PROCESS OPTIMISATION AND QUALITY EVALUATION OF PASSION FRUIT BASED PROBIOTIC DRINKS

By

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(2018-16-002)

DEPARTMENT OF COMMUNITY SCIENCE

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680 656

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THESIS

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KERALA, INDIA

2020

DECLARATION

I, hereby declare that the thesis entitled "**Process optimisation and quality evaluation of passion fruit based probiotic drinks**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed during the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Introduction

1. INTRODUCTION

The deeply entwined relationship between food and health benefits has been a fertile field for research since the dawn of the scientific age. This in turn has triggered the development of functional food products. FFC (2011) defined functional food as "Natural or processed food that contains known or unknown biologically active compounds; which, in defined, effective non- toxic amounts, provide a clinically proven and documented health benefit for the prevention, management, or treatment of chronic disease." Probiotic food is an example for such type of food which provide various beneficial effects on human body.

Probiotics are live microbial supplement, which beneficially affect the host by improving the intestinal microbial balance. Addition of probiotics to food provides several health benefits like decreasing the number of pathogenic gastrointestinal microorganisms, reducing the serum cholesterol level, improving the gastrointestinal function, strengthening immune system, protection of proteins and lipids from oxidative damage and has anticarcinogenic and antimutagenic effects.

The growing demand for probiotics has widened the scope for innovation and development of new probiotic products. The widely used probiotic strains are *lactobacilli*, *bifidobacterium* and *streptococci*. *Lactobacillus acidophilus* is one of the most common probiotic bacteria which have beneficial effects on the microbiota of the gastrointestinal tract.

Probiotic products are usually marketed as dairy products. This initiated the development of non dairy based probiotic products. The presence of vitamins, minerals, antioxidant compounds, dietary fibres and minerals, makes fruits and vegetables ideal vehicles for probiotic culture. However, some intrinsic characteristics of fruits and vegetables like high concentration of organic acids, high amount of water and low pH pose challenge to the maintenance of the viability of probiotics.

The incorporation of probiotics to underutilised fruits can improve their acceptability and market potential. Such products may also have better profile of nutrients and therapeutic value. Yellow passion fruit (*Passiflora edulis flavicarpa*), which is native to tropical America, is considered as an underutilized fruit crop and

considered to be a good source of vitamins, like A and C, and minerals. Considering these factors, passion fruit can act as a potential matrix for the incorporation of probiotics. If a probiotic product is developed from this fruit, it would definitely attract consumer attention and improve its economic value.

Hence, the present study entitled “Process optimisation and quality evaluation of passion fruit based probiotic drink” was undertaken with the following objectives

1. To standardise passion fruit drinks with *L. acidophilus*
2. To evaluate the nutritional, organoleptic and shelf life qualities of the developed drinks.

Review of literature

2. REVIEW OF LITERATURE

The literature pertaining to the study entitled "Process optimisation and quality evaluation of passion fruit based probiotic drinks" is presented under the following headings.

2.1. Nutritional and therapeutic importance of passion fruit

2.2. Value added products of passion fruit

2.3. Probiotics: A general review

2.3.1. Probiotic - History and definition

2.3.2. Types of probiotic organism

2.3.3. *Lactobacillus acidophilus* as probiotics

2.3.4 Market potential of probiotics

2.4. Probiotic beverages

2.4.1. Fruit beverages

2.4.2. Vegetable beverages

2.4.3. Milk based beverage

2.4.4 Other probiotic beverages

2.1. Nutritional and therapeutic importance of passion fruit

India is the second largest producer of fruits in the world. There is a difference between the production and net availability due to the improper post harvest operations. Many fruits are grown in the homesteads of Kerala which are underutilized and considered as minor crop. In India, studies conducted on passion fruit and its post harvest handling is limited. So, the research on this aspect has better scope in extending the shelf life of fruits, minimizing the loss by enhancing the storage life and also improving the nutritional quality of the product which have better market in future (Kishore *et al*., 2010).

Passion fruit (*Passiflora edulis*), a native of tropical America (Brazil) belonging to the family passifloracae, is a minor fruit in India. It bears delicious fruits of two types, purple (*Passiflora edulis f. edulis*) and yellow (*Passiflora edulis f. flavicarpa*)

(Vanderplank, 1991). It is a perennial, climbing, woody vine producing round or ovoid fruits which have smooth, waxy dark purple/yellow coloured rind with fine white specks in mesocarp. The fruit have orange coloured pulpy juice with large number of small, slightly hard, dark brown seeds covered with mucilaginous substance. Rao *et al*. (2014) observed that high acidic nature, low juice content and presence of large number of seeds makes it unsuitable for table purpose. The juice is delicious with good flavour, intense aroma and sweet-acid taste and is well known for its excellent blending quality.

Passion fruit stands out not only for its exotic and unique flavour and aroma but also for its amazing nutritional and medicinal properties. The fruit contains crude fiber (22.1%), total phenol (3.32 ± 0.6 GAE/100 g), pectin (12.5%,), starch, protein, polysaccharides (20.62g/100g), flavonoids (1180.67 ± 16.73 mg/100 g) and other substances (Wen *et al*., 2008). Vitamin C values have been reported as 40mg in 100g of natural passion fruit juice (Souci *et al*., 2000). Deng *et al*. (2013) concluded that this fruit is rich in aroma and nutrients and have more than 135 aromatic compounds which also contains citric acid, L-malic acid, L-lactic acid, L-ascorbic acid and other seven kinds of organic acids. Passion fruit contains iron, copper, manganese, zinc, selenium and 21 kinds of trace elements. Histidine, arginine, glutamic acid and 17 kinds of amino acids are also present in this fruit. (Wang *et al*., 2015). A 100g of fruit contains 75.8 per cent water, 63 kcal, 9.5g carbohydrates, 0.4g lipids, 2.4g proteins, 1.5g dietary fibre, 3.9g organic acids and 0.9g minerals.

The fresh whole fruit have a very low shelf life of one week. Because of this perishable nature, passion fruits produced during a particular season results in abundance in the market and become scarce during other seasons. Its use is limited to fresh consumption, pulp, juices and blended beverages which can be stored for only 2-3 months (Jena, 2013). This opens up avenues for development of other value added products like preserve, fruit juice, fruit wine, jam and jellies, fruit vinegar and so on. Passion fruit peel can be processed into animal feed or extracted pectin, dietary fiber and so on (Lin, 2014).

Passion fruits are not only nutritious but also has a variety of health care functions, such as refreshing, solve thirst, help digestion, improve renal function, eliminate fatigue and other effects (Liu *et al*., 2017). Passion fruit contains anti inflammatory, anticonvulsant, antimicrobial, anticancer, antidiabetic, antihypertensive,

antisedative, antioxidant properties and is used in treating conditions like osteoarthritis, asthma and also act as colon cleanser. The different parts of the plants have also been used for treatment of ulcers, haemorrhoids, as sedatives, remedy for insomnia, digestive stimulant and remedy for gastric carcinoma (Thokchom and Mandal, 2017).

2.1.1. Acidity

Passion fruit has been widely consumed because of its high aroma and acidity, especially as juice, and has also been used in a wide variety of products such as ice-creams, mousses, alcoholic beverages and others. According to Lancashire (1997), passion fruit is a high acid fruit (pH ~3.2), due to the predominanace of two acids, citric acid (~93-96% of total) and malic acid (3-6%) and also determined the amount of different acids of 2 different types of passion fruits, purple and yellow passion fruit to be 13.1 meq/100 and 55meq/100 respectively.

Titratable acidity in seven passion fruit (*Passiflora* spp.) cultivars: *P. edulis* cultivars Purple, Frederick, Yellow, Pink, *P. edulis f. flavicarpa, P. maliformis* and *P. quadrangularis* was determined by Ramaiya *et al*. (2012), and reported that yellow type (*P. edulis f. flavicarpa*) recorded the highest titratable acidity (3.03 ± 0.19%) whereas *P. quadrangularis* recorded lowest titratable acidity (0.88 ± 0.05%).

The titratable acidity of organically produced passion fruit was found to have higher concentration of citric acid content (4.32g/100 ml) than that of conventionally cultivated fruit (3.81 g/ 100 ml) (Janzantti *et al*., 2012).

Patel *et al*. (2014) observed the conversion of organic acid to sugars and decrease in the biosynthesis in later stages of maturity resulted in a decrease in the acidity of the fruit.

2.1.2. TSS

Dasilva *et al*. (2005) reported an increasing amount of TSS during maturation of passion fruits ranging from 10.2°Brix to 16.8°Brix upon ripening. According to Cerqueira *et al*. (2011), the soluble solids content depends on the maturity stage and it generally increases progressively during the ripening process due to the hydrolysis of polysaccharides to maintain the respiration rate.

The total soluble solids of different genotypes of passion fruit (*Passiflora* spp.); Megha purple, Nagaland purple, Kerala yellow, RCPS- 1, Panama yellow was 15^{o} Brix, 14.8^{o} Brix, 16.2^{o} Brix, 16.6^{o} Brix and 17.2^{o} Brix, respectively (Patel *et al.*, 2014).

Mahajan *et al*. (2006) estimated the reduction of TSS on high temperature storage which is due to faster metabolic rates of the fruit during storage. Similar result was observed by Kishore *et al*. (2010), who reported that TSS reduction during high temperature storage was due the hydrolytic changes of carbohydrates present in the fruit.

Neves *et al*. (2013) concluded that the reason for the intense pulp colour of ripe passion fruit have a direct correlation with the TSS content of the fruit.

2.1.2. Sugars

Passion fruit is a tropical fruit that has a low glycemic index (GI) value of 30. The sugar present in fruit does not cause a steep increase in blood sugar and also insulin levels will be maintained (Galdeano and Perdigon, 2004) by the slow absorption into the bloodstream.

Chinnici et al. (2005) concluded that the sugar content in fruit can influence the physiochemical properties like pH, total acidity, microbial stability and also can provide valuable information on food wholesomeness. According to Ramaiya *et al*. (2012), total sugars of purple and yellow types have higher concentration *ie*., 142.85 ± 0.17 g/kg and 139.69 ± 0.12 g/kg respectively compared to the other seven different cultivars: *P*. *edulis* cultivars Purple, Frederick, Yellow, Pink, *P*. *edulis f*. *flavicarpa*, *P*. *maliformis* and *P*. *quadrangularis.*

According to Adeyeye and Aremu (2017), passion fruit have different sugars: dextrose (0.54 g/100g), fructose (0.59 g/100g), maltose (1.00 g/100g), hydrated lactose (0.71 g/100g) and anhydrous lactose (0.78 g/100g).

2.1.4. Protein

According to Ramaiya *et al*. (2012), passion fruit have good protein content compared to other commercial fruits like pineapple, orange, papaya and apple which have comparatively lower values. The protein ranged from 1.13±0.11 % to 2.81±0.19 % in *P*. *quadrangularis* and *P*. *edulis* (purple) respectively.

Malacrida and Jorge (2012) concluded that the passion fruit seeds have higher protein percentage (12.23%) which was similar to that of some cereal grains like corn (10.2%), oats (11.3%) and wheat (12.2%) also the passion fruit seeds have high percentage of carbohydrates and fibre (48.73%).

Passion fruit concentrate contain 2.29 g of protein and also an antifungal protein has been isolated from seeds of the passion fruit (*Passiflora edulis*) known as passiflin. Passiflin specifically inhibits the fungus *Rhizoctonia solani* and also suppresses proliferation of breast cancer (Agizzio *et al*., 2003).

2.1.5. Dietary fibre

Dietary fibre helps the bowel function and are considered prebiotic; soluble fibres retard intestinal passage, gastric emptying and glucose uptake, helping to reduce blood cholesterol and insoluble fibres accelerate intestinal transit, increasing the fecal volume, slowing down glucose hydrolysis, contributing to the reduction of some colon diseases and serve as a substrate for beneficial microorganisms such as probiotics (Yapo and Koffi, 2008).

Passion fruit is a rich source of fibre which keeps the bowel healthy and moving. Passion fruit lowers cholesterol and risk for diabetes, heart disease, and certain kinds of cancer. Lopez- Vargas *et al*. (2013) determined the amount of total dietary fibre, insoluble dietary fibre and soluble dietary fibre as 71.79 g/ 100g, 52.34 g/ 100g and 19.49 g/ 100g respectively.

Since the consumption of insoluble fibres is beneficial to intestinal peristalsis by increasing fecal bulk and decreasing transit time, passion fruit could be a good source of insoluble fibre with desirable physiological effects (Gordon, 1989). According to Ferrari *et al*. (2004), passion fruit seeds contain 64.8 per cent of total fibre in their composition. Insoluble dietary fibre (IDF) (84.9g/ 100g) was the predominant fibre fraction (98.8%) of total dietary fibre.

2.1.6. Vitamin and Minerals

Passion fruit is known to be rich in minerals like magnesium, calcium, iron phosphorous, potassium and sodium. These minerals maintain bone density, help in speedy recovery of bones and prevent osteoporosis. Potassium helps in vasodialation which in combination with copper and iron helps in RBC production and eventually

improves the metabolism of body. Minerals regulate the metabolism of several enzymes, osmotic pressure, muscular and neurological activity, facilitate the transfer of essential compounds through membranes and, in some cases, are part of the constituent elements of body tissues (Novaes *et al*., 2017).

According to De Souza *et al*. (2012), the major minerals present in passion fruit are phosphorus (34.95 mg), potassium (375.42 mg), calcium (4.76 mg), magnesium (19.82 mg) and iron (1.06 mg) in 100g of pulp.

Vitamin A helps to improve vision, vitamin C acts as antioxidant and the rich content of riboflavin (Vitamin B6) and niacin (Vitamin B3) in passion fruit helps in regulating the thyroid activity in the body and also prevents hardening of the arterial walls of the heart (atherosclerosis), keeping heart functions running smoothly. The phenolic compounds and alkaloids can also help in relieving anxiety and treating insomnia to a certain level (Septembre-Malaterre *et al*., 2016).

Passion fruit is a tropical species rich in vitamin C, B2 and B3, β-carotene, as well as in minerals and fibres (de Oliveira *et al*., 2017). Staughton (2020) estimated the quantity of vitamin A, vitamin C, Vitamin B2 (riboflavin) and vitamin B3 (niacin) as 64 µg, 30mg, 0.13mg and 1.5 mg in 100g of fruit respectively.

The iron content of passion fruit is 1.60 mg which helps in purification of blood and also blood production. This also helps in enhancing immunity by increasing haemoglobin in our red blood cells (Correa *et al*., 2016).

2.1.7. Antioxidants

Antioxidants are compounds that inhibit oxidation and scavenge the free radicals that lead to damage of the cells of organisms (Jiang *et al*., 2010). Antioxidant compounds like phenolic acids, polyphenols, flavonoids, beta-carotene, lutein, lycopene, selenium, vitamin A, vitamin B, vitamin C, etc. are the scavengers of free radicals (Murshid, 2013).

Passion fruit (*Passiflora edulis*) is also a rich source of bioactive compounds, which reduce oxidative stress. Phenolic compounds in passion fruit have different therapeutic effects like immuno modulation, anticarcinogenic and antioxidant activities (Dasilva *et al*., 2012). Passion fruit contains a high amount of Vitamins A and C, and

other phenolic compounds that helps in the prevention of cancer. Free radicals are one of the prominent causes of cancer which can be neutralized by antioxidants present in the fruit and protect us from cell damage and cancer. The flavonoids in Passion fruit further enhance its potency against various types of cancer (Tadimalla, 2019).

According to Staughton (2020), antioxidants present in passion fruits help in prevention of plaque formation and artery blockages is caused by accumulation of cholesterol and other substances in blood vessels. It relaxes the tension of blood vessels and promotes increased blood flow. This reduces the strain on the heart and increases overall cardiovascular health.

According to Ramaiya *et al*., (2012), the total antioxidant activity (TAA) of seven different passion fruit cultivars range from 409.13 to 1964.90 μmol Trolox/litre which was determined by using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method.

2.1.7.1. Ascorbic acid

Vitamin C, or ascorbic acid (AA), is a hydrosoluble and thermolabile vitamin (Zhang and Hamauzu, 2004). Genovese *et al*. (2008) determined that two forms of ascorbic acid (L-ascorbic acid and L-dehydro ascorbic acid) is mainly found in organically cultivated passion fruit and in these L- ascorbic acid is the major portion (64 mg/ 100g).

According to Sema and Maiti (2006) the major antioxidants present in passion fruit are vitamin C, beta carotene and polyphenols. Ascorbic acid content in fresh passion fruit juice from different cultivars was determined by Uchao *et al*. (2008) and *P*. *edulis* (Purple) showed the highest mean ascorbic acid content (0.32 ± 0.72 g kg−1) compared to other *Passiflora* cultivars. According to Ramaiya *et al*. (2012) vitamin C is an important antioxidant which supports the immune system and helps in healthy aging. Beta carotene is converted to Vitamin A within the body which is essential for good eyesight.

As per Valente *et al*. (2011), Vitamin C is a water soluble vitamin and is one of the key antioxidant nutrient which plays a crucial role in preventing losses and maintaining required level of antioxidants in our body. Ascorbic acid helps in production of collagen, hormones, plays vital role in immunity and works as anti- histamine during nasal congestion due to activation of histamine.

2.1.7.2. Carotenoids

Food colour is due to the presence of pigments, among which carotenoids (red and yellow compounds found in fruits, flowers, leaves and animal fats) plays an important role. Holden *et al*. (1999) determined the presence of carotenoids such as β-cryptoxanthin, prolycopene, cis-z-carotene, z-carotene, β-carotene, a-carotene and 13 - cis-b-carotene in yellow passion fruit juice. Carotenoid content depends on fruit origin and on the enzymatic reactions that produce these pigments.

Carotenoids are unstable compounds due to the presence of highly conjugated double-bond structure. They are synthesized from the initial stage of fruit formation and then degrade towards the end of maturity (Mendes-Pinto, 2009).

The total carotenoid content in passion fruit ranges between 27,600 to 35,400 µg/100g. The accumulation of carotenoids in passion fruit is variable according to the stage of maturity and systems of cultivation (Pertuzatti *et al*., 2015).

In passion fruit 13 different carotenoids were identified including zeta-, beta- and alpha- carotene, beta- cryptoxanthin and lycopene (Sema and Maiti, 2006).

Franco *et al*. (2013) reported that carotenoids come from carotenes in which hydroxyl, carbonyl, epoxy or carboxyl groups have substituted for atoms of hydrogen or for carbon oxygenated derivatives.

Garcia-Ruiz *et al*. (2017) reported that β-carotene is the main dietary source of vitamin A, essential for normal growth and development, immune system function and vision which has antioxidant properties that can decrease the risk of developing chronic degenerative diseases such as cardiovascular disease and cancer..

2.1.7.3. Total phenols

Evans and Miller (1996) identified that non-nutritive phyto-chemicals found in passion fruit are poly-phenolic compounds that have antioxidant properties. Phenolic compounds are secondary metabolites widely found in fruits, mostly represented by flavonoids and phenolic acids. Phenolic compounds can avoid the oxidative damage that leads to ageing and age-related diseases by scavenging the free radicals from cell metabolism. Polyphenols are plant compounds that have antioxidant and anti-

inflammatory effects. They may reduce the risk of chronic inflammation and conditions like heart diseases (Kurosumi *et al*., 2007).

Phenolic compounds are the most important natural antioxidants found in fruit that protect cells against oxidative stress. Some phenolic compounds have been characterized in *Passiflora* spp. pulp, including piceatannol and caffeic, p-coumarin, and ferulic acids (Gil *et al*., 2014). Piceatannol is a polyphenol that may improve insulin sensitivity with excess weight, potentially reduce type 2 diabetes risk when taken as a supplement (Kitada *et al*., 2017) Phenolic components act as antioxidant, anti mutagenic agent, free radical scavenging agent and also prevention of cancer and cardiovascular disease.

Song *et al*. (2018) determined the phenolic content of fresh inedible portion of passion fruit (288 mg /100 g) and fresh edible portion of passion fruit (1018 mg/100 g). The content of phenolics in the edible portion was higher than that in the inedible portion which may be due to genetic variation, environmental conditions or extraction methods.

Sano *et al*. (2011) isolated scirpusin B, a major polyphenolic compound present in passion fruit especially in seeds after piceatannol. This polyphenol have vasorelaxant effects and also exert antioxidant activity. This can be for curing cardiovascular diseases.

2.1.7.4. Flavonoids

Flavonoids are group of bioactive compounds, classified into subgroups based on their chemical structure: flavanones, flavones, flavonols, flavan-3-ols, anthocyanins and isoflavones. Their regular consumption reduces risk of a number of chronic diseases like cancer, cardiovascular disease (CVD) and neurodegenerative disorders (Kozlowska and Szostak-Wegierek, 2014).

Dhawan *et al*. (2004) concluded that C- glucoside flavone is the major flavonoid in *P*. *edulis* pulp extracts. Total flavonoid content in *P*. *edulis* pulp is significant in comparison with other beverages like orange juice and sugarcane juice and the major flavonoid found was isoorientin (Zeraik and Yariwake, 2010). Other

flavonoid compounds, such as orientin, isovitexin, luteolin 6-*C*-chinovoside and luteolin 6-*C*-fucoside, are also found in the fruit of *P. edulis* (Li *et al*., 2011).

Passion fruit is a good source of bioflavonoids, i.e., chrysin, apigenin, kaempferol, quercetin, apigenin and genistein, etc., which have therapeutic potentials as antioxidants, immuno-modulators, antianxiety agents and anticarcinogens. Chrysin form complexes with fluorine which is a very common industrial pollutant. Chrysin as well as other flavonoids of passion fruit appear to exert a beneficial effect in chronic exposure to industrial toxins including fluorine compounds (Liwiec *et al*., 2000).

2.3. Probiotics: A general review

2.3.1. Probiotic - History and definition

'Probiotic' is a Greek word which means 'for life'. Elie Metchnikov was credited with the development of food containing beneficial bacteria and postulated that intestinal auto intoxication and subsequent aging can be prevented by altering the gut microbiota with beneficial microbes. He developed fermented milk with organism called "Bulgarian bacillus" (Metchnikoff, 1907).

Alfred Nissle during 1917 isolated a non-pathogenic strain of *Escherichia coli* from the faeces of two soldiers who were not affected with the outbreak of dysentery (shigellosis) during World War I. He discovered *E. coli* Nissle strain 1917, which helped in the inhibition of colonisation of pathogenic bacteria and was then further used in different medical field. With the secretion of certain bacteriocins, this strain inhibits the adherence of pathogenic bacteria (Lodinova *et al*., 1967).

Another probiotic bacteria *Bifidobacterium* from a breast fed infant was isolated by Henry Tissier and named the bacterium as *Bacillus bifidus communis*. He claimed that this organism displaces the probiotic bacteria causing diarrhoea in infants (Crociani *et al*., 1995).

Lilly and Stillwell (1965) were the first to use the word 'probiotics' to describe the production of one protozoan by the growth of another. "Probiotics are live microbial feed supplements which beneficially affect the host animal by improving microbial balance" was the most used definition by Fuller (1989) and later Fuller (1999) used the word to describe the beneficial effects of the tissue extracts that stimulated microbial growth and animal feed supplements by contributing to their intestinal flora balance.

FAO/WHO (2001) defined probiotics as 'live microorganisms which when administered in adequate amount confer a health benefit on the host'.

Schrezenmeir and de Vrese (2001) proposed that 'a preparation of or a product containing viable defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and that exert beneficial health effects on this host'.

