STUDIES ON FERMENTATION OF POMEGRANATE JUICE BY LACTIC ACID BACTERIA

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CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON FERMENTATION OF POMEGRANATE JUICE BY LACTIC ACID BACTERIA"** submitted by **Ms. SHUBHADA, N., ID. NO UHS15PGM622** for the degree of **MASTER OF SCIENCE (HORTICULTURE)** in **POST HARVEST TECHNOLOGY** of the University of Horticultural Sciences, Bagalkot is a record of research work done by her during the period of her study in this university under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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BAGALKOT **(SHUBHADA, N.)**
JUNE, 2017

Affectionately dedicated

to

My Beloved Parents

and Chairman

CONTENTS

LIST OF ABBREVIATIONS

Symbols	**Abbreviations**
%	Per cent
ºC	Degree Celsius
CD	Critical Difference
cv.	Cultivar
et al.,	Et al (and his co-workers)
g	Gram
i.e	That is
kg	Kilogram
mg	Milligram
ml	Millilitre
S.Em	Standard Error of Mean
viz.	*Videlicet* (namely)
cfu	Colony forming unit
hr	Hour
Anon.	Anonymous

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1. INTRODUCTION

Pomegranate (*Punica granatum* L.) is an ancient favourite table-fruit of the tropical and subtropical regions of the world, belonging to the family Punicaceae. The word pomegranate is derived from pomum (apple) and granates (seeded). Morphogicaly it is known as Balusta. As a commercial crop, pomegranate is grown on a large scale in Maharashtra, Karnataka, Andra Pradesh, Uttar Pradesh, Gujarat, Rajasthan and Tamil Nadu. Apart from India, it is also grown in Burma, China, Japan and USA (Regde and Pai, 1999).

India is the world leading country in pomegranate production. The estimated global cultivated area under pomegranate is around 3.0 lakh hectares and production is 3.0 million tonnes. In India, total area under pomegranate is 130.80 thousand hectares, out of which 90,000 ha is in Maharashtra only and total production in India is 1345.70 thousand MT. Production of pomegranate in Karnataka is around 134.20 thousand MT from an area of 16.60 thousand hectares (Anon, 2015). The important cultivars grown in India are Kesar, Dholka, Kabul, Alandi, G-137, Ganesh, Khandari, Mridula and Jyothi (Wasker and Sushant, 2004). Sizeble area under this crop in Karnataka is in the districts of Bijapur, Bagalkot, Chitradurga, Koppal, Kolar, Belgaum, Tumkur and Banglore.

Pomegranate juice has been reported to possess numerous health benefits which are attributed to a wide range of phenolic compounds and antioxidants in the juice (Pokorny and Schmidt, 2003). Pomegranate fruit is also rich in vitamin C. One pomegranate fruit provides about 40 percent of the daily requirement of vitamin C. The processed products of pomegranate such as jellies, syrup and especially pomegranate juices are well accepted among consumers. Pomegrante juice is consumed throughout the world for its pleasant and unique aroma, flavour and colour (Ferrara *et al.*, 2011).

Pomegranate fruits with larger size and attractive colour fetches higher price in market. However, the fruits with physical defects such as spoiled, cracked and other non-marketable pomegranate fruits which are often not desired by consumers are being wasted in large quantities on the farm as well as in market yards. The utilization of such fruits for processing and converting them to value added products is one of the better management strategies to avoid wastage of fruits and to get better revenue to farmers.

The utilization of such second quality fruits for development of fermented beverages has been revealed as a new and promising alternative to generate extra revenues.

Fermentation is one of the oldest forms of food preservation technology in the world. Fermentation is a potent tool in the development of new products with modified physical and chemical properties, improved flavour and nutrition besides therapeutic value. Considerable research efforts have been attempted towards fermented beverages from fruits and vegetables (Gobbetti and Rossi, 1989; Beuchat and Nail, 1978). Fermented foods are more nutritious than unfermented ones due to synthesis or release of nutrients by some of the microorganisms employed (Joshi *et al.*, 1990). The term fermentation was used for the production of wine in early days, but at present it encompasses the foods made by the application of microorganisms including lactic acid bacteria (LAB). Food industry is targeting for development of more healthy products due to consumer’s demands for decrease of chemical preservatives. Lactic acid fermentation is one of the oldest methods of preserving fruits and vegetables without use of chemical preservatives which contributes desirable physical and flavour characteristics.

