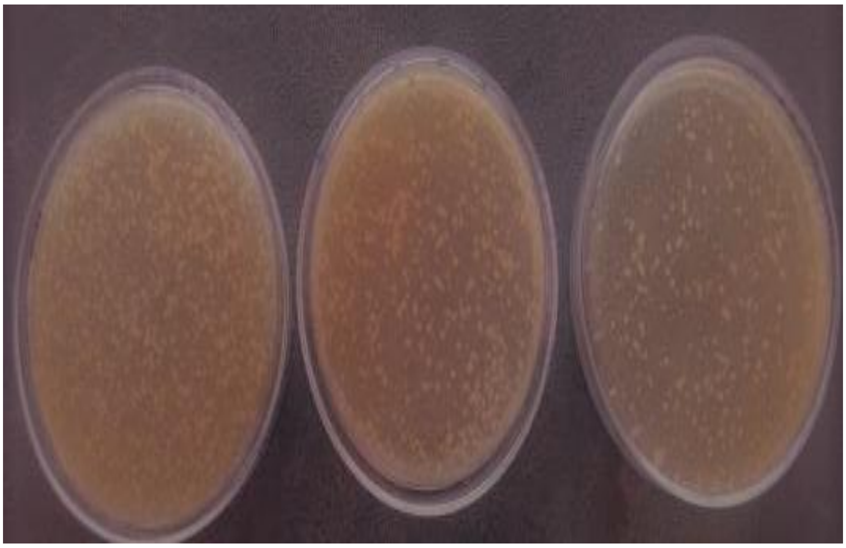

Plate 14: Cell viability on MRS agar plates of probiotic smoothie on 1st day

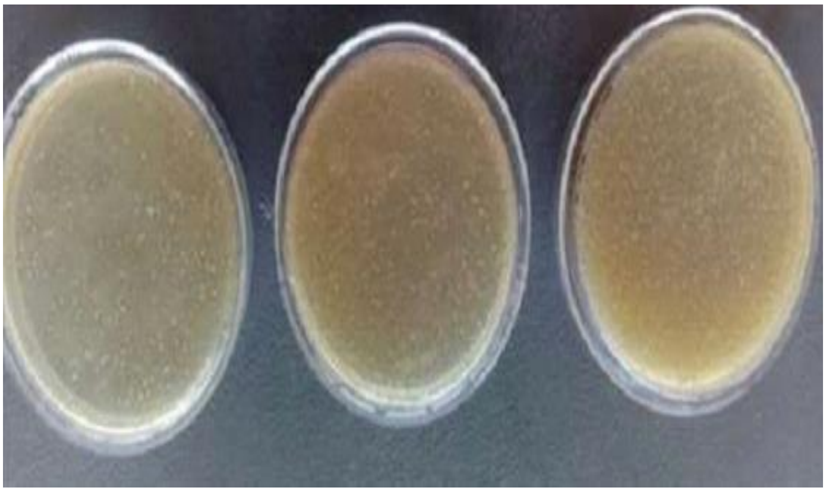

Plate 15: Cell viability on MRS agar plates of probiotic smoothie on 6th day

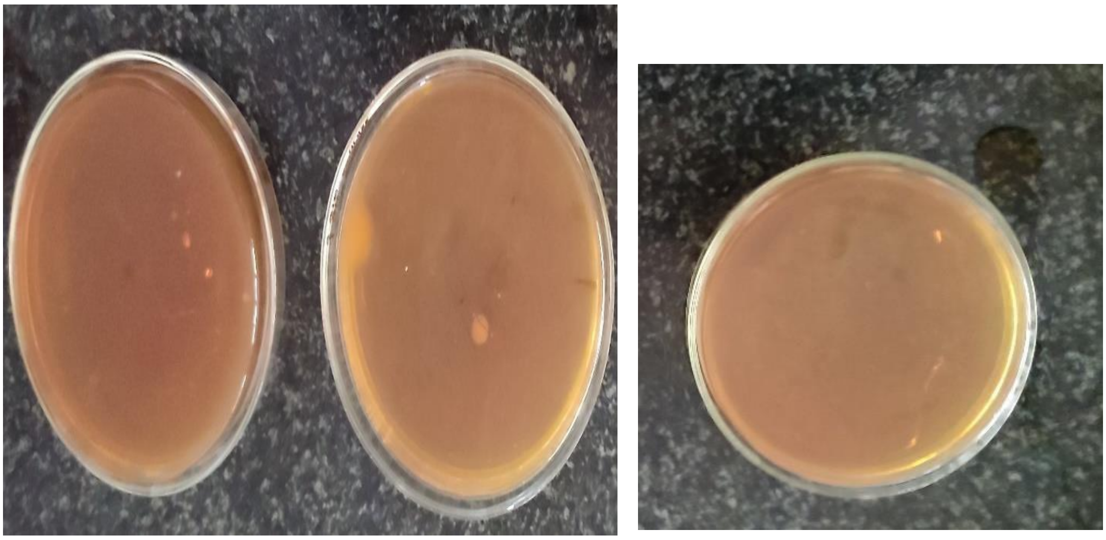

Plate 16: TPC count of smoothie

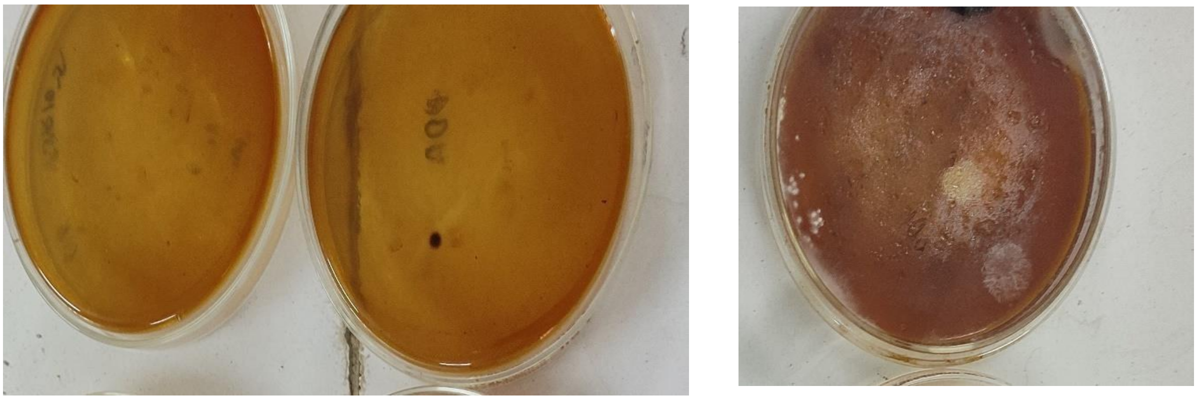

Plate 17: Yeast and mould count of smoothie

s

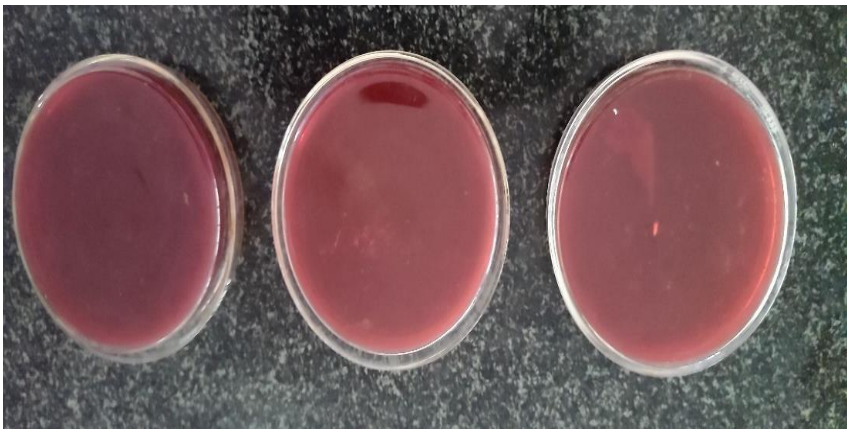

Plate 18: Coliform count of smoothie

Table 28: Techno-economical feasibility of smoothie

Particular	Price for 1Lit / Kg(Rs)	Quantity (in ml /g)	Cost (rs)
Milk	40/lit	400 ml	16
Psyllium husk	100/kg	6.0 gm	0.60
Banana	30/kg	400gm	12
Sugar	40/kg	80gm	3.2
Honey	350/kg	50gm	17.50
water	1/lit	70 ml	0.07
Total raw material cost			49.30
Processing cost (10% of Raw Material Cost)			4.93
Packaging cost (5% of Raw Material Cost)			2.46
production cost of 100 ml of smoothie			5.66
Production cost of 1 lit of smoothie			**56.6**
Production cost of 10 lit of smoothie			566

The table 28 depicts the price of 1 kg/ml of raw material and the cost of producing 1 lit of smoothie from sample M2. The cost of production for 100 ml of milkshake is Rs.5.66, 1 lit of milkshake is Rs.56.6 and the cost of production for 10 kg of sample is Rs.566, assuming a 98 percent yield of product. Overall, the cost of the product was in affordable range on the whole as it bears various health benefits.