2.3.2. Types of probiotic organism

Bacteria and yeast can be considered as common probiotic organisms. Among the organisms *Lactobacilli*, *Bifidobacteria* and *Saccharomycetes* are the common ones.

According to Krishnakumar and Gordon (2001) and Heyman and Menard (2002) the major organism which are used for preparation of probiotics include *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus casie*, *Lactobacillus lactis*, *Lactobacillus helviticus*, *Lactobacillus salivarius*, *Lactobacillus rhamnosus*, *Lactobacillus johnsonii*, *Lactobacillus reuteri*, *Lactobacillus fermentum*, *Lactobacillus delbrueckii*, *Lactobacillus gasseri*, *Lactobacillus plantarum*, *Bifidobacterium bifidum*, *B.longum*, *B.breve*, *B.infantis*, *B.lactis*, *B.aadoescentis* and *Escherichia coli*.

Saccharomyces cerevisiae (wine, bread, beer), *Saccharomyces bayanus* (wine) and *Saccharomyces boulardii* are types of yeast used for medicine prepartion as probiotic. During kefir preparation *Saccharomyces* yeasts form symbiotic matrices with probiotic bacteria (Witthuhn *et al*., 2004).

Suvarna and Boby (2005) included other probiotic species into the list like *Streptococcus thermophillus*, *Enterococcus faecium*, *Enterococcus faecalis*, *Sacchromyces boulardi*, *Sacchromyces cerevisiae*, (Anuradha and Rajeshwari, 2005). Majority of the probiotics are gram positive bacterias (Khetarpaul, 2005).

Lactobacillus genus bacteria are gram positive, facultative, anaerobic or microaerophilic rod-shaped bacteria. Major part of the lactic acid bacteria (LAB) group includes *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Oenococcus*, *Pediococcus*, *Strept ococcus* and *Leuconostoc* species. The LAB produce an acid environment which inhibits the growth of harmful bacteria (Makarova *et al*., 2006).

Lactobacillus acidophilus, *L. casei*, *L. paracasei*, *L. rhamnosus*, *L. delbrueckii* subsp. *bulgaricus*, *L. brevis*, *L. johnsonii*, *L. plantarum* and *L. fermentum* are commonly used as probiotic products even though they are indigenous inhabitants of the human intestinal tract (Kailasapathy and Chin, 2000).

Strains of *bifidobacteria* that are used as probiotics are *Bifidobacterium infantis*, *B. adolescentis*, *B. animalis* subsp *animalis*, *B. animalis* subsp *lactis*, *B. bifidum*, *B. longum* and *B. breve* (Ruiz *et al*., 2013).

Escherichia coli Nissle 1917 (EcN) together with other probiotics can be used to treat constipation (Chmielewska and Szajewska, 2010)

Bacillus coagulans is another probiotic organism which is combined with other organism for preventing antibiotic-associated diarrhoea (Hempel *et al*., 2012).

2.3.3. *Lactobacillus acidophilus* as probiotics

Lactobacillus acidophilus is a well tested probiotic which is safe for human consumption and provide several health benefits.

Gilliland *et al*. (1985) reported that while growing inside the intestine, *L. acidophilus* itself take up the cholesterol and subsequently reduce the absorption into the blood stream.

The reason for reduction in cholesterol upon consumption of *L. acidophilus* is due to deconjugation of bile acids, which prevents the absorption of lipids (Klaver and Meer, 1993).

Michetti *et al*. (1995) reported that secretory components of *L. acidophilus* have antimicrobial effect on *Helicobacter pylori* which causes peptic ulcer.

Lactic acid bacteria present in acidophilus milk helps in reducing lactose malabsorption by increasing the lactase activity in small intestine (Onwulata *et al*., 1989).

Study published by Annals of Internal Medicine concluded that consumption of *L. acidophilus* can reduce the reccurence of vaginal infection which is caused by *candida* (Hilton *et al*., 1992).

Udani (1999) reported that consumption of *L. acidophilus* helps to prevent traveller's diarrhoea and does not have any side effect. These organism rapidly hydrolyze lactose and produce lactic acid and also some bacteriocins which makes a hostile environment for other organism.

Ishida *et al*. (2005a) suggests that oral intake of *L. acidophilus* L-92 helps in reduction of symptoms of perennial allergy rhinitis which causes hay fever like symptoms. Similarly, study done by Ishida *et al*. (2005b) concluded that, incorporation of the same strain helps in reduction of symptoms of Japanese cedar pollen allergy.

According to *in vivo* study done by Maroof *et al*. (2012), oral administration of *L. acidophilus* alters the cytokine production in tumor bearing group by protecting TH cells, activating antitumoral cells and increasing lymphocyte proliferation.

El deeb *et al*. (2018) concluded that *L. acidophilus* LA-EPS-20079 pentasaccharide have direct effect on cytotoxic action on the tumors cells through apoptotic mechanisms and also stimulate the immune response of the cells.

According to Pakdaman *et al*. (2015), lactic acid bacteria *L. acidophilus* helps to digest lactose present in fermented dairy products and is beneficial to people suffering from lactose intolerance.

Schiffrin *et al*. (1995) documented the enhancement in the activity of non specific immune phagocyte of granulocyte population in blood of individuals consuming *L. acidophilus*. Weiss *et al*. (2010) opined that *L. acidophilus* have the capacity to develop viral defence phenotype in bone marrow derived murine dentritic cells.

Nuclear factor kappa B (NF-kB) and endoplasmic reticulum (ER) stress are the factors responsible for pathogenesis of inflammatory bowel disease (IBD). *L. acidophilus* acts as immunomodulator by interfering ER stress and suppressing NF-Kb, will regulate the stress and have significant effect on regulating IBD (Kim *et al*., 2019).

Significant decrease in abdominal pain and flatus was observed in individuals consuming *L. acidophilus* for 4 weeks (Sinn *et al*., 2008) and similar result was observed by Sadrin *et al*. (2020).

Yadav *et al*. (2008) observed delayed onset of hyperglycemia, dyslipidemia, hyperinsulinemia, glucose intolerance and oxidative stress on supplementation with probiotic dahi (*L*. *acidophilus*) and also reported lower risk of complications of diabetes.

Andreasen *et al*. (2010) reported that *L*. *acidophilus* decreased the insulin resistance and inflammatory markers in humans. Similarly, Vajro *et al*. (2011) concluded that incorporation of specific strains of *Lactobacilli* and *Bifidobacteria* help to cure obesity and diabetes, and also suggest that the probiotic mediated modulation of the gut flora can be a potential therapy against the same.

2.3.4 Market potential of probiotics

Probiotics are live microorganisms when consumed in adequate amounts provide health benefits on the host (Salminen *et al*., 1998). Foods containing these live microorganism fall in the category of functional foods. These products are gaining widespread acceptability throughout the world.

Japan is considered as the place of origin of the term functional food and the concept of developing food which medically beneficial was evolved during 1980s. The term defines the fortified food which have health effects on consumer (Stanton *et al*., 2001).

Japan provided functional food legal status which are described as FOSHU (Foods for Specific Health Use) which has been licensed for a label to that effect (Berner and O'Donnell, 1998).

According to Hilliam. (1998), probiotic dairy products especially probiotic yoghurts and milk was the most active area of functional food market. Sixty five per cent of European functional foods market was probiotic yoghurt and milk which had value of US $889 million, followed by spreads which accounts 23 per cent and had value of US $320 million during 1997. Similar study was done by Stanton *et al*. (2001) and result showed that the market for functional food market in the United Kingdom, France, Germany, Spain, Belgium, Netherlands, Denmark, Finland and Sweden was highest for yoghurt.

According to the report of 'Probiotics Market' (2009-2014), published by Markets and Markets, the global probiotics market has a worth of $32.6 billion in 2014

and Europe and Asia accounts for nearly 42 and 30 per cent of the total revenues respectively. According to the report, Europe forms to be the largest probiotic market with an estimate of $13.5 billion by 2014. Asia is the second largest market, growing with an approximate CAGR of 11.2 per cent to reach $9.0 billion by 2014.

In 2012, the probiotic market had an estimate worth of $26 million which increased to approximately $1.7 billion by 2017 and by 2018 the global probiotic market was estimated at USD 48.38 billion (Betz *et al*., 2015).

Based on probiotic products, the market is divided into three categories, probiotic food and beverages, probiotic dietary supplements and probiotic animal feed. The food and beverage are further divided into dairy, non-dairy, cereals, baked food, meat and dry probiotic food. The dietary supplement can be in the form of food supplements, nutritional supplements and infant formula (Sanders,1998).

Probiotics in India generally comes in two forms, milk (62 %) and fermented milk products (38 %). The value of indian probiotic market is $2 million as per 2010 estimate and was expected to reach $8 million by 2015. The existing probiotic market in India have three divisions urban chain, young adults and people with special needs such as pregnancy, lactation, immunodeficiency and geriatry *etc*. (Raja and Arunachalam, 2011).

The probiotic market is expanding both in food and nutrition supplement industries. Food manufacturers are encouraged by the market growth, high margins and growing consumer interest for further development of products (Bimbo *et al.* 2017).

Probiotic foods are also used as animal feed supplements for cattle, poultry and piggery. Sporolac (*Sporolactobacilli*), *Saccharomyces boulardii* and yogurt (*L. bulgaricus* + *L. thermophillus*) are the common animal feeds which are rarely used by human beings.

Japan launched world's best probiotic drink 'Yakult' which contain more than 6.5 billion beneficial bacteria (*Lactobacillus casei*). It provides health benefits by boosting immunity, digestion and prevent infections (Thompson and Moughan, 2008).

The major probiotic products available in India are Prolife (Amul), Yakult, probiotic tea, coffee, yogurt, Organic low fat Kefir, b-Active, ViBact, inLife, NesVita, Neo, Acidophilus Plus, Healthvit, Bio-K Plus, Vista Nutritions, Doctor‟s Best, Nature

Made and Ultimate (Lakshmy *et al*, 2018). At present the major players in Probiotics in India are Chr.Hansen (India) Pvt. Ltd., Danone Foods and Beverages (India) Pvt Ltd, Mother Dairy Fruits and Vegetables Pvt.Ltd, Nestle India Ltd., Zytex Biotech Pvt.Ltd. Micrbax (India) Ltd., Yakult Danone India Pvt. Ltd. and Shree Additives Pharma and Foods Ltd.

India's best known dairy brand is Amul which developed sugar free probiotic diabetic ice cream of different flavours which have 50 per cent less fat and half of the calorie than normal ice cream (Das *et al*., 2007). This ice cream bagged The International Dairy Federation Marketing Award 2007 in the nutri-marketing category (Hickey, 2014).

2.4. Probiotic beverages

2.4.1. Fruit beverages

The ingredients like energy sources (glucose), growth factors (yeast extract and protein hydrolysates), antioxidants, minerals, vitamins *etc*. make the food suitable substrate for probiotic growth (Dave and Shah, 1998).

Tuorila and Cardello (2002) reported that there is an interest in the development of fruit juice based probiotic beverages, because they have taste profiles that are appealing to all age groups and are considered as healthy and refreshing foods. Luckow and Delahunty (2004) states that an increase in consumer demand for non dairy based probiotic products in recent years. Non dairy based food products include soy based products, nutrition bars, cereals and variety of juices as a means of probiotic delivery to the consumer (Ewe *et al*., 2010). Since fruit juices are rich in nutrients such as antioxidants, vitamins and minerals, this can be used as an alternative source for the incorporation of probiotics and also does not contain starter cultures that compete with probiotics for nutrients.

Number of fruits and vegetables are used in the development and commercialisation of non-dairy fermented probiotic beverages. Several tropical fruits are widely used as substrates for the fermentation by different strains of lactic acid bacteria (Panghal *et al*., 2018). Fruit juices are also extremely healthy, having high content of antioxidants, vitamins, minerals, dietary fibre and many other beneficial nutrients, and hence could serve as a good medium for probiotic production.

Mohammadi and Mortazavian (2011) found that there is alteration in taste and aroma of the probiotic food product due to the production of different metabolites such as organic acids during fermentation and extended storage. Neffe-Skocińska *et al*. (2018) stated that minimum therapeutic level of viable probiotic microorganisms should be at least 10^6 CFU/g of viable cells throughout the product shelf-life.

Ding and Shah (2008) investigated the survival of eight different strains of free and microencapsulated probiotic bacteria in orange and apple juices during six weeks storage. They reported that encapsulated probiotic bacteria survived in fruit juices throughout the storage period, whereas free probiotic bacteria showed a reduction in viability within five weeks of storage. The microencapsulation technique does not only improve the survival of probiotics in fruit juices (Krasaekoopt *et al*., 2008), but may reduce the off-flavour of the product.

In the study conducted by Krasaekoopt and Tandhanskul (2008), the addition of probiotic beads significantly affected the turbidity of grape juices which was increased from 6.50 to 7.20. This may be because, the white colour of the beads contrast with the deep purple colour of grape juice, but in case of orange juice, probiotic beads did not affect the turbidity.

According to Luckow and Delahunty (2004), masking the off flavour produced by probiotic organism on fermentation can be done by combining the juice with tropical fruit juices like pineapple, mango and passion fruit. The sample population preferred mask juice than the control juice, followed by no mask juice.

Nualkaekul *et al*. (2011) reported that orange and pineapple juices showed highest cell survival after 6 weeks of storage at 4° C with a pH of 3.8. A decrease in cell viability was observed in pomegranate and strawberry juices may be due to presence of high levels of phenolic compounds. They also concluded that cell survival on refrigerated storage was because of high levels of pH, citric acid, protein and dietary fibre.

Pereira *et al*. (2013) worked on cashew apple juice and found that there is higher loss in ascorbic acid content, antioxidant activity and total phenolic content of non fermented juice than fermented juice upon storage and also browning reaction and nutritional breakdown by enzymes were reduced. Sensory analysis revealed that the

product was acceptable, with a percentage above 80 for the sweetened juice at the end of 42 days of storage period.

Ellendersen *et al*. (2012) reported good sensory acceptance for gala apple probiotic beverage fermented with *L*. *casei*. The juice was characterised with caramel colour, apple aroma and acidic apple taste after 10 hrs fermentation at 37^0C. The product was organoleptically evaluated and an acceptance index of 96 per cent was observed after a storage period of 28 days at a temperature of 7^0 C.

According to Kumar *et al*. (2011), probiotic RTS prepared by a combination of fermented carrot juice and lime juice (25:75) with *Streptococcus lactis* and *Lactobacillus plantarum* (2 percent bacteria), showed better result after 45 days of refrigerated storage with pH ranging from 4.22- 4.46, TSS ranging from 15-20^0 Brix and had an overall acceptability of 4.6 ± 0.81. It was also suggested that it can replace the synthetic beverages in market as it does not contain any additives, yet have longer shelf life and better organoleptic qualities.

Apple juice fortified 20 per cent β-glucan oat flour along with fresh *L. rhamnosus* cells, showed much better survival of cells when stored 4^0C (Saarela *et al*., 2006). Rakin *et al*. (2007) suggested that, for the enhancement of *L*. *acidophilus* growth, reduction in fermentation period and enriching the juice with vitamins, minerals, amino acids and antioxidants can be done.

According to Majid *et al*. (2018) oxygen concentration and oxygen permeability of the packaging should be maintained at low levels to reduce the losses of culture viability upon storage. Different methods like vacuum packaging, addition of antioxidants or oxygen scavengers like ascorbic acid can be done to reduce the oxygen content of the package.

2.4.2. Vegetable beverages

Probiotic tomato juice was developed by Yoon *et al*. (2004), by incorporating *Lactobacillus acidophilus, L*. *plantarum* , *L*. *casie* and *L*. *delbrueckii* and the cell count after fermentation ranged from $10^6 – 10^8$ cfu/ml after 4 months storage in 4^0 C. They also suggest that this probiotic drink can be served to vegetarians as well as consumers who are allergic to dairy products.

Yoon *et al*. (2005) concluded that beet root served as a matrix for *L. acidophilus* and *L. plantarum* and was able to maintain the required number of beneficial bacteria (10^9 cfu/ ml) after a storage period of four weeks.

Profir *et al*. (2015) developed vegetable probiotic blended beverage of beetroot, carrot and celery and concluded that fermented vegetable juices can be considered as functional food due to the proper viability of probiotics even after a storage of 21 days.

Uzma *et al*. (2019) opined that probiotic carrot based beverage have potential health benefits and also had good organoleptic attributes and nutritive value. It was observed that, on storage for four weeks the probiotic viability was reduced but was maintained within the limits (10^7 -10^9 cfu/ ml).

Nagasivudu *et al*. (2016) encapsulated *Lactobacillus plantarum, Lb. fermentum, Lb. casei, Lysinibacillus sphaericus* and *Saccharomyces boulardii* using alginate coated chitosan beads and incorporated in tomato juice and carrot juice. They concluded that encapsulation enhanced the stability of culture than free cells. After storing for 5 to 6 weeks at 4^0 C the viability of the organism was higher in encapsulated tomato juice.

Beetroot acts as a good medium for probiotic growth without any supplementation. Mixed culture of *L. plantarum, L. rhamnnosus* and *L. delbreckii* was incorporated in to the juice and found out that the drink was rich in antioxidants, total phenols and flavonoids (Panghal *et al*., 2017).

Porto *et al*. (2018) blended beet juice with orange juice and added lyophilised *L. acidophilus* probiotic culture. The juice prepared was organoleptically acceptable and showed purchase intention because of its colour. The viability of the organism was maintained at minimum level even after storage of 28 days.

Vanajakshi *et al*. (2015) developed blended probiotic beverage with moringa leaves paste and beetroot juice and the product was found to have high nutritive value and was rich in calcium and iron. Adjusting the pH to neutral level improved the shelflife of the product to 30 days at 4^0 C.

2.4.3. Milk based beverage

Among the probiotic products developed milk based products were the first and the most popular was yoghurt and buttermilk (Bourlioux and Pochart, 1988).

Gilliland *et al.* (1985) reported that Harry and Leo developed first probiotic food with *L. acidophilus* in milk. As the product had low sensory acceptance it increased the popularity of yoghurt. Many efforts have been made to give conventional yogurt additional beneficial properties by adding value added ingredients such as probiotics, prebiotics and various plant extracts.

Mital and Garg (1992) worked on development of acidophilus milk and reported the major health benefits of the same as it helps to control intestinal infection, control serum cholesterol level, prevention of colon cancer and also enhances the availability of nutrients.

According to Kim and Gilliland (1983), sweet acidophilus milk was fermented cold milk with high concentration *Lactobacillus acidophilus* cells. They reported that this improve lactate activity without the tart and acid taste which is predominant in fermented products.

Holocomb *et al*. (1991) suggested that yoghurt developed by *Bifidobacterium bifidum* and *L. acidophilus* improved the dietetic properties and are also used for manufacturing probiotic ice cream.

Hekmat and Reid (2006) reported that yoghurt act as good vehicle to transfer these beneficial microorganisms to consumers as they found good acceptability of probiotic yogurt containing *L. rhamnosus* GR-1 and *L. reuteri* RC-14 among consumers.

Yakult, a probiotic fermented milk drink with *Lactobacillus casei* strain Shirota (LcS) have the health benefits like reduction of infectious gut related disease and immune modulating effect (Spanhaak *et al*., 1998).

Castro *et al*. (2013) developed strawberry flavoured probiotic dairy beverage and concluded that increase in whey content in probiotic dairy product does not degrade the organoleptic quality of the product.

According to Robinson and Tamime (2007) and Jayawardana *et al*. (2015) there are different probiotic dairy beverages having different types of fermentation. Lactic acid fermentation is responsible for cultured milk, Bulgarian milk, drinkable yoghurt, acidophilus milk and yakult.

Kefir is a probiotic milk beverage similar to yoghurt where lactose is converted to lactic acid and alcohol (Tamai *et al*., 1996). Different cultures like *Candida Leuconostoc,* C*ryptococcus* and *Lactobacillus* can be used for preparation of kefir (Witthuhn *et al*., 2005). Kefir is also used as a leavening agent for bread preparation (Plessas *et al*., 2005), and also as starter culture for cheese preparation (Goncu and Alpkent 2005).

2.4.4 Other probiotic beverages

Rathore *et al*. (2012) developed probiotic beverage with single and mixed cereal substrates fermented with lactic acid bacteria and found that there was significant difference in the production of lactic acid in mixed culture fermentation of mixed cereal than single cereals and also the sensory quality of the product was considerably lower than other probiotic products.

Probiotic beverage with rice and millet grains fortified with pumpkin and sesame seed milk was developed by Hassan *et al*. (2012) which had high nutritive value and also had shelf life of 15 days in refrigerated condition.

Aparna (2015) developed probiotic honey beverage in combination with aloe vera pulp and soy milk which had 91 per cent of consumer acceptance and a shelf life of 10 days.

Probiotic beverage of soybean hydrolysed extract, colostrum and honey which was fermented with kefir grains increased the functional quality, antioxidant activity and also had higher level of lactic acid bacteria and yeast count was observed. The sensory quality of the product was better than traditional kefir beverage (Fiorda *et al*., 2016).

Remya (2020) developed probiotic shake mix with food mixture of raw jackfruit flour, defatted soy flour, jackfruit seed flour, tomato and papaya pulp and skimmed milk powder. Shake mixes were nutritionally and organoleptically acceptable without the presence of fungi, yeast and insect infestation throughout the

storage period. The probiotic count of the developed instant shake mixes varied from 10.14 to 10.19 log cfu/g and it maintained the probiotic viability throughout the storage period.

Recent study was done on jeruslem artichoke tubers, where the water extract of the same was fermented with lactic acid bacteria and concluded that high growth of probiotic organism was observed without any preliminary treatment and addition of additives. Inulin present in the raw material acted as prebiotic factor (Plotnikova *et al*., 2020).

Materials and methods

3. MATERIALS AND METHODS

The various methods followed and materials used for the thesis entitled 'Process optimisation and quality evaluation of passion fruit based probiotic drinks" are discussed under the following heads.

3.1. Collection of raw materials

3.2. Standardisation of passion fruit based drinks

3.2.1. Standardising the combination of juices in the passion fruit based drinks

3.2.2. Acceptability of the prepared passion fruit based drinks

3.2.2.1. Selection of panel members for the organoleptic evaluation

3.2.2.2. Preparation of score cards for the organoleptic evaluation

3.2.2.3. Organoleptic evaluation of prepared passion fruit based drinks

3.2.2.4. Selection of the most acceptable combination of passion fruit based drinks

3.3. Optimisation of conditions for the growth of *L. acidophilus* in the passion fruit based drinks

3.3.1. Optimisation of substrate concentration

3.3.2. Optimisation of time of incubation

3.3.3. Optimisation of temperature

3.3.4. Optimisation of population of inoculum concentration

3.4. Development of passion fruit based probiotic drinks

3.4.1. Incorporation of culture to the selected combinations of passion fruit based drinks

3.4.2. Physico-chemical qualities of the selected drinks

3.5. Storage studies of the developed probiotic drinks

3.5.1. Organoleptic evaluation of the probiotic drinks

3.5.2. Viability of *L. acidophilus* in passion fruit based probiotic drinks

3.5.3. Enumeration of total microflora

3.6. Cost of production of the developed passion fruit based drinks.

3.7. Statistical analysis

3.1. Collection of raw materials

Ripe passion fruit (*Passiflora edulis*) (yellow type) were collected from Cashew Research Station, Madakkathara, KAU as well as from the local households. Pineapple and mango were collected from Pineapple Research Station, Vellanikkara and mango orchard of the Department of Fruit Science, Kerala Agricultural University, Vellanikkara respectively. Other ingredients needed for the study were purchased from the local market.