The quality of fermented beverage depends upon a number of factors like cultivars, adequate sugar level, acid content, colour, aroma and strains used (Ethiraj and Suresh., 1993). The biochemical and nutritional parameters of fermented beverages can be enhanced by blending juices from two or more fruits which are having unique quality traits. There is high potential for the development of blended fermented products using different fruit juice. In the present study, efforts were made to develop fermented pomegranate beverage by blending with kokum rind extract.

Several minor fruits are grown which are known for their therapeutic and nutritive values with an excellent flavour and attractive colour and have a great potential for processing. Kokum (*Garcinia indica* choisy) is one of such minor fruits which belong to family clusiaceae and found in tropical rain forests of Western coastal region of India and also grown as home garden tree (Subashchandran, 2005). It is found in forest lands, riversides and wasteland. In Karnataka the tree mainly found in Uttara Kannada, Udupi, Dakshina Kannada Districts and Kasaragod area of Kerala. Majority of the yield (rind) is used only for syrup and juice preparation during summer months.

Remaining part is not harvested and goes as waste. These fruits can be utilised to produce value added products like fermented beverages. In kokum, the predominant fermentative microorganisms are both lactic acid bacteria and yeasts. Kokum contains low sugar content, so when lactic acid bacteria are involved, acids are formed which turns the product sourer and consumer doesn‘t relish the product. Hence it is required to add an external source of sugar to facilitate the fermentative activity of bacteria. When kokum juice is blended with the raw materials rich in carbohydrates like pomegranate juice, it improves the quality with respect to flavour, chemical characters and nutrient content through fermentation.

Several health benefits of fermented beverages or value added juices from kokum could attract health conscious. It has been found that rind of the fruit contains hydroxy citric acid (HCA), garcinol and the coloring pigment anthocyanin. Hydroxy citric acid which is claimed to have fat-reducing properties is often used to reduce obesity (Lopes, 2007). Garcinol exerts strong antioxidant activity (Padhye *et al.*, 2009) and anti- inflammatory effect (Liao *et al.*, 2005). It is also a remedy for diarrhea, dysentery, piles and tumors. It facilitates digestion, purifies the blood and reduces cholesterol (Mishra *et al.*, 2006).

Lactic acid bacteria are among the most important probiotic microorganisms typically associated with the human gastrointestinal tract (Klewicka *et al*., 2004; Mousavi *et al*., 2011; Yoon *et al*., 2004, 2005, 2006). Probiotic products are usually marketed in the form of fermented milks and yoghurts. However, with an increase in the consumer vegetarianism, there is a demand for the non dairy probiotic products. Furthermore, lactose intolerance and the cholesterol content are two major drawbacks related to the fermented dairy products (Heenan *et al*., 2004; Yoon *et al*., 2006). There are a wide variety of traditional non-dairy fermented beverages produced around the world. The non dairy probiotic beverages may be made from a variety of raw materials, such as cereals, millets, legumes, fruits and vegetables. In this direction, fermentation of pomegranate juice as single substrate or mixed with kokum juice for beverage production may results in high nutritional value deriving from both the substrates and the culture used.

Fermentation of fruit juices using lactic acid bacteria have been reviewed by Dushyantha *et al*. (2010), Mousavi *et al.* (2011), Costa *et al.* (2013), Filannino *et al.* (2013), Shukla *et al*. (2013) and Kumar *et al*. (2015). The fermented beverage with both probiotic and prebiotic components could be a good health drink. Saccharides are a good source of food for probiotic lactic acid bacteria, being utilized mostly as prebiotics (Rastall and Maitin *et al*., 2002; Teitelbaum and Walker *et al*., 2002; Wang, 2009). Instead of adding cane sugar, prebiotics like, honey and lactose can be blended for the development of symbiotic fermented beverage. Honey with fruits and vegetables gained lot of importance with respect to nutrition and health points. Since, honey is a fructooligosaccharide and very rich in sugars, different concentration of honey is added to pomegranate and kokum blended juice in the present study. Similarly, lactose is also important prebiotic substance hence; this can be used to improve the cell viability in the fermented juice. Keeping in view of the above facts in the present investigation attempts were made in order to produce good quality fermented beverage from pomegranate juice with the blend of kokum and with addition of prebiotics. The study was undertaken with the following objectives.