CHAPTER-V

SUMMARY AND CONCLUSIONS

CHAPTER V

SUMMARY AND CONCLUSION

In the present investigation entitled under "Studies on use of psyllium husk as a prebiotic in food products". Efforts were made to select the better acid in modification among HCL and H_2SO_4 and the acid with better functional properties was used in the preparation of milkshake and smoothie. The prepared probiotic milkshake and smoothie was analyzed for sensorial, physicochemical and microbial quality parameters for storage study.

The proximate studies showed that the moisture content of native psyllium husk was 6.91 ± 0.07 per cent on whole weight basis, Fat content per cent 1.91 ±0.03 and protein content was 2.31 ±0.01 per cent. And crude fibre, ash and total carbohydrate content of native psyllium husk were found to be 3.2 ± 0.02per cent, 2.64 ± 0.03 per cent and 86.82 ±0.7per cent respectively. The dietary fiber was found to be76.59 ±0.4 and calculated energy value was 371 Kcal/100g.

Mineral composition of psyllium husk was analysed and the results showed that iron and copper content of native psyllium husk was found to be 7.88 mg/100 g and 0.713 mg/100 g respectively while manganese and zinc content was found to be 0.650 mg/100 g and 0.356 mg/100 g. Iron content was found highest among the minerals evaluated.

The evaluation of functional properties were studied and the observed values shows the hydration capacity of native psyllium husk was found to be 3.2 ±0.03 ml/g while the oil absorption capacity and water up-taking rate was 1.19 ±0.02 ml/g and 2.21 ±0.02 mg/g x min respectively.

Efforts have been also made to study the effect of acid modifications with concentration @ 0.75 per cent of 34 to 37 per cent hydrochloric acid (HCl) and Sulphuric acid (H_2S0_4) in ethanol as a solvent. The effect of HCL and H_2S0_4 treatment for psyllium husk solvent ratio @ 1:6 on functional properties such as hydration capacity, oil absorption capacity and water up-taking rate were estimated. The studies have revealed that functional properties were improved after modification by both the acids. The results showed that the hydration capacity of HCL and H_2S0_4 modified psyllium husk was 0.8±0.04 ml/g and 1.5±0.03 ml/g. And the oil absorption capacity of the modified psylllium husks were 0.3±0.02 ml/g and 0.4±0.04 ml/g. The estimated water up taking rate was found to be 1.54±0.05 mg/g x min and 1.62±0.07 mg/g x min.