Pure cultures of the probiotic strain *L. acidophilus* MTCC 10307 needed for the study was obtained from Institute of Microbial Technology (IMTECH) Chandigarh.

3.2. Standardisation of passion fruit based drinks

3.2.1. Standardising the combination of juices in passion fruit based drinks

Drink combinations were prepared using ripe passion fruit, pineapple, tomato and mango juice. For the preparation of passion fruit based drink, the standardized procedure of FSSAI (2010) was followed (Plate. 1). Various combinations used for the preparation of the drink are given in Table 1. The quantity of ingredients used for preparation of drink was taken by calculating the acidity and TSS of the sample and then adding other ingredients in accurate quantity to maintain FSSAI limits.

Table 1. Proportion of ingredients in the passion fruit based drinks

Combinations	**Treatments**				
	T_1	**T_2**	**T_3**	**T_4**	**T_5**
PFJ+ MJ	90%+ 10%	80%+ 20%	70%+ 30%	60%+ 40%	50%+ 50%
PFJ+ PJ	90%+ 10%	80%+ 20%	70%+ 30%	60%+ 40%	50%+ 50%
PFJ+TJ	90%+ 10%	80%+ 20%	70%+ 30%	60%+ 40%	50%+ 50%

(PFJ- Passion fruit Juice, PJ- Pineapple Juice, TJ- Tomato Juice, MJ- Mango Juice)

Passion fruit

Passion fruit pulp

Straining of juice

Sugar syrup preparation

Passion fruit drink

Plate 1. Preparation of passion fruit drink

3.2.2. Acceptability of the prepared passion fruit based drinks

3.2.2.1. Selection of panel members for the organoleptic evaluation

Triangle test suggested by Jellinek (1985) was carried out in the laboratory. Based on the results of triangle test, a panel of fifteen judges (between 18-35 years) were selected.

The acceptability trials of fruit drinks were done by this panel.

3.2.2.2. Preparation of score cards for the organoleptic evaluation

The score cards were prepared for the evaluation of fruit drinks and this is given in Appendix I and II.

3.2.2.3. Organoleptic evaluation of prepared passion fruit based drinks

The prepared fruit drinks underwent a series of sensory evaluation by a panel of 15 selected judges using the nine point hedonic scale. The sensory evaluation were carried out and quality attributes like appearance, colour, flavour, texture, taste and overall acceptability were evaluated.

Table 2. Actual quantity of ingredients used to prepare passion fruit drinks (200 ml)

Treatment	Juice content (ml)		Sugar(g)	Water(ml)
	Passion fruit juice	Other juice		
T_0	26	-	14.43	159.53
T_1	23.4	2.6	15.14	158.83
T_2	20.8	5.2	15.39	158.58
T_3	18.2	7.8	15.69	158.28
T_4	15.6	10.4	16.1	157.87
T_5	13	13	16.55	157.42

3.2.2.4. Selection of the most acceptable combination of passion fruit based drinks

On the basis of organoleptic scores, the fruit drinks with maximum quality attributes were selected for further study.

3.3. Optimisation of conditions for the growth of *L. acidophilus* in the passion fruit based drinks

3.3.1. Optimisation of substrate concentration

From the selected combinations of passion fruit based drinks (one each from three combinations of pineapple, tomato and mango) 25 ml, 50 ml and 75 ml were measured and was pasteurized at 80^0C for 20 minutes and allowed to cool. The pasteurized drink was then inoculated with 4 µl of *L. acidophilus* culture. The samples were incubated at 37^0C for 15 hours. After 15 hrs the samples were enumerated for the viable counts of *L. acidophilus*.

The viability of probiotic organism in fruit drinks were assessed using MRS (De Man Rogosa and Sharpe) medium. One ml of the sample was measured and transferred to a test tube containing 9 ml sterile distilled water (10^{-1} dilution). This was then serially diluted upto 10^{-9} dilutions. The microbial enumeration was done by pour plate method using MRS agar and the results are expressed as 10^9 cfu/g (Plate 2).

3.3.2. Optimisation of time of incubation

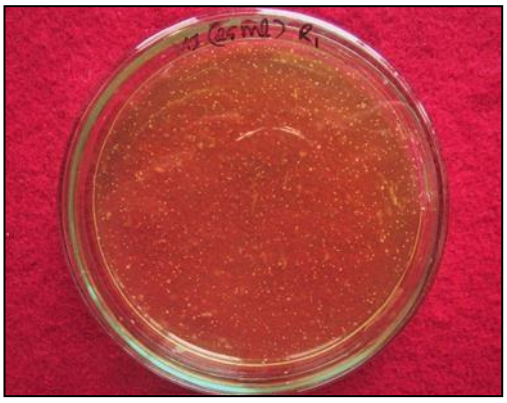
The best substrate concentration with maximum number of colonies was taken and pasteurized at 80^0C for 20 minutes and allowed to cool. It was then inoculated with 4 µl of *L. acidophilus* culture. The samples were then incubated at 37^0 C for 1, 2 and 3 hours. After this, the viability of probiotic organism was enumerated.

3.3.3. Optimisation of temperature

The passion fruit drink with optimum substrate concentration was selected, pasteurized and then inoculated with 4 µl of the culture and incubated at varying temperatures of 37^0 C, 38^0 C and 39^0 C for optimum time of growth of the organism. The fruit drinks were kept for incubation and then tested for the viability of the *L. acidophilus*.

MTCC 10307

Mother culture



***Lactobacillus acidophilus* in MRS media**

Plate 2. Probiotic strain sub culturing

3.3.4. Optimisation of population of inoculum concentration

Each fruit drink combinations with best substrate concentration was pasteurized and then inoculated with 3 µl, 4 µl and 5 µl of *L. acidophilus* and kept for incubation at optimum temperature for optimum period of time. Fruit drinks were then enumerated for the total number of viable cells of *L. acidophilus*.

3.4. Development of probiotic drinks

After the process of optimisation of variables, the selected fruit drinks from each set were incorporated with optimum amount of culture.

3.4.1. Incorporation of culture to the selected combinations of passion fruit based drinks

The selected fruit drink from each set (25ml) was pasteurised at 80^0C for 20 minutes and allowed to cool. The pasteurised drink was then inoculated with 4µl *L. acidophilus* and incubated for a period of 1 hour at 37^0 C. The probiotic passion fruit based drinks along with their control were then packed in food grade plastic bottles and stored under refrigerated condition.

3.4.2. Physico-chemical qualities of the drinks

Analysis of each parameter was carried out in three replications and the methods used are discussed below

3.4.2.1. Acidity

To determine acidity of the fruit drink, the method suggested by Ranganna (1986) was followed. Titratable acidity was determined by titrating the fruit juice against 0.1 N sodium hydroxide (NaOH) using one per cent phenolphthalein solutions as indicator. The titre values were recorded when the solution turned pink. Titratable acidity was expressed as per cent citric acid equivalent using the formula.

$$\% \text{ titratable acidity} = \text{Titre value} \times \text{Normality of NaOH} \times \text{Volume made up} \times \frac{\text{Equivalent weight of acid} \times 100}{\text{Volume of sample taken for estimation} \times \text{Weight of sample taken} \times 1000}$$

3.4.2.2. TSS

Total soluble solids (TSS) of the fruit drinks were determined using a hand refractometer. The readings were taken at room temperature and expressed as degree brix (Ranganna, 1986).

3.4.2.3. Reducing and total sugars

25 ml of sample was transferred to a conical flask. It was then neutralised with 1N sodium hydroxide solution in the presence of phenolphthalein. Clarification of the neutralised mixture was done by the addition of 2 ml of lead acetate. The excess amount of lead acetate was removed by adding 2 ml of potassium oxalate. It was then allowed to stand for 10 minutes for the settlement of the precipitate. The solution was filtered through Whatman's No.1 filter paper. It was then made upto 250 ml. Aliquot of the solution was titrated against a boiling mixture of fehlings solution A and B using methylene blue as indicator. End point of the reaction is the appearance of brick red colour (Ranganna, 1986). The reducing sugars present in the food mixtures were computed using the formula as follows.

$$\text{Reducing sugar (\%)} = \frac{\text{Fehling's factor x dilution x 100}}{\text{Titre value x weight of the sample}}$$

3.4.2.4. Total sugar

The total sugar was determined using the method suggested by Ranganna (1986). From the clarified solution used for the estimation of reducing sugar, 50 ml was taken. This solution was gently boiled after adding citric acid and water. The volume was made upto 250 ml after neutralizing the solution with sodium hydroxide. The aliquot of this solution was titrated against Fehling's solution A and B. The total sugar content was expressed as percentage.

$$\text{Total sugars (\%)} = \frac{\text{Fehling's factor x 250 x dilution x 100}}{\text{Titre value x 50 x weight of the sample}}$$

3.4.2.5. Protein

0.2 ml sample was taken into test tube and volume was made upto 1ml by adding 0.8 ml distilled water. To the test tube 5 ml of alkaline CUSO4 reagent was added and incubated at room temperature for 10 minutes. To the test tube 0.5 ml of folin's phenol reagent was added. The contents were mixed well and the blue colour developed was read at 640 rpm after 15 minutes. From the standard graph the amount of protein in juice was calculated (Sadasivam and Manickam, 1992).

3.4.2.6. Carbohydrate

The carbohydrate content was measured colourimetrically using anthrone reagent (Sadasivam and Manikkm, 1992). 0.1 ml of juice sample was hydrolysed with 5 ml of 2.5 N HCl, cooled and the residue was neutralized with solid sodium carbonate. Made up the content to 100 ml standard flask and centrifuged. Pipetted 0.1 ml of supernatant by the addition of 1 ml distilled water and 4 ml anthrone reagent. Heated the contents for eight minutes on cooling and the intensity of colour from green to dark green was read at 630 nm. The amount of total carbohydrate present in the sample was estimated from the standard graph and is expressed in grams.

3.4.2.7. Energy

The energy content was worked out from the amount of total carbohydrate, protein and fat present in the sample.

Total carbohydrate, protein and fat were estimated by the method as described in 3.4.2.5 and 3.4.2.6. Finally multiply the amount of total carbohydrate, protein and fat by 4, 4 and 9 respectively. Then the results are added together to get the energy. Energy content was expressed as kilo calorie (Kcal) (Sadasivam and Manickam, 1992).

$$\text{Energy (Kcal)} = (\text{CHO} \times 4) + (\text{Protein} \times 4) + (\text{Fat} \times 9)$$

3.4.2.8. β carotene

The sample (2 ml) was taken in a 100 ml glass stopper flask and added 10 ml of water saturated butanol (WSB). The contents of the flasks were mixed vigorously for 1 minute and kept undisturbed for 16-18 hrs (overnight) at room temperature. Dark condition was maintained for the complete extraction of β-carotene. The contents were

again subjected to shaking and filtered completely through the Whatmann no.1 filter paper into a 100 ml volumetric flask. The optical density (O.D) was measured at 440 nm (Sadasivam and Manickam, 1992).

3.4.2.9. Total ash

Total ash was determined by the procedure of AOAC (1994). A clean and dry crucible was accurately weighed first and noted down. About three to five millilitre of the sample was placed in the crucible and again weighed so as to get the accurate weight of the sample. The crucible containing the sample was placed in an electric burner in a partially open manner for the sample to get charred with initial expulsion of smoke. After this, the crucible was placed in a muffle furnace and heated to 500- 600^0 C for 2-3 hours. Crucible was carefully removed from the furnace and cooled to room temperature and weighed again to get the reading.

$$\text{Ash content (\%)} = \frac{(Z-X)}{(Y-X)} \times 100$$

Where,

X- Weight of empty crucible in grams

Y- Weight of crucible + sample in grams

Z- Weight of crucible + ash in grams (after complete ashing)

3.4.2.10. Calcium

Calcium content of the selected fruit drinks were estimated by Atomic Absorption Spectrophotometric method using the di acid extract prepared from the sample (Perkin-Elmer, 1982). A sample of 0.20 ml was predigested with 10 ml of 9:4 mixture of nitric acid and perchloric acid and made up the volume to 50 ml and used directly in Atomic Absorption Spectrophotometer for the estimation of calcium and expressed in mg 100 g^{-1} of sample.

3.4.2.11. Iron

Iron content present in selected fruit juices were determined using the method suggested by Perkin-Elmer (1982). One millilitre of the sample was pre-digested using 9:4 ratio of nitric and percholoric acid (10 ml). The prepared di acid extract of the fruit drink sample was used for estimation of iron in Atomic Absorption Spectrophotometer. Iron content present in the sample was expressed as mg 100 g^{-1} of the sample.

3.4.2.12. Potassium

The potassium content present in the prepared fruit drink was estimated using the procedure suggested by Jackson (1973). The di acid extract of the fruit drink was directly read in the flame photometer and the potassium content was expressed in mg 100 g^{-1} of sample.

3.4.2.13 Phosphorus

The method suggested by Jackson (1973). Phosphorus content was estimated by colorimetrically which gives yellow colour with nitric acid and vandate molybdate reagent. One millilitre sample was pre-digested with 12 ml of 9:4 diacid and volume made up to 100 ml. The five ml of predigested aliquot, five ml of nitric acid, vandate molybdate reagent was added in to the volumetric flask and made upto 50 ml with distilled water. After 10 minutes the optical density was red at 470nm. The phosphorus content was expressed in mg 100g -1.

3.5. Storage studies of the developed probiotic drinks

The fruit drinks were stored in food grade plastic bottles kept under refrigerated condition and stored for a period of 15 days and the organoleptic evaluation and enumeration of population of *L. acidophilus* was done at 7^{th} and 15^{th} day of storage.

3.5.1. Organoleptic evaluation of the probiotic fruit drinks

The developed fruit drinks were subjected to organoleptic evaluation by the panel of selected judges. The procedure of organoleptic evaluation is mentioned in 3.2.2.3.

3.5.2. Viability of *L. acidophilus* in passion fruit based probiotic drinks

The viable count of *L. acidophilus* present in the developed passion fruit based probiotic drinks were enumerated by serial dilution and plate count method as detailed by Agarwal and Hasija (1986). For enumerating the probiotic bacteria (*L. acidophilus*), ten millilitre of the developed fruit juice was mixed with 90 ml distilled water and mixed thoroughly. One ml of this mix was transferred to a test tube containing 9 ml of distilled water. This form 10^{-2} dilution. Similarly the dilutions upto 10^{-9} were made. The viable counts of *L. acidophilus* were enumerated as mentioned in 3.3.1.

3.5.3. Enumeration of total microflora

3.5.3.1. Enumeration of total microflora

The microbial population present in the fruit drinks were estimated using serial dilution plate count method as suggested by Agarwal and Hasija (1986). The microbial analysis was carried out in selected fruit drinks of each set initially, 7^{th} day and 15^{th} day of storage.

The sample was prepared by mixing 90 ml of distilled water with 10 ml fruit drink. This is 10^{-1} dilution. The serial dilutions were carried out in the prepared water blank. To 9 ml of water blank transfer one ml of the prepared fruit drink and this forms a dilution of 10^{-1}. This is then diluted to 10^{-2} followed by 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} using serial dilution techniques. Bacteria, fungi and yeast count were assessed using Nutrient Agar (NA) for bacteria, Potato Dextrose Agar (PDA) for fungi and Sabouraud's Dextrose Agar (SDA) media for yeast respectively and results were given as cfu/g.

3.5.3.2. Enumeration of bacterial colony

Total number of bacterial colony was enumerated in 10^{-5} dilution in nutrient agar medium. In a sterile petri dish, pour one ml of 10^{-5} dilution using a micropipette. To petri dish pour about 20 ml of the nutrient agar medium which is uniformly spread in petri dish by rotating in clockwise and anticlockwise directions. For bacterial colony the enumerated petri dishes were incubated for 48 hrs at room temperature. The total number of bacterial colonies were counted and expressed as cfu/g.

3.5.3.3. Enumeration of fungal colony

Total number of fungal colony was enumerated in 10^{-3} dilution in Potato Dextrose Agar medium. In a sterile petri dish, pour one ml of 10^{-3} dilution using a micropipette. To petri dish pour about 20 ml of the Potato Dextrose Agar medium and uniformly spread. For fungal colony enumeration, the petri dishes were incubated for 4 to 5 days at room temperature. The total number of fungal colonies counted and expressed as cfu/g.

3.5.3.4. Enumeration of yeast colony

Total number of yeast colony was enumerated in 10^{-3} dilution in Sabouraud's Dextrose Agar medium. In a sterile petri dish, pour one ml of 10^{-3} dilution using a micropipette. To petri dish pour about 20 ml of the Sabouraud's Dextrose Agar medium which is uniformly spread in the petri dish by rotating. For enumeration of yeast population, the petri dishes were incubated for 4 to 5 days in room temperature. The total number of yeast colonies were counted and expressed as cfu/g.

3.6. Cost of production

Cost analysis of the products were done to assess the extent of expenses for the preparation of products. The cost of production was worked out based on the market rates of different ingredients used for the preparation of the products. The cost was calculated for 200 ml.

3.6. Statistical analysis

The observations were tabulated statistically as completely randomised design (CRD). The scores of organoleptic evaluations were assessed by Kendall's coefficient of concordance and the differences among treatments in nutritional qualities were assessed using Duncan's multiple range test (DMRT).

Results

4. RESULTS

4.1 Standardising the combination of juices in passion fruit based drinks

The passion fruit based drinks were prepared as per the standard procedure of FSSAI (2010) as mentioned in section 3.2.1 and all the prepared drinks were organoleptically evaluated by a panel of fifteen selected judges. The organoleptic qualities like appearance, colour, flavour, texture, taste and overall acceptability of all the drinks were evaluated using a nine point hedonic scale. Results of the organoleptic evaluation of different drinks are given in Table 3, 4 and 5.

In passion fruit based drink set 1, different combinations of mango juice (MJ) and passion fruit juice (PFJ) were tried, in which the percentage of passion fruit juice varied from 50 to 90 per cent and mango juice varied from 10 per cent to 50 per cent. On observing the mean scores for the organoleptic evaluation of passion fruit based mango drinks, it was evident that the treatment T_5 scored maximum score for the organoleptic attributes except for texture and T_0 had the highest score of 8.04 for texture. This combination (T_5) secured a mean score of 8.84, 8.71, 8.48, 8.02, 7.84 and 8.37 for appearance, colour, flavor, texture, taste and overall acceptability respectively and the total score of this treatment was 50.26. The overall acceptability of the drinks were in the order of 8.1, 7.64, 7.80, 8.00, 8.03 and 8.37 for T_0, T_1, T_2, T_3, T_4 and T_5 respectively. Among the treatments, T_1 was the least acceptable combination.

In the second set of experiment, mango juice was replaced with pineapple juice (PJ) and the other proportion of ingredients remained the same. The mean scores obtained by the fruit drinks are given in Table 4.

Table 4 revealed that in the second set of passion fruit based drinks (PFJ+PJ), treatment T_3 was the most acceptable among the judges than the other four treatments. For the samples, the mean scores for overall acceptability was in the order of 8.10, 8.08, 8.16, 8.52, 8.11 and 7.88 and the mean rank score were 3.40, 2.63, 3.77, 4.90, 2.83 and 2.47 for the treatments T_0, T_1, T_2, T_3, T_4 and T_5 respectively. Mean scores for the sensory parameters among the treatment were found to increase upto T_3 and slightly decreased after that. Total score of this treatment was 51.16 and the mean scores for appearance, color,

flavor, texture, taste and overall acceptability of T_3 was in the order of 8.93, 8.02, 8.48, 8.68, 8.53 and 8.52 respectively as evident from the table.

he set 3 (Table 5) was a combination of tomato juice (TJ) and passion fruit juice (PFJ). Here also the percentage of tomato juice varied from 10 per cent to 50 per cent. The mean scores of organoleptic evaluation of the fruit drink T_3 of set 3 (TJ+ PFJ) were in the order of 8.88, 8.02, 7.63, 8.81, 7.84 and 7.83 respectively for appearance, colour, flavour, texture, taste and overall acceptability. The mean scores for overall acceptability of the treatments T_0, T_1, T_2, T_3, T_4 and T_5 were 8.10, 7.60, 7.79, 7.83, 7.53 and 7.21. The maximum total score was obtained by the treatment T_3 (49.01). Here, the acceptability of fruit juice tends to increase from T_1 to T_3 and then decreased.

The mean scores obtained by each treatment of three sets on organoleptic evaluation were statistically analysed using the Kendall's coefficient of concordance and the mean ranks were also analysed. Based on the mean scores and mean rank scores, the best treatment from each of the three sets were selected for further studies. In set 1 (PFJ+MJ) T_5 scored the maximum score and was selected for further studies. In set 2 and 3, the treatment T_3 was selected based on the organoleptic evaluation.

Table 6 shows the comparative evaluation of organoleptic qualities of the selected passion fruit based drinks. Based on the evaluation, the PFJ+ PJ (T_3) had the highest total score (51.16) and also overall acceptability (8.52) followed by PFJ+ MJ (T_5) with total score of 50.26. The least accepted combination was PFJ+ TJ which had total score of 47.01 and overall acceptability of 7.83.