The functional properties of both the modified psyllium were improved when compared to the native psyllium husk. Furthermore, the native psyllium husk treated with HCL showed better results of functional properties when compared to the native psyllium husk treated with H_2SO_4. And for the preparation of products HCL treated psyllium husk was used as it showed improved functional properties**.**

The textural profile of native and HCL modified psyllium husk was measured. Two particulars, native PSH and selected acid modified PSH were taken for analysis. The hardness of psyllium husk was reduced from 85.5±1.1 gm to 48.3±3.1 gm and adhesiveness was also reduced from 10.8±0.75 gm to 8.4±0.27 gm.

Milkshake

The whole mix of ingredients were stirred at the time of boiling. Ageing of mix was done at 6-10°C for 2-3 hours followed by addition of encapsulated beads aseptically. Then deep freezing of mix was done for -2 to -6 °C and stored at 18°C. The final product of milkshake was prepared with 630 ml milk, 270 gm apple pulp and 100 gm sugar and and encapsulated LAB culture having (10^8, $10^9$and 10^{10} cfu/gm) containing equal proportions of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* and modified psyllium husk.

The organoleptic evaluation of four samples of milkshake was carried out. As per the score of hedonic scale M_2 sample with encapsulated 10 per cent probiotic culture (10_9) cfu/gm and modified psyllium husk had shown maximum consumer acceptability among all samples. The maximum score of milkshake sample was obtained by sample M_2 (i.e. 8.1). Therefore the accepted milkshake sample with highest hedonic score was further analyzed for its physicochemical properties, proximate analysis and microbial analysis.

The proximate composition of milkshake sample was evaluated and the moisture content in psyllium husk was 81.91±0.7 percent and total carbohydrate content found to be 8.21±0.1 percent. It was noticed that Ash, Total fat, Total protein and Crude fiber content were found to be 0.92±0.01 percent, 3.89±0.04 percent, 2.73±0.03 percent, 2.25±0.01 percent respectively. Calculated energy value was found 79 Kcal/100ml. And the viscosity of the selected sample was found to be 0.56 Pa-s and the value was in range of acceptance.

The mineral analysis of the selected milkshake sample showed that the zinc content was the highest with 0.595 mg followed by iron and copper with the values of

0.453 mg, 0.279 mg respectively. The manganese content was the lowest for the sample with 0.153 mg.

The sample was assessed to microbial study during storage period. Microbial examination of the final accepted sample showed no growth of coliforms. Yeast and mold was not detected during the first week, but in the second and third week growth of 2.1 x 10^4 and 2.9 x10^4 (cfu/g) can be seen. And the TPC growth in the first week was found to be 1.6 x104 in the first week, growth in the second and third was found to be 3.5 x10^4 and 4.2 x10^4 (cfu/g) respectively.

Furthermore, this milkshake formulation is technologically feasible and economically suitable for commercialization. Furthermore, the cost of producing 1 lit milkshake is Rs.65.3.

Smoothie

Blending of ingredients was done until obtaining fine thin paste of mixture. , Inoculum at 10 per cent of encapsulated beads were aseptically added to 100ml smoothie. Then the smoothie was refrigerated to 4-10°C for 3hrs and stored at 10°C. The formulation of smoothie was done with 400 ml milk, 400 gm banana pulp, 80 gm sugar, 70 ml water and 50 gm and encapsulated LAB culture having (10^8, 10^9 and 10^{10} cfu/gm) containing equal proportions of *Lactobacillus acidophilus* and *lactobacillus bulgaricus* and modified psyllium husk.

The organoleptic evaluation of four smoothie samples was carried out. As per the score of hedonic scale M_2 sample with encapsulated 10 per cent probiotic culture (10_9) cfu/gm and modified psyllium husk had shown maximum consumer acceptability among all samples. The maximum score of milkshake sample was obtained by sample M2 (i.e. 8.0). Therefore the accepted smoothie sample with highest hedonic score was further analyzed for its physicochemical properties, proximate analysis and microbial analysis.

The prepared smoothie sample was evaluated for its proximate composition and the smoothie sample contained 77.2±0.6 per cent moisture, 4.31±0.06 per cent crude protein. It was observed that the carbohydrate content of prepared smoothie was 11.98±0.2 per cent It was observed that ash content of smoothie was 1.15±0.01 percent. It was observed that fat content and crude fiber of smoothie was 2.85±0.02 and 2.38±0.02 per cent. And the viscosity of the selected sample was found to be in acceptable range with 0.71 Pa-s.

The mineral analysis of the selected smoothie sample showed that the zinc content was the highest with 0.768 mg followed by iron and copper with the values of 0.527 mg, 0.215 mg respectively. The manganese content was the lowest for the sample with 0.196 mg.

The sample was estimated to microbial study during storage period. Microbial examination of the final accepted sample showed no growth of coliforms. Yeast and mold was not detected during the first week, but in the second and third week growth of 1.7 x 10^4 and 2.6 x10^4 (cfu/g) can be seen. And the TPC growth in the first week was found to be 1.2 x10^4 in the first week, growth in the second and third was found to be 2.9 x10^4 and 3.5 x10^4 (cfu/g) respectively.

Moreover, this smoothie composition is both technologically and economically feasible for commercialization. In addition, the cost of creating one litre smoothie is Rs.56.6.

Future studies need to be carried out by performing clinical studies, So that modified psyllium husk can be utilized as a versatile food additive in different food products.

Conclusion

Milkshake

From the present research and study it is proven that milkshake can be prepared with encapsulated beads containing modified psyllium husk, *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* cultures (10^9) Cfu/gm. The prepared milkshake with improved nutritional properties can be explored commercially as an innovative beverage with dietary fiber to improve gut health, which dairy products are lacking.

Smoothie

The developed technology of smoothie by incorporation of encapsulated beads with modified psyllium husk, *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* cultures is a new avenue for dairy industries as it is a low calorie beverage with several health benefits.

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APPENDIX

APPENDIX-I

VASANTRAO NAIK MARATHWADA KRISHI VIDYAPEETH
COLLEGE OF FOOD TECHNOLOGY, VNMKV, PARBHANI

Organoleptic Evaluation of milkshake and smoothie

Date : / /2021

Name of Evaluator :--

Designation : --

You are requested to evaluate the given samples and organoleptic qualities of the given samples. Express the acceptability of product by rating as per the given score point.

Hedonic Rating Scale

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

Sample Code	**Sensory Attributes**					
	Appearance	**Colour**	**Taste**	**Flavour**	**Consistency**	**Overall Acceptability**
Control						
T1						
T2						
T3						
T4						
Remark:						

Signature of the Evaluator

CURRICULUM VITAE

Name of candidate	:	Hemanth M
Date of Birth	:	30/05/1998
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Permanent address	:	Ramamurthy nagar, Bangalore (D) , Karnataka (S)
Mobile No	:	8310050587
Email id	:	Hemanthmj63@gmail.com
Title of thesis	:	STUDIES ON USE OF PSYLLIUM HUSK AS A PREBIOTIC IN FOOD PRODUCTS

Academic qualification

Course/ Degree	Name of college/Institute	University/ Board	Year of passing	Percentage (%)/ CGPA	Class/ Grade
SSLC	NSVK English Medium High School, Bangalore	Karnataka Secondary Education Examination Board	2013	81.28	First class
PUC	New horizon pre university college, Bangalore.	Government of Karnataka , Department of Pre-University Education	2015	81.33	First class
B. Tech (Food Science and Technology)	College of Agriculture, Hassan.	UAS, Bengaluru	2019	7.25	First class

Place: Parbhani
Date : 22/11/2021

Signature of the candidate
Hemanth M