Table 3: Mean score and mean rank scores for the organoleptic qualities of passion fruit based drinks (PFJ+MJ)

Treatment		Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
Set 1	T_0	8.57 (3.93)	8.48 (4.30)	7.88 (3.07)	8.04 (4.47)	7.82 (4.47)	8.10 (4.13)	48.89
	T_1	8.35 (2.80)	8.00 (2.57)	7.82 (2.93)	7.26 (2.53)	6.77 (2.80)	7.64 (4.03)	45.84
	T_2	8.33 (2.97)	8.02 (2.37)	7.88 (3.00)	7.64 (3.07)	7.17 (2.77)	7.8 (3.60)	46.84
	T_3	8.55 (3.47)	8.17 (3.07)	7.95 (3.27)	7.91 (3.7)	7.46 (3.40)	8.00 (3.0)	48.04
	T_4	8.57 (3.53)	8.24 (3.47)	7.97 (3.23)	7.82 (3.73)	7.56 (3.77)	8.03 (2.93)	48.19
	T_5	8.84 (4.30)	8.71 (4.43)	8.48 (4.50)	8.02 (4.43)	7.84 (4.80)	8.37 (4.3)	50.26
	Kendalls W value	0.12	0.37	0.31	0.38	0.37	0.23	

*Figures in parenthesis indicates mean rank scores; ** Significant at 1% level

T_0 – PFJ (Control); T_1-10% MJ+ 90% PFJ; T_2- 20% MJ+ 80% PFJ; T_3- 30% MJ+ 70% PFJ; T_4-40% MJ+ 60% PFJ; T_5 – 50% MJ+50% PFJ

PFJ-Passion fruit juice, MJ-Mango juice

Table 4: Mean score and mean rank scores for the organoleptic qualities of passion fruit based drinks (PFJ+PJ)

Treatment		Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
Set 2	T_0	8.57 (4.20)	8.48 (4.33)	7.88 (3.07)	8.04 (3.43)	7.82 (2.77)	8.10 (3.40)	48.89
	T_1	8.60 (3.40)	8.17 (3.07)	7.97 (3.27)	8.06 (3.53)	7.60 (2.83)	8.08 (2.63)	48.48
	T_2	8.57 (3.27)	8.24 (3.47)	7.97 (3.33)	8.11 (3.48)	7.91 (3.93)	8.16 (3.77)	46.03
	T_3	8.93 (4.60)	8.02 (2.37)	8.48 (4.40)	8.68 (4.33)	8.53 (4.73)	8.52 (4.9)	51.16
	T_4	8.40 (2.97)	8.71 (4.20)	7.82 (2.93)	7.95 (3.27)	7.68 (3.20)	8.11 (2.83)	48.67
	T_5	8.40 (2.57)	8.00 (2.57)	7.91 (3.0)	7.73 (2.10)	7.44 (2.53)	7.88 (2.47)	47.36
	Kendalls W value	0.24	0.37	0.28	0.35	0.43	0.48	

*****Figures in parenthesis indicates mean rank scores; ** Significant at 1% level

T_0 – PFJ (Control); T_1-10% PJ+ 90% PFJ; T_2- 20% PJ+ 80% PFJ; T_3- 30% PJ+ 70% PFJ; T_4-40% PJ+ 60% PFJ; T_5- 50% PJ+50% PFJ

PFJ-Passion fruit juice, PJ-Pineapple juice

Table 5: Mean score and mean rank scores for the organoleptic qualities of passion fruit based drinks (PFJ+TJ)

Treatment		Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
Set 3	T_0	8.57 (3.93)	8.48 (4.30)	7.84 (3.07)	8.04 (4.47)	7.82 (4.47)	8.10 (4.13)	48.89
	T_1	7.53 (3.40)	7.62 (3.07)	7.57 (3.27)	7.82 (3.37)	7.46 (2.83)	7.60 (2.63)	45.6
	T_2	7.68 (3.27)	7.75 (3.37)	7.68 (3.30)	8.02 (3.40)	7.84 (3.73)	7.79 (3.77)	46.76
	T_3	8.88 (4.60)	8.02 (4.17)	7.63 (4.43)	8.81 (4.27)	7.84 (3.93)	7.83 (3.90)	49.01
	T_4	7.64 (3.10)	7.68 (2.63)	7.53 (2.97)	7.64 (3.20)	7.17 (3.20)	7.53 (2.83)	45.19
	T_5	7.46 (2.47)	7.34 (2.53)	7.22 (2.97)	7.26 (2.10)	6.77 (2.53)	7.21 (2.47)	43.26
	Kendalls W value	0.25	0.34	0.29	0.34	0.36	0.38	

*****Figures in parenthesis indicates mean rank scores; ** Significant at 1% level

T_0 – PFJ (Control); T_1-10% TJ+ 90% PFJ; T_2- 20% TJ+ 80% PFJ; T_3- 30% TJ+ 70% PFJ; T_4- 40% TJ+ 60% PFJ; T_5- 50% TJ + 50% PFJ

PFJ-Passion fruit juice, TJ-Tomato juice,

The selected treatments along with their combination of ingredients are given in Table 7. The total mean score of the selected combination was 50.56 for set.1, 51.16 for set. 2 and 47.01 for set. 3. The selected combinations were taken for further studies.

Table 7. Selected combinations of passion fruit based drinks

Set	Combination	Treatment	Total score of organoleptic evaluation
1	50% PFJ+ 50% MJ	T_5	50.26
2	70% PFJ+ 30% PJ	T_3	51.16
3	70% PFJ+ 30% TJ	T_3	49.01

PFJ-Passion fruit juice, MJ- Mango juice, TJ- Tomato juice, PJ- Pineapple juice

4.2. Optimisation of conditions for the growth of *L. acidophilus*

From the prepared fruit drinks, best one from each set was selected for probiotic optimisation process. The selected fruit drinks were inoculated with the probiotic strain *L. acidophilus* at various conditions and the optimum growth conditions were concluded from the results. Variables such as substrate concentration, time of incubation, temperature and population of *L. acidophilus* for inoculation were optimised.

4.2.1. Optimisation of substrate concentration

Each of the selected fruit juices were taken in three different quantities like 25 ml, 50 ml and 75 ml and pasteurised for 20 min at 80^0C and were inoculated with 4µl of 24 hour old culture of *L. acidophilus*. The drink was then incubated for 15 hours at 37^0C and then enumerated for the viable count of *L. acidophilus.* Results are given in the Table 8.

Table 6. Comparitive evaluation of organoleptic qualities of the selected passion fruit based drinks

Treatment	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
Control PFJ	8.57 (3.93)	8.48 (4.30)	7.84 (3.07)	8.04 (4.47)	7.82 (4.47)	8.10 (4.13)	48.89
T_5 (set.1) PFJ+ MJ	8.84 (1.93)	8.71 (2.80)	8.48 (2.70)	8.02 (2.47)	7.84 (1.70)	8.37 (2.97)	50.26
T_3 (set. 2) PFJ+ PJ	8.93 (2.07)	8.02 (1.60)	8.48 (1.63)	8.68 (1.77)	8.53 (2.60)	8.52 (1.57)	51.16
T_3 (set. 3) PFJ+ TJ	7.88 (2.13)	8.02 (1.60)	7.63 (1.67)	7.81 (1.68)	7.84 (1.70)	7.83 (1.47)	47.01
Kendalls W value	0.38	0.48	0.39	0.21	0.28	0.415	

*****Figures in parenthesis indicates mean rank scores; ** Significant at 1% level

T_0 – Control; T_1-10% TJ+ 90% PFJ; T_2- 20% TJ+ 80% PFJ; T_3- 30% TJ+ 70% PFJ; T_4- 40% TJ+ 60% PFJ; T_5- 50% TJ + 50% PFJ

PFJ-Passion fruit juice, MJ-Mango juice, PJ-Pineapple juice, TJ-Tomato juice,

Table 8. Viable count of *L. acidophilus* in fruit drink with different substrate concentrations

Quantity of substrates (ml) / Treatment (Fruit drink)	25	50	75
	Viable counts ($\times 10^9$cfu/ml)		
PFJ (Control)	186 (13.26)	98 (12.99)	24 (12.38)
PFJ+ MJ (Set. 1)	225 (13.35)	110 (13.04)	33 (12.51)
PFJ+ PJ (Set. 2)	234 (13.36)	142 (13.15)	63 (12.79)
PFJ+ TJ (Set. 3)	286 (13.45)	158 (13.19)	86 (12.93)

All values are means of three independent enumerations
PFJ- Passion fruit juice, PJ- Pineapple juice, TJ- Tomato juice, MJ - Mango juice
Figures in parenthesis indicates log cfu/ml

From the Table 8, it can be concluded that 25 ml of the substrate concentration showed the maximum growth of the probiotic organism. The viable count of probiotic organism varied from 24 to 286 $\times 10^9$ cfu/ml (12.38 log cfu/ml to 13.45 log cfu/ ml). Number of colonies of 25 ml drinks of control, set. 1, set. 2 and set. 3 were 186×10^9 cfu/ml (13.26 log cfu/ml), 225×10^9 cfu/ml (13.35 log cfu/ml), 234 $\times 10^9$ cfu/ml (13.36 log cfu/ ml) and 286 $\times 10^9$ cfu/ml (13.45 log cfu/ ml) respectively. Minimum probiotic growth was observed in 75 ml substrate concentration for all the sets. PFJ+TJ (set. 3) have the highest count of colonies among the different treatments.

4.2.2. Optimisation of time of incubation

As the maximum probiotic growth was observed in 25 ml substrate concentration, it was then taken for optimising the time of incubation. The fruit drink (25 ml) was pasteurized and 4µl of 24 hour old culture of *L. acidophilus* was added to the drink and was kept for 1, 2 and 3 hours at 37^0 C. After incubation, the drink was enumerated for the viable count of *L. acidophilus.* The results of the above said experiment is depicted in the Table 8.

Table 9. Viable count of *L. acidophilus* in fruit drinks with different time of incubation

Time (hrs) / Treatment (Fruit drinks)	1	2	3
	Viable counts (× 10^9cfu/ml)		
PFJ (Control)	181 (13.25)	78 (12.89)	22 (12.34)
PFJ+MJ (Set. 1)	211 (13.32)	91 (12.95)	24 (12.38)
PFJ+PJ (Set. 2)	230 (13.36)	141 (13.14)	54 (12.73)
PFJ+ TJ (Set. 3)	242 (13.38)	148 (13.17)	77 (12.88)

All values are means of three independent enumerations
PFJ- Passion fruit juice, MJ-Mango juice, PJ- Pineapple juice, TJ- Tomato juice, Figures in parenthesis indicates log cfu/ml

Table 9 represents the viable count of *L. acidophilus* at different time of incubation at 10^9 dilution. The combination PFJ+TJ followed by the combination PFJ+PJ and then PFJ+MJ showed the maximum probiotic activity. Their viable counts were in the order of 242, 230 and 211 × 10^9cfu/ml (13.38, 13.36, 13.32 log cfu/ ml) respectively at 1 hour of incubation. The best treatment was PFJ+TJ with viable count of 242 x 10^9 cfu/ml kept for 1 hour and the least count was observed in the control sample kept for 3 hours (22×10^9 cfu/ml).

4.2.3. Optimisation of temperature for fermentation

The selected substrate (25 ml) was pasteurized and 4 µl of 24 hour old culture of *L. acidophilus* was added. This was then incubated for one hour which was optimised, in three different temperatures (37^0 C, 38^0 C and 39^0 C). The optimum temperature was found to be 37^0 C and the results are given in Table 9.

It is clear from Table 10 that, maximum number of probiotic colonies were found in PFJ+ TJ with 235×10^9 cfu/ml (13.37 log cfu/ ml) followed by PFJ+MJ with 223×10^9 cfu/ ml (13.34 log cfu/ ml) and PFJ+PJ (216×10^9 cfu/ ml) (13.33 log cfu/ ml) (Plate 5). The viable count varied from 39 to 235×10^9 cfu/ ml in fruit drinks. The least viable count was observed in control sample with 39×10^9 cfu/ ml.

The best set was PFJ+TJ which had viable count of 235 x 10^9 cfu/ml (13.37 log cfu/ ml)which was kept at a temperature of 37^0 C for 1 hour and least count was observed in PFJ+TJ kept at 39^0 C.

Table 10. Viable count of *L. acidophilus* in fruit drinks at various temperatures

Temperature (^{0}C) / Treatment (Fruit drinks)	37	38	39
	Viable counts ($\times 10^9$cfu/ml)		
PFJ (Control)	192 (13.28)	158 (13.19)	39 (12.59)
PFJ+ MJ (Set 1)	223 (13.34)	185 (13.26)	63 (12.79)
PFJ+ PJ (Set 2)	216 (13.33)	166 (13.22)	87 (12.93)
PFJ+ TJ (Set 3)	235 (13.37)	197 (13.29)	43 (12.63)

All values are means of three independent enumerations
PFJ- Passion fruit juice, MJ-Mango juice, PJ- Pineapple Juice, TJ- Tomato juice
Figures in parenthesis indicates log cfu/ml

4.2.4. Optimisation of population of *L. acidophilus* for inoculation

Fruit drinks (25 ml) were pasteurised and inoculated with 3, 4 and 5 µl of 24 hour old culture of *L. acidophilus* and incubated at 37^0 C for 1 hour. After incubation, the fruit drinks were again enumerated at 10^9 dilution for the number of probiotic organism. Table 11 describes the results.

The probiotic count ranged from 28 to 245×10^9 cfu/ml (12.44 to 13.38 log cfu/ ml) in all the three sets. The probiotic count tend to increase from 3 µl to 4 µl and

decrease from 4 µl to 5 µl. The maximum growth was observed in 4µl concentration of the combination PFJ+TJ 245 $\times 10^9$ cfu/ml (13.38 log cfu/ ml) followed by PFJ+PJ 230 $\times 10^9$ cfu/ml (13.36 log cfu/ ml), PFJ+ MJ 227 $\times 10^9$ cfu/ml (13.35 log cfu/ ml) and control 188×10^9 cfu/ml (13.27 log cfu/ ml) (Plate. 5). The least count was observed in control sample with 5 µl inoculum

Table 11. Viable count of *L. acidophilus* in fruit drinks at various inoculum concentration

Concentration of inoculum (µl) / Treatment (Fruit drinks)	3	4	5
	Viable counts ($\times 10^9$cfu/ml)		
PFJ (Control)	69 (12.83)	188 (13.27)	28 (12.44)
PFJ+MJ (Set 1)	71 (12.85)	227 (13.35)	46 (12.66)
PFJ+ PJ (Set 2)	73 (12.86)	230 (13.36)	32 (12.50)
PFJ+ TJ (Set 3)	86 (12.93)	245 (13.38)	41 (12.61)

All values are means of three independent enumerations
PFJ-Passion fruit juice, MJ-Mango juice, PJ-Pineapple juice, TJ-Tomato juice, Figures in parenthesis indicates log cfu/ml

Thus, it can be concluded that for all the treatments incubation with 25 ml substrate concentration inoculated with 4µl of 24 hour old culture of *L.acidopillus*, which was incubated at 37^0 C for 1 hour resulted in the production of fruit drink with maximum probiotic organisms. This is in line with the desired value of probiotic organisms to be present in any probiotic foods, as specified by FSSAI (2010).

4.3. Development of probiotic drinks

After optimisation, passion fruit based probiotic drinks were developed as per the conditions optimised in the previous section of this chapter. To 25 ml of blended juices, 4µl of probiotic culture was added. This blended juice was then incubated at 37^0 C for one hour. The prepared drinks were packed in food grade containers and stored in refrigerated condition (Plate. 3 and Plate 4).

4.3.1. Physico-chemical analysis of the developed probiotic drinks

The physico-chemical analysis of the probiotic drinks along with their respective controls were done and the results are presented in Table 12 and 13.

4.3.1.1. Titratable acidity

The titratable acidity of the selected probiotic fruit drinks and their respective controls ranged from 1.60 to 3.18 per cent. Probiotic passion fruit drink (PFJ) showed comparatively higher acidity of (3.18 %), followed by non probiotic passion fruit drink (PFJ) with 3.02 per cent titratable acidity and probiotic passion fruit and pineapple drink with 2.28 per cent. Titratable acidity was higher in probiotic juice compared to non probiotic drinks. The least value of titratable acidity 1.60 per cent was observed in non probiotic PFJ+ MJ *ie*, non probiotic passion fruit and mango drink. It was observed that there was significant increase in titratable acidity of probiotic drinks compared to non probiotic drinks (Table 12).

4.3.1.2. TSS

The TSS content of probiotic drinks of PFJ, PFJ+MJ, PFJ+PJ and PFJ+TJ was 12.3, 12.5, 12.8 and 12.3 respectively. The TSS content of non probiotic PFJ, PFJ+MJ, PFJ+ PJ and PFJ+TJ were 14, 13.2, 13.5 and 13.1 respectively (Table 12). There was significant reduction in the TSS content of probiotic drinks compared to non probiotic drinks.

4.3.1.3. Total sugar

The total sugar content of the developed drink ranged from 14.28 to 17.1 g/100g. Maximum sugar content was observed in non probiotic passion fruit and mango drink (PFJ+MJ) with 17.1 g/100g of total sugar followed by non probiotic passion fruit drink with 16.96g/ 100g, non probiotic PFJ+MJ with 16.66 g/ 100g and non probiotic passion fruit and pineapple drink (PFJ+ PJ) with total sugar contents of

Passion fruit probiotic drink

Passion fruit and mango probiotic drink

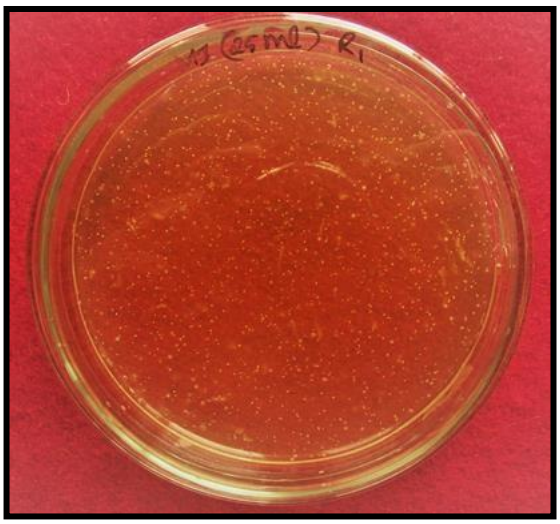
Passion fruit and pineapple probiotic drink

Passion fruit and tomato probiotic drink

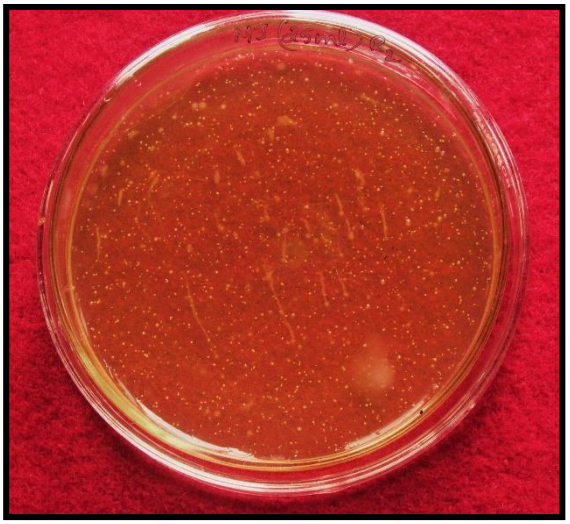
Plate 3. Passion fruit based probiotic drinks

Selected probiotic drinks

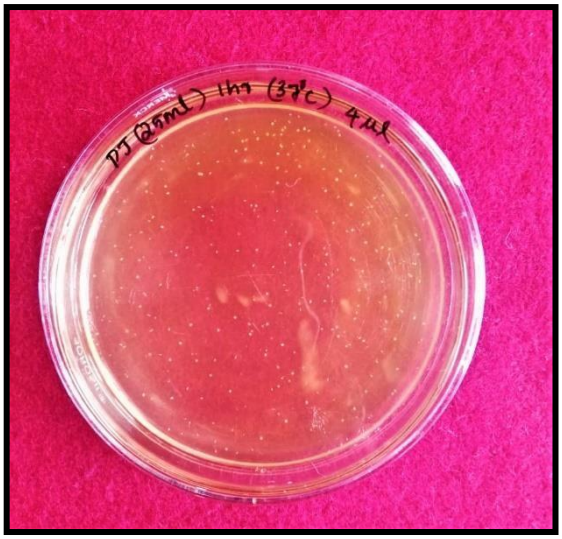
Plate 4. Selected probiotic drinks



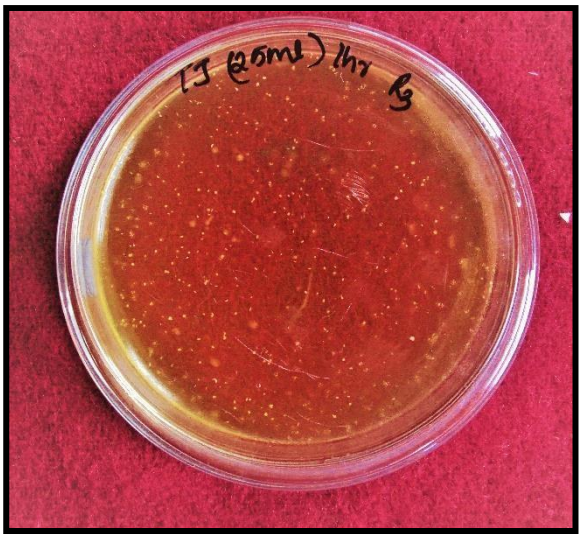
Passion fruit drink



Passion fruit and mango drink



Passion fruit and pineapple drink



Passion fruit and tomato drink

Plate 5. Viability of *L. acidophilus* in passion fruit based probiotic drinks

16.29 g/ 100g. Least quantity of total sugar was observed in probiotic PFJ+TJ drink with 14.28 g/ 100g of total sugar. The probiotic drinks showed a significantly lower content of total sugar compared to non probiotic drinks.

4.3.1.4. Reducing sugar

Table 12 reveals that the reducing sugar content of probiotic drinks of PFJ, PFJ+ MJ, PFJ+ PJ and PFJ+TJ were 3.28, 4.08,3.52 and 3.08 g/ 100g respectively. The reducing sugar content of non probiotic drinks of PFJ, PFJ+ MJ, PFJ+ PJ and PFJ+TJ were 3.57 g/100g, 4.40 g/100g, 4.53 g/100g and 4.18 g/100g respectively (Table 12), which shows that there is significant decrease in reducing sugar content upon probiotication.

4.3.1.5. Protein

The protein content of the selected probiotic fruit drinks and their respective controls ranged from 0.36 to 1.37 g/100g. Probiotic passion fruit and tomato drink combination (PFJ+TJ) showed comparatively higher protein content of (1.37 g/100g), followed by probiotic passion fruit and pineapple drink (PFJ+PJ) with 1.06 g/100g protein content and probiotic passion fruit and mango drink with 0.70 g/100g. Protein content was significantly higher in probiotic juice compared to control samples which are non probiotic drink. The least value of protein content 0.36 g/100 g was observed in control PFJ+ MJ *ie,* non probiotic passion fruit and mango drink.

4.3.1.6. Carbohydrate

The carbohydrate content of the selected probiotic fruit drinks and their controls ranged from 12.19 to 16.85 g/100g. Non probiotic passion fruit and pineapple drink combination (PFJ+PJ) shows comparatively higher carbohydrate content of 16.85 g/100g, followed by non probiotic passion fruit and tomato drink (PFJ+TJ) with 14.74 g/100g and non probiotic passion fruit and mango drink with 14.72 g/100g. Carbohydrate content was higher in non probiotic juice compared to probiotic samples. The least value of carbohydrate content 12.19 g/100 g was observed in probiotic passion fruit juice (PFJ) (Table 12). There was significant difference in carbohydrate content of probiotic and non probiotic drinks.

4.3.1.7. Total energy

The total energy of the developed drinks ranged from 51.24 to 69.56 Kcal/ 100g. Maximum energy was observed in non probiotic passion fruit and pineapple drink (PFJ+PJ)

Table 12. Physicochemical quality analysis of probiotic passion fruit drinks

Treatments		Titratable acidity (%)	TSS (^{0} Brix)	Total sugar (g/100g)	Reducing sugar (g/ 100g)	Protein (g/ 100g)	Carbohydrate (g/100g)	Energy (Kcal/100g)
PFJ	Non probiotic	3.02^b	14^a	16.96^a	3.57^a	0.45^a	13.7^a	56.6^a
	Probiotic	3.18^a	12.30^b	15.70^b	3.28^b	0.62^b	12.19^b	51.24^b
	CD Value (0.05)	0.023	1.61	0.161	0.023	0.023	0.023	0.023
	Significance	S	S	S	S	S	S	S
PFJ+ MJ	Non probiotic	1.60^b	13.20^a	17.10^a	4.40^a	0.36^b	14.72^a	60.32^a
	Probiotic	1.98^a	12.50^b	16.66^b	4.08^b	0.70^a	13.32^b	56.08^b
	CD Value (0.05)	0.023	0.22	0.161	0.023	0.023	0.023	0.023
	Significance	S	S	S	S	S	S	S
PFJ+ PJ	Non probiotic	2.03^b	13.50^a	16.29^a	4.53^a	0.54^a	16.85^a	69.56^a
	Probiotic	2.28^a	12.80^b	14^b	3.52^b	1.06^b	15.90^b	67.84^b
	CD Value (0.05)	0.029	0.0227	1.60	0.023	0.023	0.023	0.023
	Significance	S	S	S	S	S	S	S
PFJ+ TJ	Non probiotic	1.67^b	13.10^a	15.20^a	4.18^a	0.61^b	14.74	61.40^a
	Probiotic	2.68^a	12.30^b	14.28^b	3.08^b	1.37^a	13.94	61.24^b
	CD Value (0.05)	0.023	0.0227	0.161	0.023	0.023	0.023	0.023
	Significance	S	S	S	S	S	S	S

PFJ- Passion fruit juice, MJ-Mango juice, PJ- Pineapple juice, TJ- Tomato juice

Values with different superscript differ significantly at 5%

DMRT Column wise comparison

Table 13. Physicochemical quality analysis of probiotic passion fruit drinks

Treatments		Ascorbic acid (mg $100g^{-1}$)	β-carotene (mg $100g^{-1}$)	Total ash (%)
PFJ	Non probiotic	13.10^{a}	1.02^{a}	1.40
	Probiotic	10^{b}	0.52^{b}	1.52
	CD Value (0.05)	1.61	0.023	
	Significance	S	S	NS
PFJ+ MJ	Non probiotic	13.70^{a}	2.20^{a}	1.52
	Probiotic	10^{b}	1.10^{b}	1.60
	CD Value (0.05)	1.61	0.023	
	Significance	S	S	NS
PFJ+ PJ	Non probiotic	12.80^{a}	1.09^{a}	0.92
	Probiotic	10^{b}	0.56^{b}	1.20
	CD Value (0.05)	1.61	0.023	
	Significance	S	S	NS
PFJ+ TJ	Non probiotic	13.20^{a}	1.20^{a}	2.05
	Probiotic	10.52^{b}	0.73^{b}	2.07
	CD Value (0.05)	0.161	0.023	
	Significance	S	S	NS

PFJ- Passion fruit juice, MJ-Mango juice, PJ- Pineapple juice, TJ- Tomato juice
Values with different superscript differ significantly at 5%
DMRT Column wise comparison

and the energy of other non probiotic drink was 56.6, 34.24 and 35.56 Kcal/ 100g for PFJ, PFJ+ MJ and PFJ+ PJ respectively. Least energy was observed in probiotic PFJ drink with 23.52 g/ 100g and that of other probiotic drink was 30, 33.84 and 44.68 for PFJ+ MJ, PFJ+ PJ and PFJ+ TJ respectively. A significant difference between probiotic and non probiotic drinks were observed after analysis.

4.3.1.7. Ascorbic acid

The ascorbic acid content of the selected probiotic and non probiotic fruit drinks ranged from 10 to 13.7 mg/100g. Non probiotic passion fruit and mango drink combination (PFJ+MJ) showed comparatively higher ascorbic content of (13.7 mg/100g), followed by non probiotic passion fruit and tomato drink (PFJ+ TJ) with 13.2 mg/100g ascorbic acid content and non probiotic passion fruit drink with 13.1 mg/100g (Table 13). Ascorbic acid content was higher in non probiotic drinks compared to probiotic drinks. The least value for ascorbic acid content (10 mg/100 g) was observed in probiotic PFJ, PFJ+ MJ and PFJ+ PJ. The ascorbic acid content of probiotic and non probiotic drinks were significantly different.

4.3.1.8. β-carotene

The β- carotene content of non probiotic drinks of PFJ, PFJ+ MJ, PFJ+ PJ and PFJ+ TJ was 1.02, 2.20, 1.09 and 1.20 mg/ 100g respectively, where as the β-carotene content of probiotic PFJ, PFJ+ MJ, PFJ+ PJ and PFJ+ TJ were 0.52, 1.10, 0.56 and 0.73 mg/ 100g respectively. It was observed from the above result that there was significant loss in the β-carotene content of probiotic drinks compared to non probiotic drinks.

4.3.1.9. Total ash

It was observed from the result (Table 12) that the total ash content was increasing after probiotication of different drinks. The non probiotic sample of PFJ+ TJ was having higher total ash content with 2.40 per cent. Total ash content of probiotic, PFJ+ MJ and PFJ+ PJ was 1.52 per cent, 1.60 per cent and 1.20 per cent respectively (Table 13). The least per cent of total ash (0.92 %) was in non probiotic sample of PFJ+ PJ. It was observed that, there was no significant difference among probiotic and non probiotic drinks.

4.3.1.10. Phosphorus

The phosphorus content available in non probiotic sample of PFJ, PFJ+ MJ, PFJ+ PJ and PFJ+ TJ is 5.16, 5.05, 6.71 and 6.91 mg/ 100 g respectively. This is comparatively lesser than probiotic samples. Among treatments the highest phosphorus content was observed in probiotic PFJ+TJ (6.92 mg/ 100 g) followed by PFJ+ PJ (6.72 mg/ 100 g) and PFJ (5.17 mg/ 100 g).

4.3.1.11. Potassium

From Table 14, the details of the potassium content of passion fruit based drink can be drawn. The drinks were found to have fair amounts of potassium and was expressed in milligrams per hundred gram. Highest per cent of potassium content was observed in probiotic PFJ with 45.25 mg/ 100 g and the lowest was in non probiotic PFJ+MJ with 33.02 mg/ 100g. The second highest potassium content was observed in probiotic PFJ+ TJ with 39.63 mg/ 100g.

4.3.1.12. Calcium

Tables 14 shows the result of calcium content of the developed probiotic drink. It was observed that the calcium content increased upon probiotication and the probiotic drink with maximum calcium content was PFJ+ MJ (2.65 mg/ 100 g) and minimum calcium content was PFJ with 2.04 mg/ 100g. Among non probiotic drinks PFJ+ MJ had higher calcium content with 2.64 mg/ 100g and PFJ have minimum content (2.02 mg/ 100g).

4.3.1.13. Iron

The iron content of passion fruit based probiotic drinks were found to present in the varying proportions. In probiotic drinks minimum iron content was observed in PFJ (0.22 mg/ 100g) and maximum was in PFJ+MJ (0.29 mg/ 100g). In non probiotic drinks maximum was observed in PFJ+MJ (0.28 mg/ 100g) and minimum was in PFJ (0.20 mg/100g). It was observed that there was no significant difference in total ash and mineral content among probiotic and non probiotic drinks.

4.4. Storage studies of passion fruit based probiotic drinks

4.4.1. Organoleptic evaluation of the developed passion fruit probiotic drinks

The developed fruit drinks, were subjected to organoleptic evaluation by a panel of fifteen judges using the nine point hedonic scale throughout the storage period (15 days).

4.4.1.1. Organoleptic evaluation of the passion fruit drinks (PFJ)

Table 15, gives the depicts of organoleptic evaluation of the developed drink PFJ both probiotic and non probiotic. The initial score for appearance, colour, flavor, texture and taste were 8.60, 8.47, 7.86, 8.05, 7.85 for probiotic drink and 8.58, 8.46, 7.84, 8.04, 7.83 respectively for non probiotic drink. The initial overall acceptability of the probiotic fruit drink was 8.16 and 8.15 for non probiotic drink respectively. The organoleptic attributes of the fruit drink (both probiotic and non probiotic) showed a slight decrease on storage. This is evident from the total scores of the samples. The initial total scores of the probiotic drink was 48.99 and 48.90 for non probiotic drink. On storage for 15 days, it reduced to 48.76 for probiotic and 48.71 for non probiotic drinks.

Table 15. Mean scores for organoleptic qualities of probiotic and non probiotic drinks on storage (PFJ)

Quality attributes	Storage period in days					
	Initial		7^{th} day		15^{th} day	
	Probiotic	Non probiotic	Probiotic	Non probiotic	Probiotic	Non probiotic
Appearance	8.60	8.58	8.60	8.58	8.59	8.57
Colour	8.47	8.46	8.46	8.44	8.43	8.41
Flavour	7.86	7.84	7.85	7.82	7.81	7.80
Texture	8.05	8.04	8.05	8.03	8.04	8.03
Taste	7.85	7.83	7.81	7.83	7.78	7.80
Overall acceptibility	8.16	8.15	8.14	8.13	8.11	8.10
Total score	48.99	48.90	48.91	48.83	48.76	48.71

Table 14. Mineral contents of passion fruit based probiotic drinks

Treatments		Phosphorus (mg/ 100 g)	Potassium (mg/ 100 g)	Calcium (mg/ 100 g)	Iron (mg/ 100 g)
PFJ	Non probiotic	5.16	45.23	2.02	0.20
	Probiotic	5.17	45.25	2.04	0.22
PFJ+ MJ	Non probiotic	5.05	33.02	2.64	0.28
	Probiotic	5.06	33.05	2.65	0.29
PFJ+ PJ	Non probiotic	6.71	37.23	2.51	0.24
	Probiotic	6.72	37.25	2.52	0.26
PFJ+ TJ	Non probiotic	6.91	39.62	2.48	0.24
	Probiotic	6.92	39.63	2.49	0.25

PFJ- Passion fruit juice, MJ-Mango juice, PJ- Pineapple juice, TJ- Tomato juice

4.4.1.2. Organoleptic evaluation of the passion fruit based mango drinks (PFJ+ MJ)

In Table 16, the organoleptic scores of the probiotic and non probiotic passion fruit based mango drink during storage is given. Both the probiotic and non probiotic drinks of PFJ+ MJ were highly acceptable among the judges. With respect to the appearance, colour and flavour, the drinks scored between 8.50 and 8.86 and for texture and taste, the scores were between 7.64 and 8.81. As revealed from the table, the organoleptic scores declined on storage and as a result, the total score of the fruit drink got reduced gradually. From the initial point of 50.37 and 50.17, the total scores of probiotic and non probiotic drinks reduced to 50.14 and 49.95 at the end of 15th day.

Table 16. Mean scores for organoleptic qualities of probiotic and non probiotic drinks on storage (PFJ+ MJ)

Quality attributes	Storage period in days					
	Initial		7th day		15th day	
	Probiotic	Non probiotic	Probiotic	Non probiotic	Probiotic	Non probiotic
Appearance	8.86	8.83	8.85	8.81	8.84	8.79
Colour	8.62	8.61	8.62	8.61	8.60	8.59
Flavour	8.61	8.58	8.57	8.56	8.54	8.50
Texture	8.18	8.11	8.18	8.11	8.17	8.10
Taste	7.71	7.70	7.66	7.68	7.64	7.65
Overall acceptibility	8.39	8.34	8.37	8.35	8.35	8.32
Total score	50.37	50.17	50.25	50.12	50.14	49.95

4.4.1.3. Organoleptic evaluation of the developed passion fruit based pineapple drinks (PFJ+ PJ)

The organoleptic evaluation of the fruit drink (PFJ+ PJ) was done along with non probiotic control samples and the results are given in the Table 17. The table revealed that the organoleptic attributes of the fruit juice with respect to appearance, colour, flavor, texture and overall acceptability were very much liked by the panelist. In this group of fruit drinks also, the scores tend to decrease on storage. Even after the

storage of 15 days, the overall acceptability of the fruit drinks were within acceptable levels (8.50 for probiotic and 8.47 for non probiotic) which implicates that the probiotic drinks were acceptable throughout the storage period.

Table 17. Mean scores for organoleptic qualities of probiotic and non probiotic drinks on storage (PFJ+ PJ)

Quality attributes	Storage period in days					
	Initial		7th day		15th day	
	Probiotic	Non probiotic	Probiotic	Non probiotic	Probiotic	Non probiotic
Appearance	8.91	8.90	8.89	8.88	8.88	8.87
Colour	8.04	8.03	8.03	8.02	8.00	8.01
Flavour	8.49	8.46	8.48	8.46	8.45	8.46
Texture	8.68	8.66	8.68	8.66	8.67	8.65
Taste	8.53	8.52	8.51	8.52	8.47	8.49
Overall acceptibility	8.52	8.51	8.51	8.50	8.50	8.47
Total score	51.17	51.06	51.10	51.04	50.97	50.94

4.4.1.4. Organoleptic evaluation of passion fruit based tomato drink (PFJ+ TJ)

Fruit drink of passion fruit drink added with tomato (PFJ+TJ) also showed the similar trend as that of other fruit drinks. The initial overall acceptability of the probiotic fruit drink was 8.24 and that of non probiotic drink was 8.22. During storage for 15 days, the scores got reduced and reached 8.23 for probiotic and 8.21 for non probiotic drink. At the end of storage, the total scores were 49.32 and 49.27 respectively for probiotic and non probiotic drink (Table 18).

Table 18. Mean scores for organoleptic qualities of probiotic and non probiotic drinks on storage (PFJ+ TJ)

Quality attributes	Storage period in days					
	Initial		7th day		15th day	
	Probiotic	Non probiotic	Probiotic	Non probiotic	probiotic	Non probiotic
Appearance	8.90	8.89	8.89	8.88	8.87	8.86
Colour	8.03	8.02	8.03	8.02	8.02	8.01
Flavour	7.63	7.62	7.62	7.61	7.59	7.58
Texture	8.83	8.82	8.80	8.79	8.79	8.78
Taste	7.86	7.85	7.83	7.84	7.82	7.83
Overall acceptibility	8.24	8.22	8.23	8.22	8.23	8.21
Total score	49.46	49.39	49.40	49.36	49.32	49.27

4.4.2. Viable count of *L .acidophilus* in probiotic drinks during storage

The viable count of *L. acidophilus* in the probiotic drinks were enumerated and tabulated. Table 18 represents the results. As revealed from the table, the fruit drink PFJ+ TJ (246× 10^9 cfu/g) reported maximum probiotic growth initially. As expressed in logs, the probiotic count (initially) of the developed fruit drinks ranged from 13.27 to 13.39 log cfu/g. There was reduction in the viable count of *L. acidophilus* throughout the storage period. After 15 days of storage, the viable count of probiotic organism reduced from the range of 187 to 246 × 10^9 cfu/ g. The viable count of the probiotic bacteria in log cfu are given in parenthesis. Even after 15 days of storage, the viable count of *L. acidophilus* in all the probiotic drinks was in the levels as specified by FSSAI.

Table 19. Viable count of *L .acidophilus* in probiotic drinks during storage

Treatments		**Initial**	**7**	**15**
PFJ	Probiotic	187 (13.27)	182 (13.27)	178 (13.26)
	Non probiotic	NIL	NIL	NIL
PFJ+MJ	Probiotic	226 (13.35)	218 (13.34)	212 (13.33)
	Non probiotic	NIL	NIL	NIL
PFJ+PJ	Probiotic	232 (13.36)	224 (13.34)	216 (13.35)
	Non probiotic	NIL	NIL	NIL
PFJ+TJ	Probiotic	246 (13.39)	240 (13.38)	232 (13.37)
	Non probiotic	NIL	NIL	NIL

4.4.3. Enumeration of total microflora of the developed probiotic drinks

4.4.3.1. Total microbial population of passion fruit based probiotic drinks

All the passion fruit drinks (both the probiotic and non probiotic were plated in the appropriate media for enumerating the total bacteria, fungi and yeast initially and during 7^{th} and 15^{th} day of storage and the results are presented in table 18.

Initially, total bacterial population varied from 192 to 252×10^7cfu/ g in probiotic drinks.

Table 20. Total microbial population of developed probiotic drinks

Microbial population (cfu/g)									
	Bacteria (10^7)			Fungi (10^2)			Yeast (10^3)		
	Initial	7	15	Initial	7	15	Initial	7	15
Probiotic (PFJ)	192	188	184	ND	ND	1.20	ND	ND	ND
Non probiotic (PFJ)	2.80	3.00	3.40	ND	1.20	2.00	ND	ND	ND
Probiotic (PFJ+ MJ)	232	224	218	ND	ND	1.30	ND	ND	ND
Non probiotic (PFJ+ MJ)	3.20	3.30	3.70	ND	1.10	2.10	ND	ND	ND
Probiotic (PFJ+ PJ)	238	230	222	ND	ND	1.30	ND	ND	ND
Non probiotic (PFJ+ PJ)	3.10	3.40	3.60	ND	1.10	2.10	ND	ND	ND
Probiotic (PFJ+ TJ)	252	248	238	ND	ND	2.20	ND	ND	ND
Non probiotic (PFJ+ TJ)	3.20	3.60	3.80	ND	1.20	2.40	ND	ND	ND

PFJ- Passion fruit juice, MJ-Mango juice, PJ- Pineapple juice, TJ- Tomato juice

The maximum bacterial count was observed in PFJ+ TJ probiotic drink. On storage, there was reduction in the total bacterial count of the probiotic drink. After 15 days of storage, the bacterial count of the probiotic drink ranged from 184 to 238 × 10^7cfu/g in probiotic drink.

Table 18 also gives the total bacterial count of non probiotic drink which varied from 2.8 to 3.2×10^7cfu/ g. Maximum bacterial count was observed in the fruit drink PFJ+ TJ and PFJ+ MJ, followed by the drink PFJ+ PJ. There was an increase observed in the total bacterial count of the non probiotic fruit drink on storage which varied from 3.4 to 3.8×10^7cfu/g. The minimal bacterial count was observed in PFJ drink.

4.4.3.2. Fungal and yeast count of probiotic and non probiotic drinks

There was no fungal growth in both probiotic and non probiotic drinks initially. On the seventh day of storage there was no fungal growth in the probiotic drinks, were as in the non probiotic drinks the fungal growth ranged from 1 to 1.20 × 10^2 cfu/ ml. On 15th day it was in the range of 1.20 to 2.20 × 10^2 cfu/ ml in probiotic drinks and 2.0 to 2.40 × 10^2 cfu/ ml in non probiotic drinks. Yeast growth was not observed throughout the storage period.

4.5 Cost of production for selected passion fruit based probiotic drinks

The cost of production for the selected passion fruit based probiotic drinks were calculated by considering the material cost, labour charges, fuel and electricity charges and packaging cost. The cost was calculated per 200 ml and presented in Table 21.

Table 21. Cost of production for selected passion fruit based probiotic drinks

Passion fruit based probiotic drink	Cost (200ml)
Passion fruit drink	31
Passion fruit and mango drink	33.5
Passion fruit and pineapple drink	29.5
Passion fruit and tomato drink	25.5

The production cost of probiotic drink PFJ was found to be 31 Rs/200 ml, that of probiotic PFJ+MJ was 33.5 Rs/200 ml and PFJ+PJ was 29.5 Rs/ 200 ml. Among the prepared probiotic drinks, cost of the production of probiotic PFJ+TJ (25.5 Rs/200 ml) was observed to be lowest.

Discussion

5. DISCUSSION

5.1. Standardisation of combination of passion fruit based drinks

In modern beverage processing technology, blending of two or more juices has become important which enables to produce beverages of superior quality with sensory, nutritional and medicinal properties (Bhagwan and Awadhesh, 2014). Tropical fruits are good source of vitamins, minerals and antioxidants. Among the tropical fruits, yellow type passion fruit (*Passiflora edulis* Sims f. *flavicarpa* Deg.) has wide acceptability among consumers (Zhu *et al*., 2017) because of its taste, aroma and flavour. The objective of the present study was to develop passion fruit based probiotic drinks and to evaluate its organoleptic, nutritional and shelf life qualities.

The efficacy of passion fruit as a probiotic matrix was tested in this study along with mango, pineapple and tomato in different proportions. The prepared fruit juices were subjected to organoleptic evaluation.

In the present study, passion fruit juice (PFJ), mango juice (MJ), pineapple juice (PJ) and tomato juice (TJ) were used in different combinations. From set 1 (PFJ+MJ) treatment T_5 was selected for further studies based on the organoleptic properties, and from set 2 (PFJ+PJ) and set 3 (PFJ+TJ) the treatment T_3 was selected. Table 3, 4 and 5 shows the sensory attributes of selected passion fruit based drinks. The selected combinations were 50 per cent of passion fruit juice and 50 per cent of mango juice from set 1 and in set 2 and 3, 70 per cent passion fruit juice along with 30 per cent of pineapple and 30 per cent tomato juice respectively. All the selected fruit drinks contained 50 per cent and more passion fruit juice and was found to be organoleptically acceptable.

Earlier, Shaw and Wilson (1988) prepared passion fruit orange blended nectar with sensory acceptance score between 5.1 and 6.8 and also concluded that nectar having high proportion of passion fruit had better acceptance.

According to Matsuura *et al*. (2004) blended nectar of papaya pulp (37.5%), passion fruit juice (7.5%) and arecola pulp (5%) had good sensory attributes with a sensory score of 7.

Deliza *et al*. (2005) reported that, passion fruit juice prepared in the ratio 6:9 (water: juice) and 13g of sugar in 100ml have strong fruity passion fruit aroma, sweet flavour and refreshing mouthfeel.

The result of Chakraborty *et al*. (2011) was in accordance with the present study, where passion fruit and mango fruit juice (2.57:1 ratio) was mixed with strained sugar syrup to get an acceptable product with a score above 3.50 out of 5.00. Passion fruit and mango blended squash had a longer storage life of 15 months.

Production of fermented passion fruit beverage with yeast at temperature 26 ± 1^0 C for 72 hours had a sensory score of 16.6 with pleasant smell, yellow brown colour and low turbid texture (Min *et al*., 2019).

Najumudheen (2015) produced a blended fruit syrup with pineapple and passion fruit (80:20) and concluded that this combination had the highest value in overall acceptability (7.33) than the other combinations.

Lakhanpal and Vaidya (2015), developed honey based mango nectar which showed high acceptability when compared to sugar based mango nectar even after a storage period of six months. The overall acceptability score was 7.50 for honey based mango nectar. Charan (2016), developed passion fruit nectar which had total score of 52.1, 50.9 and 47.3 respectively for first, second and third months of storage under ambient condition.

Mango and passion fruit (80:20) smoothie beverage was prepared by Gallina *et al*. (2019) and revealed that the overall acceptability of the product was 7.40 with good aroma and flavour.

5.2 Optimisation of conditions for the growth of *L.acidophilus*

In recent years, the importance of functional food is increasing, because of the increased interest in consuming foods that provide health benefits. Traditionally probiotics were used in dairy foods but nowadays, the interest in non dairy based probiotic foods are increasing (Espinoza and Navarro, 2010). According to Min *et al*. (2019), replacement of dairy products with non dairy products is mainly because of the benefits provided by bioactive molecules.

Beverages from fruits, vegetables, cereals *etc*. are the new probiotic products that serves as a good medium for probiotic organism to survive and are also equally accepted among all age group (Prado *et al*., 2008).

Probiotication of fruit juice is important to provide health beneficial products to consumers who are allergic to milk products. Even though fruit juices are established in markets, market for probiotic fruit juices are growing. Fruit juice act as a good medium for probiotic organism growth (Mattila *et al*., 2002) and also to maintain minimum therapeutic level 10^9 cfu/g or ml (WHO, 2001).

In the present study, *Lactobacillus acidophilus* MTCC 10307 was the probiotic strain used throughout the research. Several researchers have used the same strain as a probiotic entity in their studies. Pradhan *et al.* (2016) worked on comparative analysis of two bacterial strain and found out that *Lactobacillus acidophilus* MTCC-10307 (LA) and *Bacillus clausii* MTCC-8326 (BC) improved the metabolic activity and also immune responses. It was also seen that *Lactobacillus acidophilus* and *Bacillus clausii* had comparable movement in preventing disease recreated *in vitro* in murine macrophages by *Salmonella typhimurium* serovar enterica.

The present study revealed that 25 ml substrate concentration with 4µl inoculum for 1hour incubation at temperature of 37^0 C reported the maximum number of probiotic cells in all the three sets (Fig. 1, Fig.2, Fig.3). The viability of *Lactobacillus acidophilus* in the selected fruit drinks ranged from 13.26 to 13.39 log cfu/ ml. The maximum probiotic count was observed in PFJ+TJ. The maximum probiotic count was observed in the fruit juice combination PFJ+TJ followed by PFJ+PJ, PFJ+ MJ and PFJ.

Earlier similar optimization studies were conducted by Gallina *et al*. (2019) in the developed of a probiotic fermented smoothie beverage and concluded that the viable count of the probiotic organism of passion fruit and mango blend after 1, 13 and 30 days of storage was 7, 7.5 and 6.5 log cfu/ ml respectively at a temperature of 8 ± 2^0 C.

Recently, Monteiro *et al*. (2020), had also done optimization studies and suggested that passion fruit pulp act as a good medium for probiotic culture, when fermented at a temperature of 30^0C. They also conclude that, presence of phenolic compounds and other acidic molecules can be the reason for probiotic production.

This can provide health benefits because of the combination of probiotic properties and also properties of bioactive compounds.

According to Buriti *et al*. (2007), who conducted work on activity of *Lactobacillus acidophilus* in refrigerated mousses of passion fruit and guava, concluded that frozen mousse maintained the viability of *Lactobacillus acidophilus* above 6 log cfu /g after 69 days of storage at -18ºC.

An optimization study was conducted by Ranjitha *et al*. (2018) in developing a probiotic mango RTS by *Lactobacillus rhamnosus* was developed by and they opined that the cell count was 8.25± 0.21, 9.07± 0.5 and 9.1±0. 32 log cfu/ ml during 2, 4 and 6 days of incubation period respectively. Mango RTS beverage inoculated with *Lactobacillus helveticus* MTCC 5463 has the potential for development of probioticated mango beverage as they showed good sensory attributes and also higher concentration of phenolic compounds and flavonoids after fermentation.

Reddy *et al*. (2015) opined that, utilisation of sugar and reduction of pH is fastest when using *L. plantarum* when compared to other species and concluded that mango is a good substrate for probiotic growth. Mango juice does not exert inferior properties and the viability of the bacterial growth was maintained upto 21 days at a standard limit of 10^7 cfu/ 100 ml.

In the present study, passion fruit based pineapple drink (70% PFJ+30% PJ) was developed and optimised for viable count of *Lactobacillus acidophilus* which showed a cell count of 13.36 log cfu/ ml (Fig. 4). The findings were in agreement with the results of Shukla *et al*. (2013) who reported that probiotic beverage with whey and pineapple juice in the ratio of 65:35, had a good acceptability on using one per cent of inoculum of *Lactobacillus acidophilus* and can be stored for 24 days at 5 ± 1°C and 48 hrs at 30 ± 1°C.

According to Manasi *et al*. (2013) the viability of *Lactobacillus acidophilus* decreased upon refrigerated storage of probiotic pineapple juice. The initial count 3.8×10^7 cfu/ ml diminished to 1.8×10^7 cfu/ ml, however the count did’nt go below the minimum level. During storage at 30 ± 1°C, the count expanded to 9.5×108 (in 48 hrs) and afterwards declined to 2.9×107 cfu/ml after 120 hrs.

Adebayotayo and Akpeji (2016) also developed a probiotic pineapple juice, were the juice supported the viability of the organism, lactic acid production, vitamin

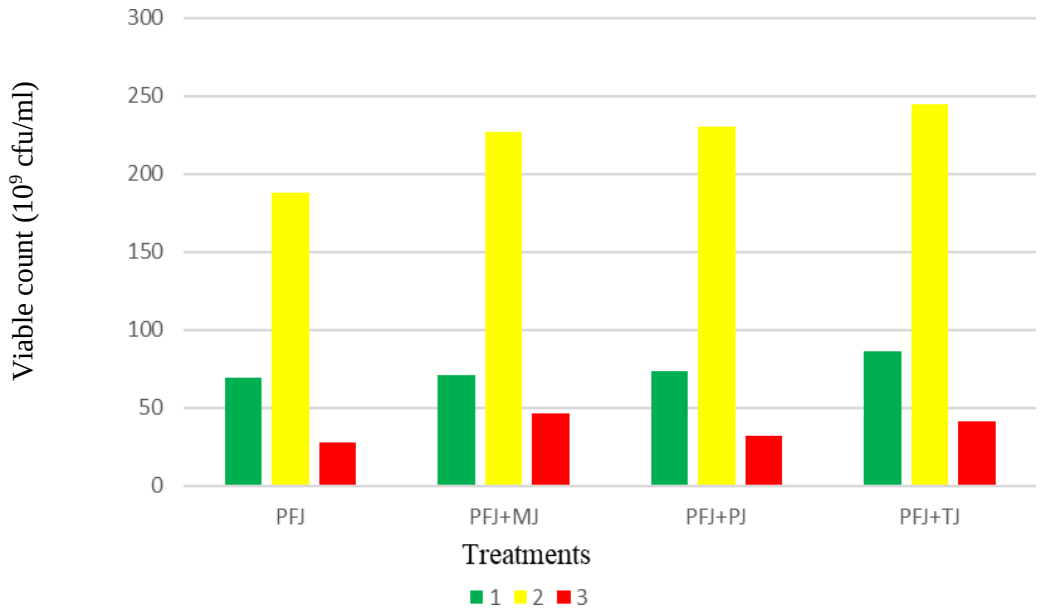


Fig. 2 Viable count of *L. acidophilus* in probiotic drinks with different time of incubation (hrs)

PFJ-Passion fruit juice, MJ-Mango juice, PJ-Pineapple juice, TJ-Tomato juice

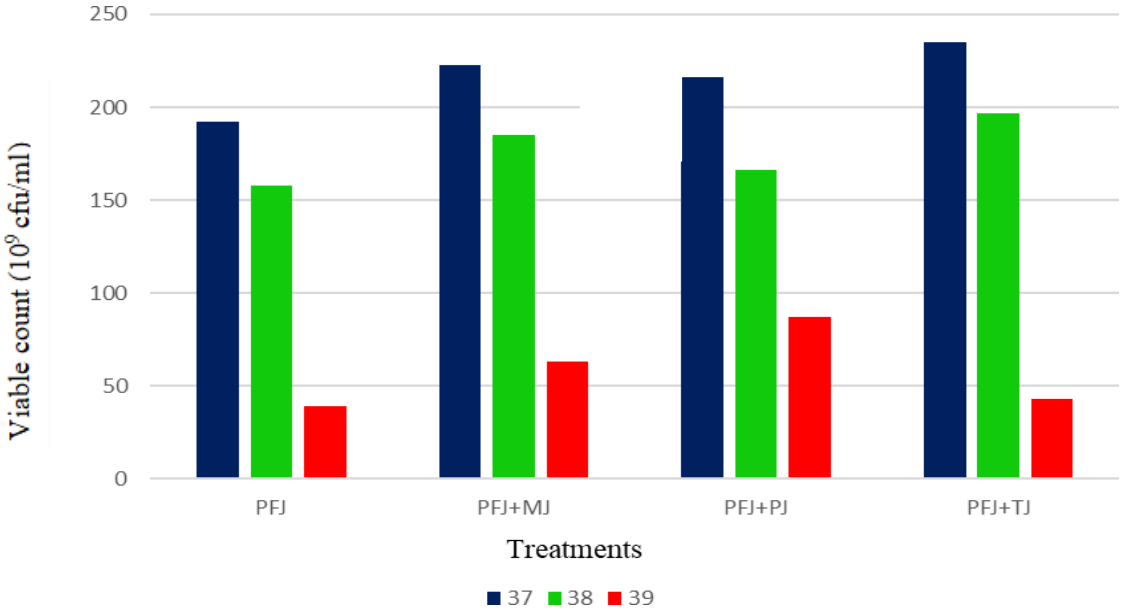


Fig.3 Viable count of *L. acidophilus* in probiotic drinks with different temperatures (^{0}C) of incubation

PFJ-Passion fruit juice, MJ-Mango juice, PJ-Pineapple juice, TJ-Tomato juice

C production and antagonistic potential of the probiotic bacteria. The lactic acid bacteria were viable throughout the storage (1.05 to 1.10 × 10^9 cfu/ml) and there was no difference in taste, colour, aroma or appearance of the final product after a storage period of four weeks.

Acevedo-Martínez *et al*. (2018) reported that fibre and carbohydrate content enhances the growth of probiotic organism and ensure a stable bacterial count of around 7 log cfu/ ml.

Klaver *et al*. (1993), reported that *Lactobacillus* and *Bifidobacterium* require free amino acids, peptides, vitamins and fermentable carbohydrates for the growth of organism as there is lack of proteolytic activity. Nguyen *et al*. (2019) investigated that without any supplements *Lactobacillus* and *Bifidobacterium* were able to grow well in pineapple juice and acts itself as a matrix for the propagation of probiotic bacteria.

Probiotic pineapple juice developed by Nguyen *et al*. (2019) showed that the cell count of the organism *Lactobacillus acidophilus* was 5.46 × 10^9 cfu/ml after 8 hours of fermentation and then reduced to 3.99 × 10^9 cfu/ml after 24 hours of fermentation. They also reported high requirements of free amino acids, peptides, vitamins, and fermentable carbohydrates for growth.

In the present study maximum viable count of *Lactobacillus acidophilus* (13.45 log cfu/ ml) was found in passion fruit drink having 70 per cent passion fruit juice and 30 per cent tomato juice.

According to Babu *et al*. (1992), growth of *L. acidophilus* was stimulated by addition of tomato juice to skimmed milk and resulted in higher viable counts, shorter generation time and improved sugar utilisation with more acid production and lower pH.

Yoon *et al*. (2004) reported that the viable cell counts of tomato juice inoculated with *Lactobacillus acidophilus* increased till third week storage and reduced on fourth week of storage. The count was 1.4±0.1 x10^9 during the first week and then increased to 2.4± 0.1 x 10^9 during third week. They concluded that the organism rapidly utilised tomato juice for cell synthesis and also lactic acid production.

The initial cell count of *Lactobacillus acidophilus* in tomato juice sample was $2.49x10^8$. After 72hr incubation, the cell counts of *L. acidophilus* increased to 2.95 x 10^8. Reports also say that the organism utilise tomato juice sugar and increase lactic acid production without any additional nutrient addition or pH adjustments (Kaur *et al*., 2016).

The viable count of *Lactobacillus sanfranciscensis* in tomato juice was estimated by Zhu *et al*. (2020). They concluded that initially the count was 8 log cfu/ml which was maintained almost to 2 weeks of storage at 4^0 C. The count reduced to 7.5 log cfu/ ml after 4 weeks of storage.

5.3. Quality evaluation of probiotic drinks

5.3.1. Physico-chemical analysis of developed probiotic drinks

5.3.1.1. Titratable acidity and TSS

In the present study, the titratable acidity values for probiotic fruit drinks ranged from 1.98 to 3.18 per cent. A significantly lower values were obtained for the non probiotic fruit drinks, 1.67 to 3.02 per cent (Fig. 5). TSS is an index of soluble solids concentration in fruit. In the present study the TSS of the probiotic fruit drinks ranged from 12.3 to 12.80^0 brix and that of non probiotic drinks ranged from 13.10 to 14^0 brix. Figure 6 shows that the non probiotic drinks were found to have more TSS than the probiotic drinks.

During probiotic fermentation, the organism convert glucose to lactic acid. This is responsible for the decrease in pH of the product. *Lactobacillus* spp. is more effective in reducing pH than yeasts and other microbes (Gautam and Sharma, 2014).

During fermentation, the probiotic organism produces lactic acid by hydrolyzing starch. This increases the titratable acidity content and decrease in starch in probiotic samples. This metabolic activity convert starch to fermentable simple sugars which is used by probiotic organisms (Adams *et al*., 2008).

Five samples of commercial whole passion fruit juice was evaluated by Pinheiro (2006) and reported that the pH ranged from 2.72-3.17, total soluble 12.5-13.3^0 Brix and acidity in grams over 100g of juice ranged from 2.96-4.02.

The titrable acidity of wine produced from mixed juice of passion fruit, mango and pineapple was 1.4 per cent after fermentation and TSS was 20^0 Brix (Nzbuherheza

and Nyiramugwera, 2014). Yan-li (2011) produced wine with combination of pawpaw and passion fruit and the pH was estimated as 4.0.

Titratable acidity increased significantly ($P \leq 0.05$) with increasing fermentation time irrespective of the medium. The mean values obtained for whey ranged from 0.394 to 1.353. The mean values obtained for whey pineapple juice blend ranged from 0.546 to 0.926. Whey- pineapple juice blend gave higher titratable acidity for 5 and 10 hours of fermentation (Shukla, 2013).

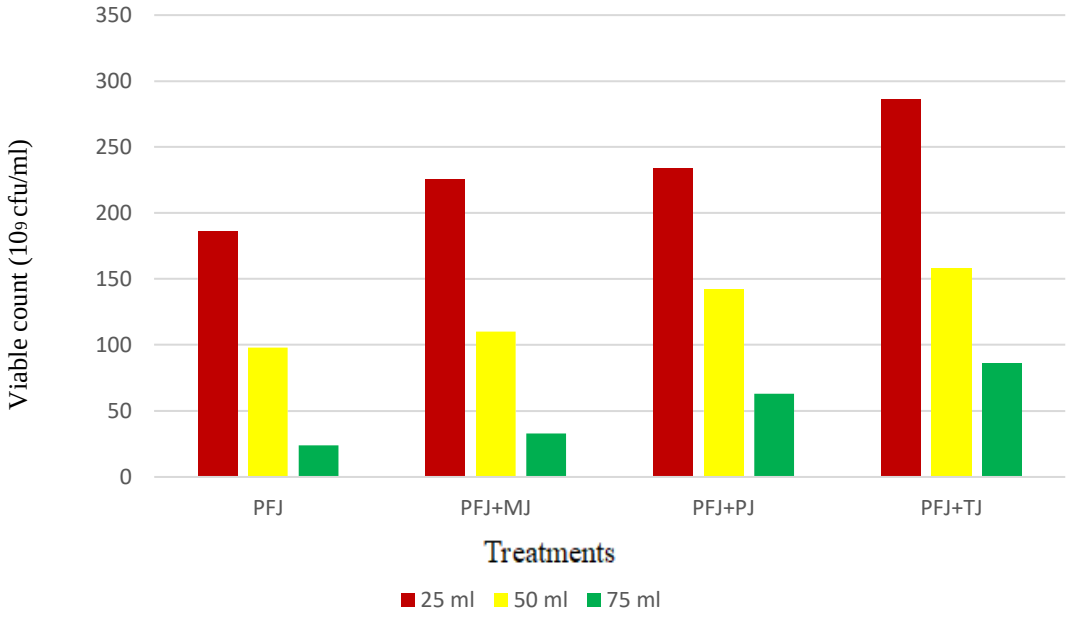


Fig. 1 Viable count of *L. acidophilus* in probiotic drinks with different substrate concentration

PFJ-Passion fruit juice, MJ-Mango juice, PJ-Pineapple juice, TJ-Tomato juice,

Lactic acid production of probiotic organism especially after incubation increase the titrable acidity of mango on preparation of probiotic mango beverage (Reddy *et al*., 2015).

The total soluble solids (TSS) in both honey and sugar enriched mango nectars (20 % pulp, 15°B TSS and 0.30 % acidity) increased during storage and this increase was more in nectar stored under ambient condition compared to those stored under refrigerated condition. After storage for six months the honey enriched mango nectar recorded maximum TSS compared to sugar based mango nectar (Lakhanpal and Vaidya, 2015).

Fernandes *et al*. (2011) concluded that upon pasteurisation of passion fruit juice, there is increase in titrable acidity (3.06 g/ 100 ml) were as the homogenised juice have 2.83 g/ 100 ml.

Nectar prepared by blending pulp of aonla and mango with 50 per cent sugar + 50 per cent stevia + 15 per cent TSS and 0.25 per cent acidity, showed decreasing trend in ascorbic acid content from 36.20 to 27.30 mg/100g during ten months of storage in glass bottles at ambient temperature (Singh *et al* ., 2014).

There was reduction in the TSS content of probiotic pineapple juice formulated by Adebayo Tayo and Akpeji (2016) from 15.28 to 12.68^0 brix after storage of 4 weeks. The reduction may be due to the utilisation of sugars for the metabolic activity of probiotic LAB in the probiotic juice samples. Similar observation was reported by Kumar *et al*. (2013), in carrot and sweet lime juice with *Lactobacillus casei.*

5.3.1.2. Total sugar and reducing sugar

In the present study, the reducing sugar content of the fruit drink decreased from 3.57-4.53 g/100g to 3.08-4.08 g/100g on probiotication. Similarly, the total sugar was also reported to reduce from 15.20- 17.10 g/100g to 14.28 16.66 g/100g (Fig. 7, Fig.8).

According to Yoon *et al*. (2004) a decrease in sugar and pH and increased acidity in tomato juice inoculated and incubated with *Lactobacillus delbrueckii, L. acidophilus, L plantarum* and *L casei* observed the sugar gets converted into acid in the presence of bacteria and thus get reduced with time, and the acidity content increase.

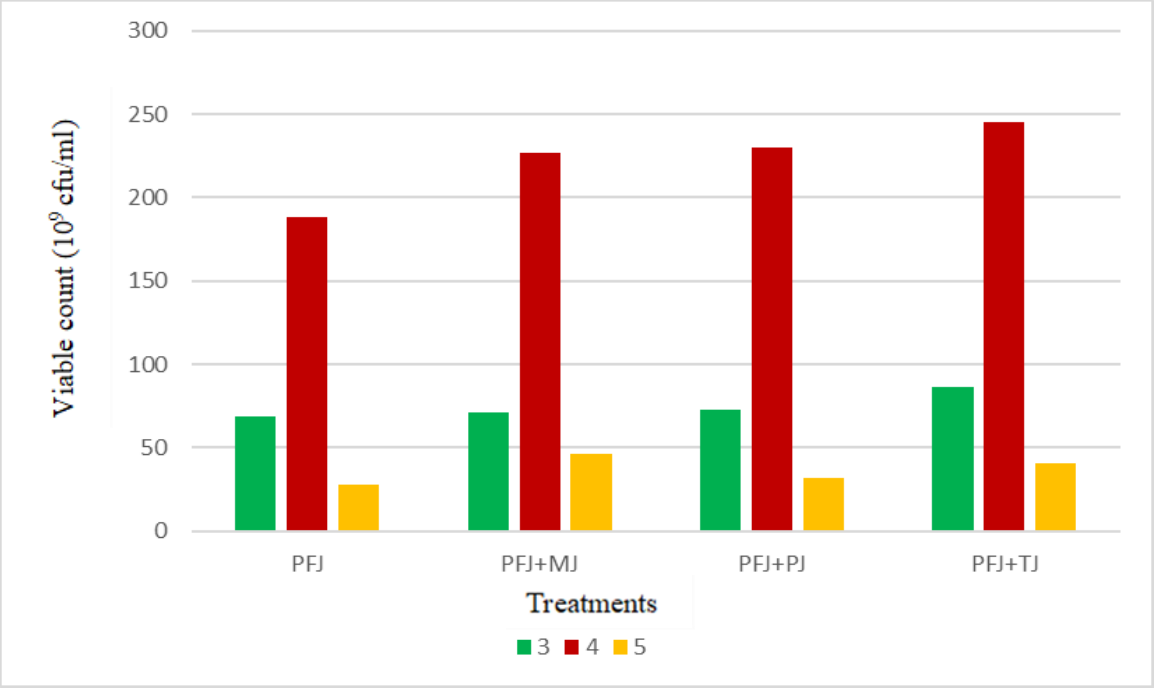


Fig. 4 Viable count of L. acidophilus in probiotic drinks with different inoculum concentrations (µl)

PFJ-Passion fruit juice, MJ-Mango juice, PJ-Pineapple juice, TJ-Tomato juice

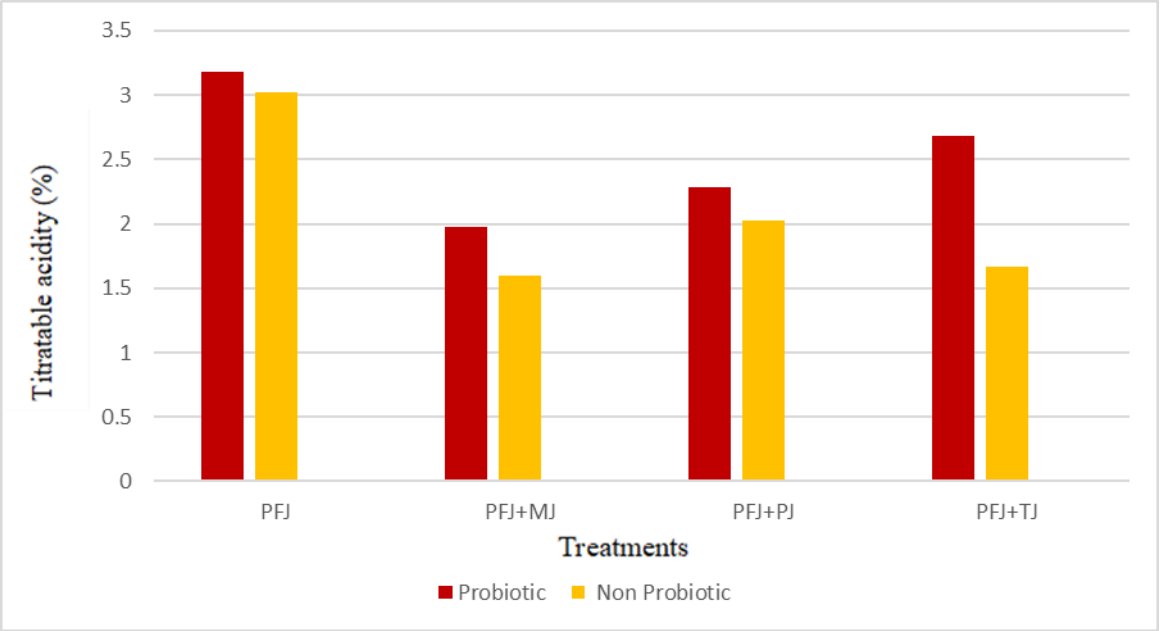


Fig. 5 Titratable acidity of probiotic and non probiotic drinks (%)

PFJ-Passion fruit juice, MJ-Mango juice, PJ-Pineapple juice, TJ-Tomato juice,

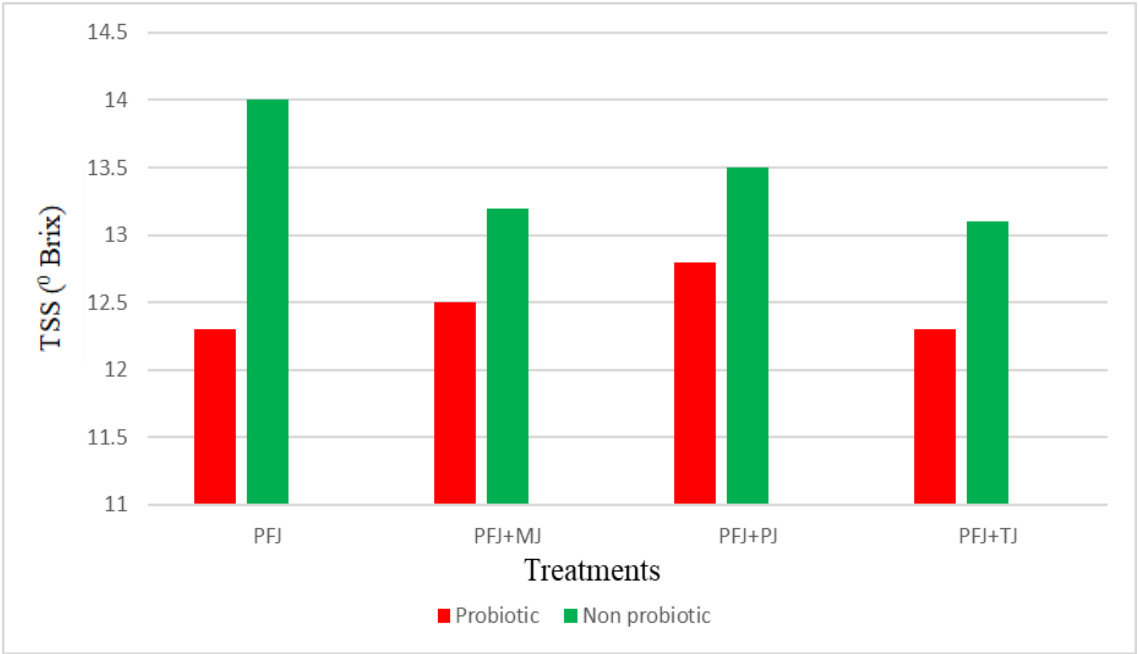


Fig.6 TSS of probiotic and non probiotic drinks (0 Brix)

PFJ-Passion fruit juice, MJ-Mango juice, PJ-Pineapple juice, TJ-Tomato juice

Fernandes *et al*. (2011) concluded that on pasteurising passion fruit juice there is difference in total sugar and reducing sugar. The pasteurized juice had 9.63 per cent total sugar and 8.33 per cent reducing sugar.

The amount of total sugars in RTS drink prepared by blending juices of passion fruit and cashew apple (50:50) along with the addition of ginger drops was 14.92 per cent (Sobhana *et al*., 2011). Decrease in total sugars and reducing sugar was observed in both honey and sugar enriched mango nectars (20 per cent pulp, 15°B TSS and 0.30 per cent acidity) during storage in sterilized glass bottles for six months at ambient and refrigerated condition. These changes in sugar content were more in nectar stored under ambient condition compared to those stored under refrigerated condition (Lakhanpal and Vaidya, 2015).

Total sugar content of watermelon and tomato probiotic drink with *L. casei* as probiotic organism *was* 20.70±4.99 mg/ml and also concluded that the probiotic cultures utilise sugar in the juice for their growth subsequently reducing the pH of the product (Sivudu *et al*., 2014).

The reducing sugar content of probiotic pomegranate beverage was 13.14 per cent were the juice was inoculated with mixed cultures of *L. bulgaricus* and *L. plantarum* in ratio 1:1 and fermented for 7 hours (Thakur and Sharma, 2017).

5.3.1.3. Carbohydrate, protein and energy

In the present study, carbohydrate content of probiotic drinks ranged from 5.29 to 9.80 g/100g and that of non probiotic drinks ranged from 6.80 to 10.6 g/100g (Fig. 9)

Stanon *et al*. (2003) reported that both genera *Lactobacillus* and *Bifidobacterium* were reported to have high requirements of free amino acids, peptides, vitamins and fermentable carbohydrates for their growth and development. *Lactobacillus* and *Bifidobacterium* strains grow well in pineapple juice meaning this matrix in itself was a suitable medium for propagation of probiotic bacteria.

Nguyen et al. (2019) concluded that Lactobacillus and Bifidobacterium require carbohydrates for their growth due to lack of proteolytic activity. Protein content of the pro biotic fruit drinks in the present study, ranged from 0.62 to 1.37 g/100g. But it ranged from 0.36 to 0.61 g/100g for non probiotic drinks. It was also

observed in the study that probiotic fermentation resulted in significant increase in the protein content of the fruit drinks (Fig 10).

As stated by Zamora and Fields (1979) the increase in protein content can be attributed to microbial synthesis of proteins from metabolic intermediates during their growth cycles. Moreira *et al*. (2017) observed an increase in protein content of probiotic mango juice. The protein content was 0.80 g/ 100g which increased to 0.97 g/ 100 g on probiotication with *Lactobacillus rhamnosus*.

Energy was comparatively lower in probiotic drinks ranging from 23.52 to 44.68 Kcal/100g while it was higher in non probiotic ranging from 29 to 44.84 Kcal/100g in the present study (Fig. 11).

The reduction in energy content of probiotic drink comparing to non probiotic drink was due to higher carbohydrate and fat content in fresh juice than probiotic juice (Rafiq *et al*., 2016).

5.3.1.4. Ascorbic acid and β carotene

Ascorbic acid content of non probiotic drinks were 12.80 to 13.70 mg/100g which reduced to 10 to 10.52 mg/100g in probiotic drinks (Fig 12)

Shukla *et al*. (2013) reported that reduction in ascorbic acid content of probiotic drinks were may be due to pasteurisation of juice and exposure to light.

The ascorbic acid content in RTS drink prepared by blending juices of passion fruit and cashew apple in different ratios such as 25:75, 50:50, 25:75 + ginger drops and 50:50 + ginger drops was 80.26 mg/100 g, 79.73 mg/100 g, 76.39 mg/100 g and 79.29 mg/100 g respectively (Sobhana *et al*., 2015).

Furtado *et al*. (2019) concluded that the mango juice probioticated with *L . acidopilus* have 15.040 mg/ 100g of ascorbic acid after a storage period of 28 days at 8^0 C.

The β carotene content of the probiotic drinks were observed to be within the range of 0.52 and 1.10 mg/100g (Fig 13). When it comes to non probiotic drinks, the values vary from 1.02 to 2.20 mg/100g, which shows significant reduction in probiotic drinks comparing to non probiotic drinks.

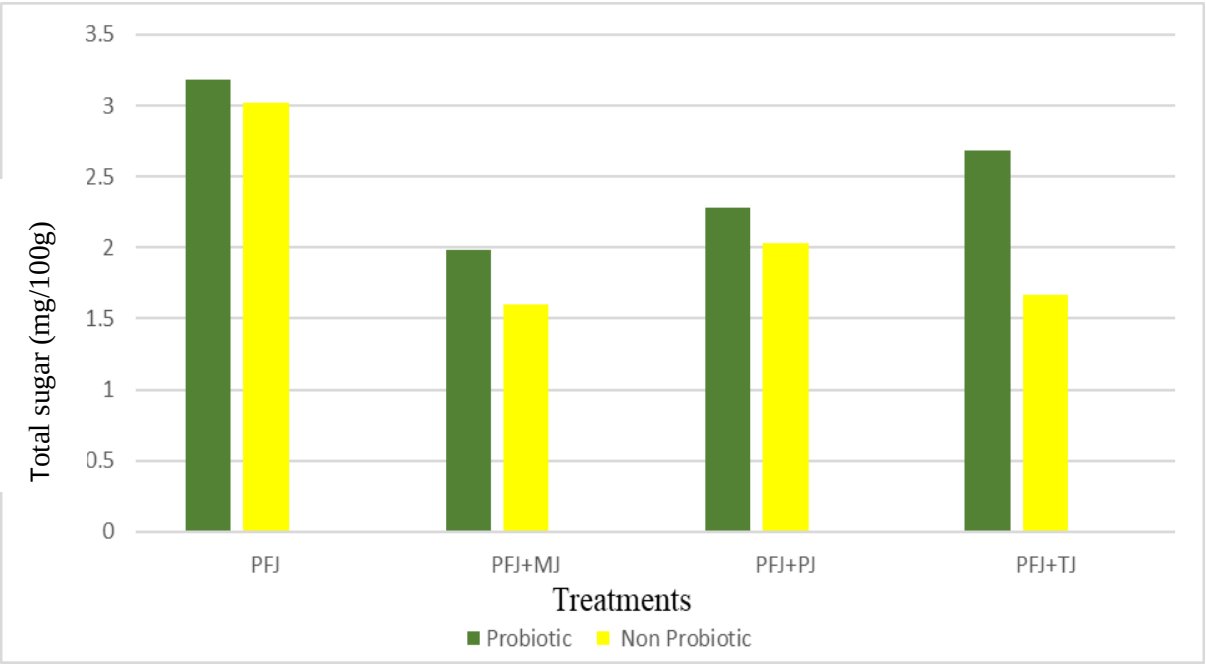


Fig. 7 Total sugar content of probiotic and non probiotic drinks (mg/100g)

PFJ-Passion fruit juice, MJ-Mango juice, PJ-Pineapple juice, TJ-Tomato juice,

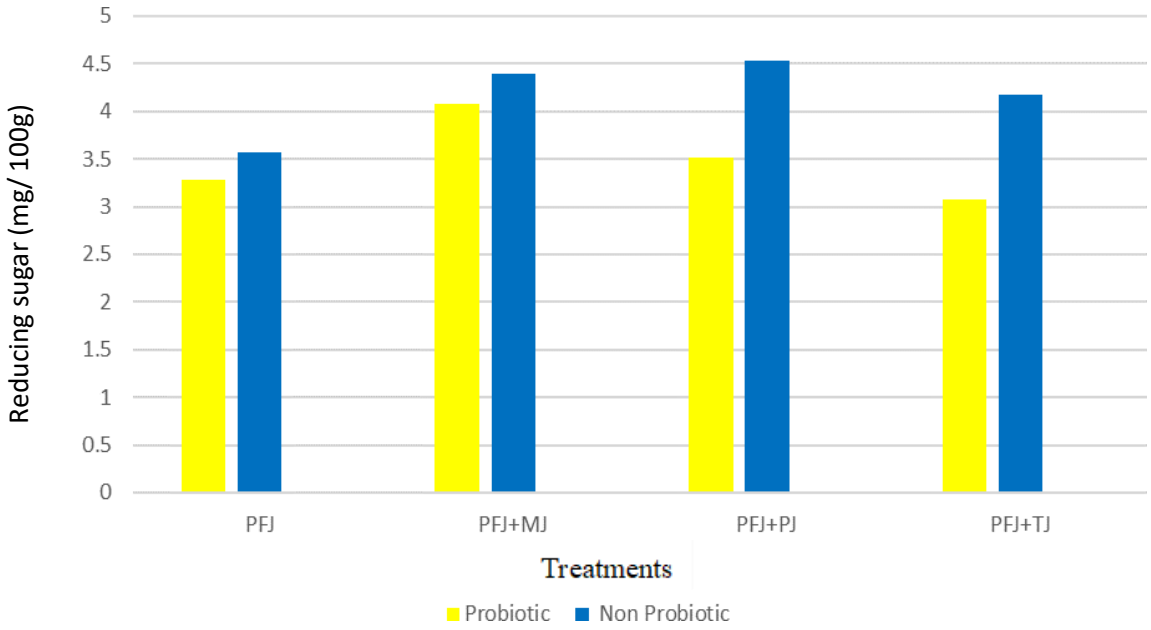


Fig. 8 Reducing sugar content of probiotic and non probiotic drinks (mg/ 100g)

PFJ-Passion fruit juice, MJ-Mango juice, PJ-Pineapple juice, TJ-Tomato juice,

This was in contrast to the findings of Sharon (2010) who reported there was no variation observed in the β carotene content of probiotic fermented and unfermented banana based food mixtures.

Kathiravan *et al*. (2013) reported a total carotenoid content of 1962.39 µg /100g in fresh yellow passion fruit juice. Total carotenoid content of passion fruit decreased during storage in different packging material. After 3 weeks of storage, maximum retention of carotenoid (0.57 mg/100g) was observed in individually shrink wrapped passion fruit with polyolefin film of 25µ thickness and the minimum (0.51 mg/100g) in individually shrink wrapped passion fruit with polyolefin film of 15µ thickness. Higher retention of carotenoids in shrink wrapped fruits may be due to lower rates of oxidation in these samples as compared to the exposed fruits in the unwrapped form (Charan, 2016).

5.3.1.5. Total ash and minerals

The present study reported non significant changes in the total ash, iron, potassium and phosphorus of probiotic and non probiotic drinks (Fig.14) . As stated by Jood and Khetarpaul (2005) probiotication may increase the bioavailability of various minerals but there need not be any change in the total mineral content in probiotic foods.

The findings are also in agreement with the results of Sharon (2010) who reported that there was no significant difference in the potassium content of probiotic fermented and unfermented banana based food mixtures.

The calcium content of probiotic drinks ranged from 8.03 to 8.80 mg/100g and that of non probiotic drink range from 7.8 to 8.10 mg/100g in the present study.

A similar finding was reported by Aparna (2015) where the calcium content of honey based beverage increased to 3.2mg/100 from 2.6 mg /100g. Similar result was observed by Suma (2009) where the calcium content of dehydrated banana drink mix increased from 14.35 to 33.16mg/100g.

5.4. Storage studies of passion fruit based probiotic drinks

5.4.1. Organoleptic evaluation of the developed passion fruit based probiotic drinks

In the present study, probiotic drinks had higher organoleptic value compared to their respective non probiotic drinks. Initially, probiotic drinks of PFJ, PFJ+MJ, PFJ+PJ and PFJ+TJ had total score of 48.99, 50.37, 51.17 and 49.46 respectively which was reduced to 48.76, 50.14, 50.97 and 49.32 after a storage period of 15 days. From the study, it was clear that there was no much differences in the overall acceptability of the probiotic and non probiotic fruit drinks.

The addition of juices like pineapple, mango and passion fruit improve the aroma and flavour of the final product and mask the probiotic off flavours after fermentation (Luckow and Delahunty, 2004).

Lactic acid bacteria are found to produce unique aroma and flavour for probiotic products. Molecules and metabolites produced during fermentation (exo polysaccharide, aromatic compounds, and organic acids) defines the sensory qualities of probiotic products. Alcohols, aldehydes, ketones, esters and fatty acids are the aromatic compounds derived after fermentation. These compounds are derived from catabolism of carbohydrates, proteins and fats in the raw material used. In tomato juice, lactic acid fermentation produce aromatic compounds like alcohols, esters, ketones, alkanes and terpenes which improves the sensory qualities of the product (Nazzaro *et al*., 2008).

Williams *et al* (2001) also opined that, during probiotic reaction, proteolytic enzyme converts amino acid and produce aromatic compounds Probiotic acidification produces acetic acid, ethanol and several other aroma compounds which subsequently enhance microbial safety and also increase shelf life of the probiotic product (Leory and De Vyust, 2004).

In line with the present study, Shukla (2013) stated that, whey based probiotic pineapple beverage, did not show much difference in sensory evaluation and also concluded that the main descriptors that characterised the probiotic product were acidity and sweetness. The mean score for overall acceptability of whey pineapple juice blend was 8.87. Highest score for overall acceptability was in drink fermented for 5 h. Flavour and taste of the product was found to be enhanced due to

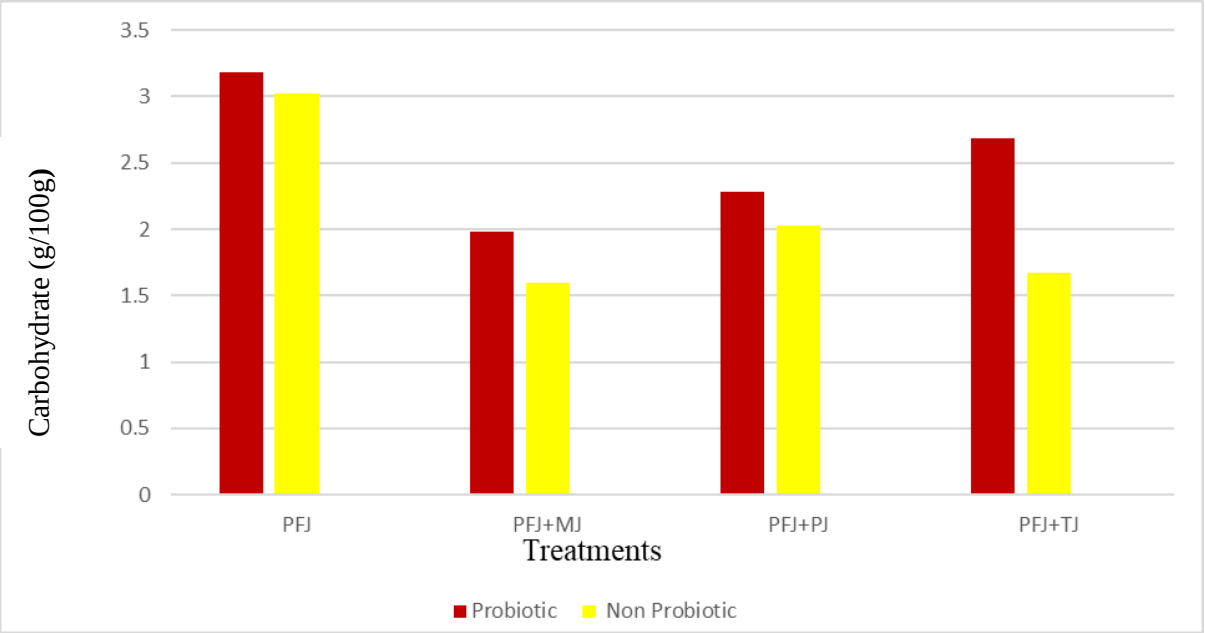


Fig. 9 Carbohydrate content of probiotic drinks (g/100g)

PFJ-Passion fruit juice, MJ-Mango juice, PJ-Pineapple juice, TJ-Tomato juice,

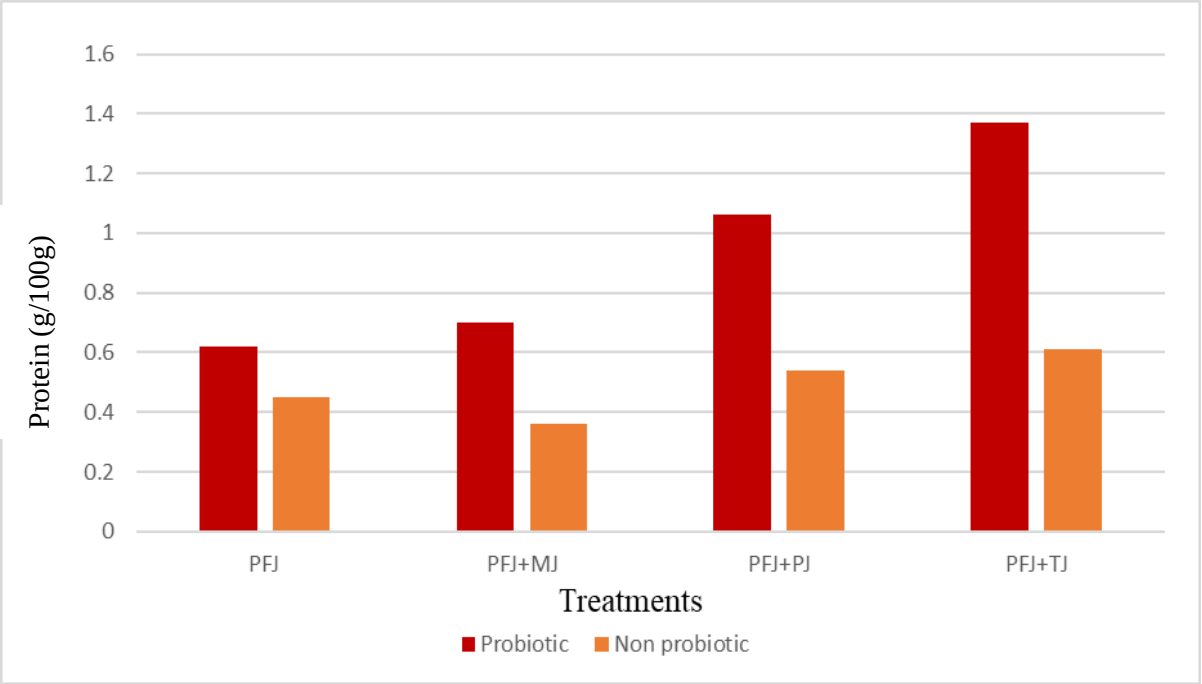


Fig. 10 Protein content of probiotic and non probiotic drinks (g/100g)

PFJ-Passion fruit juice, MJ-Mango juice, PJ-Pineapple juice, TJ-Tomato juice,

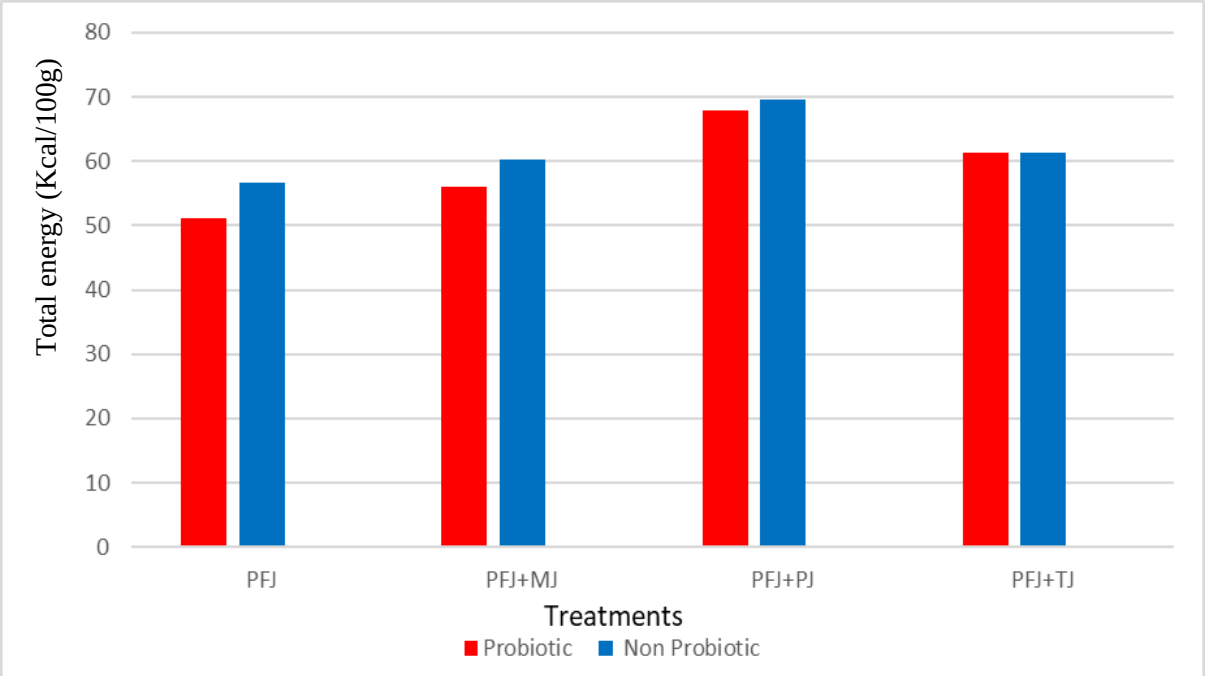


Fig. 11 Total energy of probiotic and non probiotic drinks (Kcal/100g)

PFJ-Passion fruit juice, MJ-Mango juice, PJ-Pineapple juice, TJ-Tomato juice,

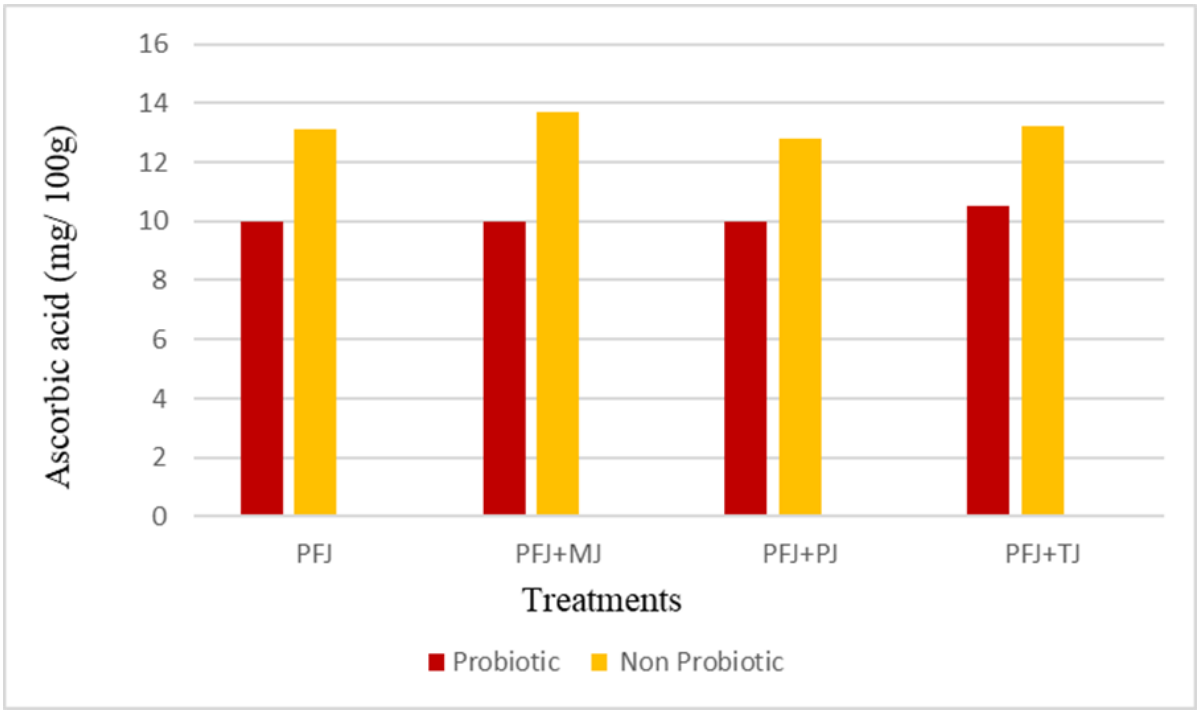


Fig 12. Ascorbic acid content of probiotic and non probiotic drinks (mg/ 100g)

PFJ-Passion fruit juice, MJ-Mango juice, PJ-Pineapple juice, TJ-Tomato juice,

probiotication. This may be due to the process of fermentation occurred in the beverage.

Similarly, Hossain *et al*. (2019) reported that, probiotic product has almost the same kind of taste, flavor, color and acceptance as fresh juice. There was no significant difference in overall acceptability among different juice samples. The sugar gets converted into acid with the help of bacteria and it get reduced with time and the acidity content get increased upon fermentation. *Lactobacillus* bacteria consume sugar for their cell synthesis during fermentation.

Gallina *et al*. (2019) developed probiotic fermented smoothie beverage with combination of mango and passion fruit. The overall acceptability, especially for the attributes like appearance, aroma and flavour, the beverages containing mango/passion fruit have scores ranging from "like moderately" to "like very much". The smoothie probiotic beverages made with mango/passion fruit were preferred in the ranking test with overall acceptability score of 7.4. It was also observed that there was an increase in acidity as well as a higher rate of syneresis and a small decline in probiotic viability after storage of 30 days.

Yoon *et al*. (2004) observed that, probiotication of tomato juice with *Lactobacillus delbrueckii*, *L. acidophilus, L. plantarum* and *L. casei* reduce the sugar content and pH subsequently increasing the acidity and concluded that the fermented tomato juice could be used to serve as a health beverage for vegetarians and consumers who are allergic to dairy products.

Mashayekh *et al*. (2015), developed blended probiotic drink of pineapple, apple and mango and concluded that the product was organoleptically acceptable for three weeks of storage and there was reduction in probiotic bacteria after 28 days of storage.

According to Ryan *et al*. (2020) the addition of mango juice was positively associated with increased consumer liking across all categories. Fourty per cent mango achieved the highest average for overall acceptance, flavour and mouthfeel. The

improved scores are likely to be the result of the mango providing sweetness and fruity flavours which mask the sour notes developed during fermentation.

5.4.2. Viability of *Lactobacillus acidophilus*

In the present study, the probiotic count (initially) of the developed fruit drinks ranged from 13.27 to 13.39 log cfu/g. There was reduction in the viable count of *L. acidophilus* with storage. After 15 days of storage, the viable count of probiotic organism reduced from the range of 13.26 to 13.37 log cfu/ ml. Even after 15 days of storage, the viable count of *L. acidophilus* in all the probiotic drinks were in the levels as specified by FSSAI (2010).

Similar findings was reported by Dogahe *et al*. (2015), where the number of live cells of probiotic bacteria in pineapple, apple and mango juice mixture during storage at temperature 4°C reduced after two weeks. The results of study are consistent with the findings of Nagasivudu *et al*. (2016) where *Lactobacillus casei* in the mixture of watermelon and tomato juice incubated at temperature 37°C, had better survival during storage at temperature 4°C.

In contrast to the present study, addition of 40 per cent of mango had a significant ($P < 0.05$) negative influence on probiotic viability (4.08 log cfu/ml) of the product developed by Ranadheera *et al*. (2010) and was not at the recommended therapeutic levels.

Sharon (2010) evaluated the probiotic capacity of banana based food mixture and found out that after storage for a period of six months the viable counts of *L. acidophilus* in the food mixtures significantly reduced which ranged from 8.84 to 9.12 log cfu/ g. Even though the viable count was within the desired level.

Aparna (2015) developed a honey aloevera pulp and soy milk beverage with *L. acidophilus* and stored in refrigerated condition and reported an increase on fourth day of storage from 21.2×10^9 cfu/ml to 42.4×10^9 cfu/ml, while on 6th day it declined to 33.6×10^9 cfu/ml.

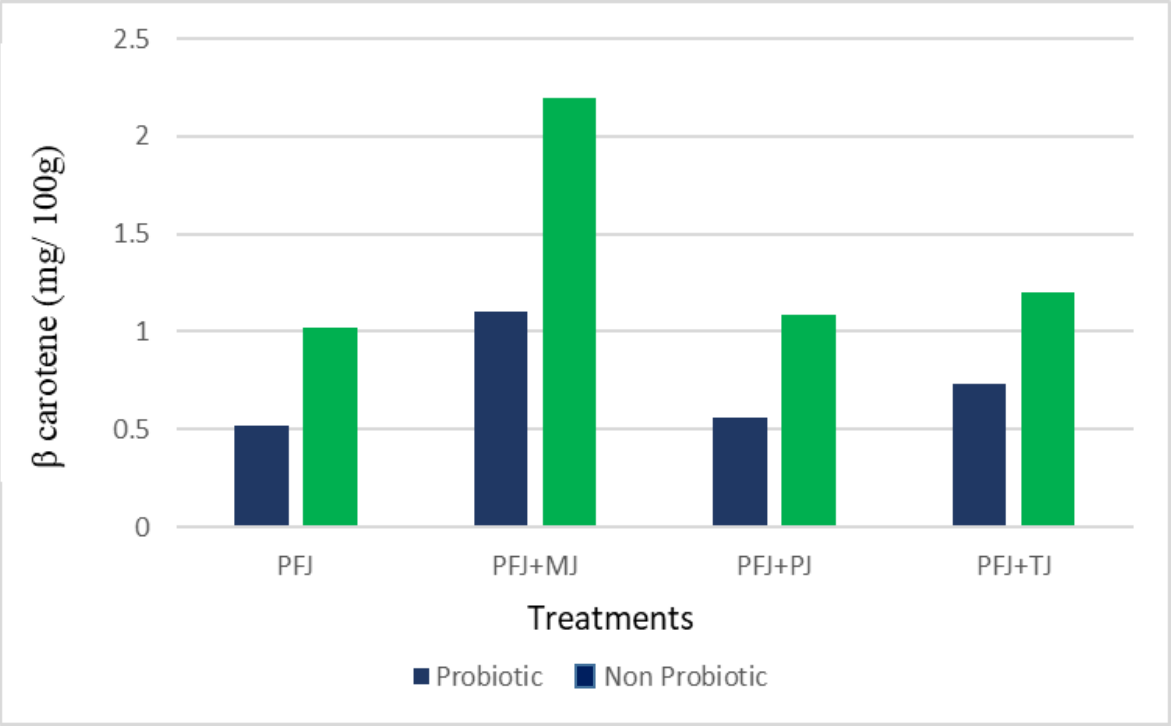


Fig. 13 β carotene content of probiotic and non probiotic drinks (mg/ 100g)

PFJ-Passion fruit juice, MJ-Mango juice, PJ-Pineapple juice, TJ-Tomato juice,

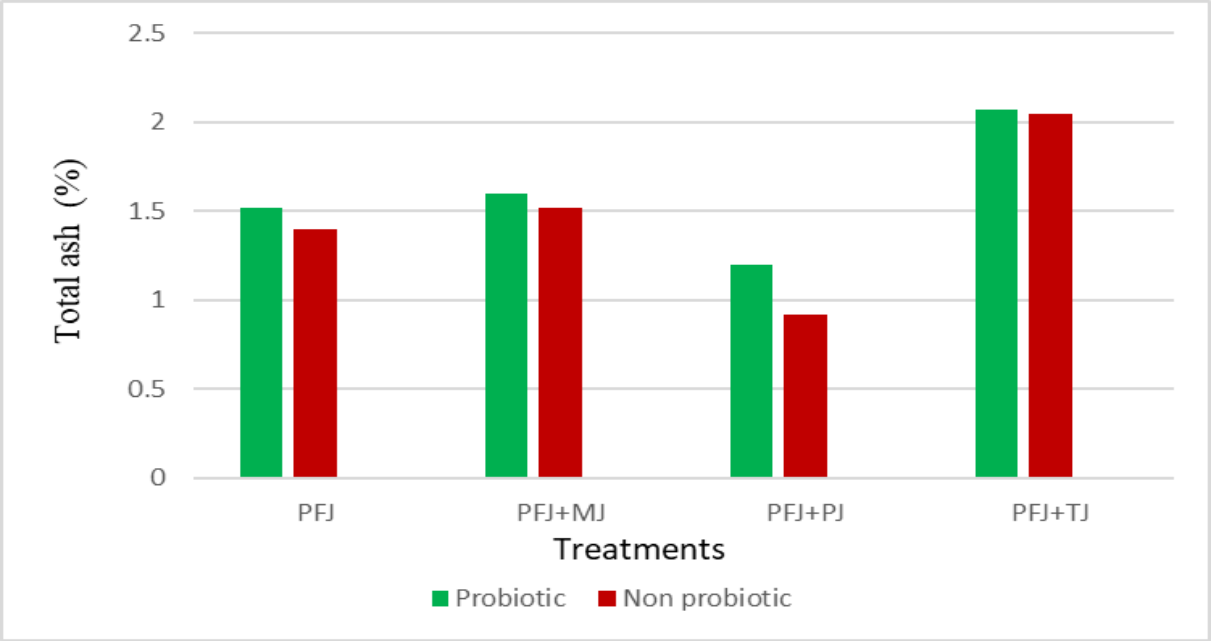


Fig. 14 Total ash content of probiotic and non probiotic drinks (%)

PFJ-Passion fruit juice, MJ-Mango juice, PJ-Pineapple juice, TJ-Tomato juice,

Remya (2020) developed shake mix with the best combination of jack fruit flour, defatted soy flour, jack fruit seed flour and tomato with probiotic strain *L. acidophilus*. The probiotic count of the developed instant shake mixes varied from 10.14 to 10.19 log cfu/g.

Ryan (2020) observed decrease in the growth of *L. acidophilus* in viability over five-week storage period at 4° C and had 7.72 log cfu/ml after storage of five weeks of mango juice.

5.4.3. Total microflora of developed drinks

In the present study, during storage, the total bacteria were found to be decreased in probiotic fruit drinks. On the other hand, the total bacterial count of the non probiotic fruit juices increased gradually. The total bacterial count in probiotic passion fruit drinks were very high due to the presence of viable *L. acidophilus* in the drinks.

Sharon (2010) reported a decrease in the total bacterial count in the banana based probiotic food mixture on storage for a period of six months (9.17 to 8.99 log cfu/g). Remya (2020) also reported a decrease in the bacterial count of jack fruit bio-yoghurts on storage.

Initially there was no fungal growth in both probiotic and non probiotic drinks and on the 15^{th} day fungal growth was observed in the range of 1.20 to 2.40×10^2 cfu/ml which was very low. Yeast growth was not observed on storage.

As per the FSSAI (2010) guidelines, the bacterial count (aerobic plate count) is not applicable in the case of fermented fruit and vegetable products. The presence of fungi in the fruit drink were in agreement with the specifications of FSSAI (2010). Hence, it can be concluded that the product was shelf stable upto 7 days of storage and was microbiologically safe for consumption upto 7 days.

5.5. Cost of production of developed probiotic drinks

Cost of the product is very important, as the product cost can decide its profit or loss. Cost of the product depends on the cost of the raw materials and other inherent cost involved in the formulation of the beverage.

The cost of production of the probiotic passion fruit based drinks ranged from Rs 25 to Rs. 34 for 200 ml. The cost was comparable with the commercially available probiotic products presently available in market ranged between Rs 25 to Rs 50 for 100 ml. The cost of honey based aloe vera probiotic drink developed by Aparna (2015) was Rs.15/ 100 ml of drink.

Summary

6. SUMMARY

The study entitled 'Process optimisation and quality evaluation of passion fruit based probiotic drinks' was carried out with the objective of developing passion fruit based drinks involving probiotic fermentation with *L.acidophillus* MTCC 10307.

The proportion of ingredients for the passion fruit based drinks were standardised with three sets of treatments. From each set, one fruit drink with maximum organoleptic scores were selected. The fruit drink containing 50 per cent passion fruit juice and 50 per cent mango juice (T_5) was selected from set 1, whereas fruit drinks containing 70 per cent passion fruit juice along with 30 per cent pineapple juice and tomato juice (T_3) were selected from set 2 and 3 by applying Kendall's coefficient of concordance based on their organoleptic qualities. The total scores obtained for the best combinations of each set was 50.26, 51.16 and 49.01 for set 1, set 2 and set 3 respectively. Standardised drinks were subjected to pasteurisation at 80^0 C for 20 minutes.

All the selected fruit drinks were then optimised for maximising the growth of *L. acidophilus* MTCC 10307. From each selected drinks, 25 ml, 50 ml and 75 ml along with their controls were taken and inoculated with 4 µl of 24 hour old culture of *L. acidophilus* and incubated for 1 hour. In all the three combinations including control 25 ml of substrate showed the maximum viable count of *L. acidophilus* ranging from 13.26 to 13.45 log cfu/ml. The selected 25 ml of each treatments were then inoculated with 4 µl culture and incubated for 1, 2 and 3 hours and 1 hour showed the best result with count ranging from 13.25 to 13.38 log cfu/ ml. To optimize the temperature, 25 ml of all treatments were taken, inoculated with 4 µl culture and kept for 1 hour at different temperature (37^0 C, 38^0 C and 39^0 C) and 37^0 C showed maximum viable count of *L. acidophilus* (13.28 to 13.37 log cfu/ ml). Inoculum optimisation was done by inoculating 3, 4 and 5 µl of culture to the selected drinks and the result showed that 4µl of culture gave maximum growth of *L. acidophilus* (13.27 to13.38 log cfu/ ml).

The selected fruit drinks along with their respective controls (non probiotic drinks) were subjected to physico-chemical analysis. In the drinks, titratable acidity ranged from 1.60 to 3.02 per cent in non probiotic drinks, were as there was a significant increase of titratable acidity in probiotic drinks (1.98 to 3.18 %). Protein

content (0.36 to 0.61 g/100 g) was observed in non probiotic drinks and a significant increase was observed in probiotic drinks (0.62 to1.37 g/100g). Significant decrease in TSS was also observed in probiotic drink (12.30 to 12.80^0 brix) compared to non probiotic drink (13.10 to 14). Total sugar and reducing sugar of probiotic drinks were in the range of 14 to 16.66 g/ 100g and 3.08 to 4.08 g/ 100g respectively and a significant increase was observed in non probiotic drink in the range of 15.20 to 17.10 g/ 100g and 3.57 to 4.53 g/ 100g respectively. The carbohydrate content was significantly decreased in probiotic drink (12.19 g/ 100g to 15.90 g/ 100g) compared to non probiotic drinks (13.7 g/ 100g to 16.85 g/ 100g) similarly, reduction in energy content was also observed in probiotic drink (51.24 Kcal to 67.84 Kcal) comparing to non probiotic drink (56.6 to 69.56 Kcal). Ascorbic acid content of non probiotic drink was between 12.80 to 13.70 mg/ 100g and that of non probiotic drink was between 10 to 10.52 mg/ 100g.

With respect to mineral content, maximum phosphorus content was observed probiotic drink and non probiotic PFJ+PJ (6.72 and 6.71 mg/ 100g), probiotic and non probiotic drink having highest potassium content was PFJ (45.25 and 45.23 mg/ 100g) Iron and calcium of probiotic drinks range between 0.22 to 0.29 mg/ 100g and 2.04 to 2.65 mg/ 100g respectively and that of non probiotic drinks were 0.20 to 0.28 mg/ 100g and 2.02 to 2.64 mg/ 100 g respectively. It was observed from the results that, there was non significant difference of total ash content between probiotic and non probiotic drinks ranging from 1.52 to 2.07 per cent in probiotic drinks and 1.40 to 2.05 per cent in non probiotic drinks.

The selected fruit drinks along with their respective controls were packed in food grade plastic bottles and kept under refrigerated condition for a period of 15 days. The quality evaluation was carried out initially and on the 7^{th} and 15^{th} day of storage. A decrease in the sensory attributes of the drinks stored under refrigerated condition was observed on the 7^{th} and 15^{th} day of storage. Initially the overall acceptability of probiotic and non probiotic passion fruit drinks (PFJ) were 8.16 and 8.15 respectively which was reduced to 8.14 and 8.13 respectively. Similar reduction was observed in PFJ+MJ, PFJ+PJ and PFJ+TJ. However, even after 15 days of storage the fruit drinks had good acceptability. The total score of probiotic PFJ, PFJ+MJ, PFJ+PJ and PFJ+TJ were 48.76, 50.14, 50.97 and 49.32 and that of non probiotic drinks were 48.71, 49.95, 50.94 and 49.27 respectively.

The viable count of *Lactobacillus acidophilus* in PFJ drink stored in refrigerated condition decreased from 13.27 to 13.26 log cfu/ml, and in PFJ+MJ the viable count declined from 13.35 to 13.33 log cfu/ml, PFJ+PJ declined from 13.36 to 13.35 log cfu/ml and that of PFJ+TJ declined from 13.39 to13.37 log cfu/ml. However, the viability of *L. acidophilus* was within the recommended level of the probiotic organism to assure health benefits. The cost of the developed fruit drinks ranged between Rs. 25.5 to 33.5 for 200 ml.

It was concluded that it is possible to produce probiotic passion fruit based drinks containing acceptable levels of *L. acidophilus* with good organoleptic properties.

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Appendices

APPENDIX – I

Score card for the organoleptic evaluation of passion fruit based drinks

Name : **Date :**

Signature :

Treatments	Appearance	Colour	Flavour	Texture	Taste	OAA

Nine point hedonic scale

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

APPENDIX – II

Score card for the organoleptic evaluation of passion fruit based probiotic and non probiotic drinks

Name: **Date :**

Signature:

	Treatments	
	Probiotic	**Non probiotic**
Appearance		
Colour		
Flavor		
Texture		
Taste		
Overall acceptability		

Nine point hedonic scale

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

PROCESS OPTIMISATION AND QUALITY EVALUATION OF PASSION FRUIT BASED PROBIOTIC DRINKS

By
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ABSTRACT OF THE THESIS
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ABSTRACT

The functions of food has extended from satisfying hunger and providing nutrients to body, to health maintenance, wellness and prevention of diseases. Probiotics are such functional foods which when incorporated to foods helps to improve its nutritional profile and therapeutic value. Hence, the study entitled "Process optimisation and quality evaluation of passion fruit based probiotic drink" was undertaken with the objective of standardising probiotic fruit drinks with different combinations of fruits with passion fruit and also to evaluate the nutritional, organoleptic and shelf life qualities of these developed passion fruit based probiotic drinks.

Passion fruit probiotic drinks were developed in combination with mango, pineapple and tomato. The proportion of ingredients were standardised with three sets of treatments, and from each set, one fruit drink combination with maximum organoleptic scores were selected. The fruit drink containing 50 per cent passion fruit juice (PFJ) and 50 per cent mango juice (MJ) (T_5) was selected from set 1, whereas fruit drink containing 70 per cent passion fruit juice and 30 per cent pineapple juice (PJ) and tomato juice (TJ) (T_3) was selected from set 2 and 3 respectively. Total scores for the selected combinations were 50.06, 51.16 and 49.01 respectively for T_5 (PFJ+MJ) and T_3 of PFJ+ PJ and PFJ+ TJ.

For all the selected fruit drinks, the conditions were optimised for attaining the maximum viable count of *L. acidophilus*. The fruit drink (25 ml) fermented with 4 µl of inoculum for 1 hour at 37^0 C gave the maximum viable count of *L. acidophilus* ranging from 13.27 to 13.38 log cfu/g. The selected fruit drinks from each set along with their respective control (non probiotic samples) were analysed for their nutritional and organoleptic qualities.

Titratable acidity ranged from 1.60 to 3.02 per cent in non probiotic drinks, where as in probiotic drinks it ranged between 1.98 to 3.18 per cent. Protein content ranging between 0.36 to 0.61 g/100 g was observed in non probiotic drinks and increased protein content was observed in probiotic drinks (0.62 to1.37 g/100g). Significant decrease in TSS was observed in probiotic drinks (12.3 to 12.8^0 brix) compared to non probiotic drinks (13.10 to 14^0 brix). Total sugar and reducing sugar of probiotic drinks were in the range of 14 to 16.66 g/ 100g and 3.08 to 4.08 g/ 100g respectively and a significant increase was

observed in non probiotic drink, 15.20 to 17.10 g/ 100g and 3.57 to 4.53 g/ 100g respectively. With respect to mineral content, maximum phosphorus content was observed in PFJ+PJ probiotic and non probiotic drinks, whereas the highest potassium content was for PFJ in both probiotic and non probiotic. Iron and calcium of probiotic drinks ranged from 0.22 to 0.29 mg/ 100g and 2.04 to 2.65 mg/ 100g respectively and that of non probiotic drinks were 0.20 to 0.28 mg/ 100g and 2.02 to 2.64 mg/ 100 g respectively.

The probiotic fruit drinks were packed in food grade plastic bottles and kept for storage studies under refrigerated condition for a period of 15 days and a decrease in the sensory attributes were observed. Initially, the overall acceptability of probiotic and non probiotic PFJ drinks were 8.16 and 8.15 respectively which reduced to 8.14 and 8.13 respectively after storage. Similar reduction was observed in every set of samples. The total score of probiotic PFJ, PFJ+MJ, PFJ+PJ and PFJ+TJ were 48.76, 50.14, 50.97 and 49.32 and that of non probiotic drinks were 48.71, 49.95, 50.94 and 49.27 respectively. The viable count of *L. acidophilus* decreased on storage from 13.27 to 13.39 log cfu/ ml to 13.26 to 13.37 log cfu/ ml.

The cost of production of probiotic fruit drinks were in the range of Rs. 25.5 to Rs. 33.5 per 200 ml. The study revealed that passion fruit can be a suitable substrate for probiotic fermentation and probiotic drinks can be successfully developed. Further research can be done for the development of innovative probiotic products from passion fruit.