Objectives of investigation

1. To develop fermented pomegranate beverage with and without kokum juice using probiotic lactic acid bacteria

2. To study the effect of different prebiotics on fermentation of pomegranate juice using probiotic lactic acid bacteria

3. To study the storage stability and biochemical properties of fermented pomegranate beverage

2. REVIEW OF LITERATURE

A comprehensive review of literature pertaining to importance of pomegranate and kokum and its value added products, conversion of fruit into safe, stable nutritious products by fermentation of blended fruit juices using lactic acid bacteria, probiotification of vegetable and fruit juices by lactic acid bacteria and bifidobacteria, prebiotic enriched food products through microbial processing has been reviewed in this chapter. The research work done on these aspects in pomegranate and kokum is very limited. Therefore, the available literature on other fruit, vegetables and fruit blended dairy products has also been reviewed.

A wide variety of traditional fermented foods have been developed over a period of time from vegetables, cereals, pulses, meat and milk. The primary microorganisms responsible in bringing about the desirable characters in the final products are those belonging to lactic acid bacteria (LAB) group.

Probiotic is the word means “for life” and it is generally used to name the bacteria associated with the beneficial effects for humans and animals. Probiotication is one of the methods to produce fermented functional foods. Addition of probiotics to food provides several health benefits including reduction in the level of serum cholesterol, improvement of gastrointestinal function, enhancement of immune system and reduction in risk of colon cancer (Burner and Donnel, 1998). The development of probiotic beverages or products in food industry has gained importance in the last two decades. Considerable research and scientific findings on probiotic products have been well documented.

2.1 Composition of pomegranate fruit

The physicochemical composition of fruit is influenced by a number of factors such as variety, location and harvesting period (Saxsena *et al*., 1987). According to El-Nemer *et al*., (1990) the edible fruit of pomegranate fruit is 52% of the total fruit weight, which contains 78% juice and 22% seeds. The fresh juice contains 85.4% moisture, 10.6% total sugars, 1.4% pectin, and 0.19% acidity, 0.70 mg ascorbic acid, 19.60 mg free amino nitrogen and 0.05 g ash per 100 ml juice.

Fawole and Opara (2013) reported that the pomegranate fruit juice of cv. Bhagwa yields juice of 54.93%. Juice contains total soluble solids 16.18(°brix), titrable acidity (% citric acid) 0.38%, sugar:acid ratio of 41.83, pH 3.57 and juice colour at absorbance 520 nm is 3.00.

2.2 Value added products from pomegranate

Anar rub (Pomegranate jam): Anar rub with fairly good keeping quality can be made by concentrating pomegranate juice and heating the mixture on a slow fire for a long period. The finished product has thick consistency and contains 70-75% TSS (Siddappa and Bhatia, 1954).

Anardana: Arils of cracked fruits are dried to yield a value added by-product known as anardana used as acidulent and condiment in Indian curries. It contains more acid (5.8- 15.4%), total sugars (9.3-17.5%) and crude fiber as compared to fresh fruit, *viz.* acid (4.1%-5.3%), total sugars (8.3%) and crude fibre (2.5%) (singh *et al.*, 1990).

Pomegranate wine: The whole fruit is pressed without crushing or juice may be extracted from pomegranate grains, which gives a yield of 76 to 85% (Adsule and Patil, 1995). Sugar is added to the juice to bring it to 22-23°brix. Potassium meta-bisulphate is added to the juice to prevent the growth of undesirable microorganisms. The juice is fermented with the starter wine yeast and the wine is aged and finished in the same manner as red grape wine.

Syrup and jelly: A syrup of 60°brix with an added acidity of 1.5% as citric acid has a bright purplish-red colour and a delightful taste and flavour. It is preserved by pasteurization or by adding sodium benzoate. An attractive jelly can be prepared by pomegranate juice (Phadnis, 1974).

Fruit juice: On whole fruit basis the juice yield is about 42% while from grains the yield is about 70% (Phadnis, 1974). The juice is clarified by heating in a flash pasteurizer at 79-82°C cooling, settling for 24 hr. racking up and filtering and decanting. The juice is preserved by heat treatment or by using chemicals (600 ppm sodium benzoate).

2.3 Composition of Kokum fruit

According to Sampathu and Krishnamurthy (1982), composition of kokum fruit per 100 g of fruit on moisture free basis is as follows moisture 80 %, protein 1.92 %, crude fibre 14.28 %, total ash 2.57%, tannin 2.85%, pectin 5.71%, acid (hydroxy citric acid) 22.8% and ascorbic acid 0.06 %.

Krishnamurthy *et al.* (1982) studied the chemical constituents of kokum fruit rind and revealed the presence of (-) hydroxycitric acid, cyanidin-3-glucoside and cyanidin-3-sambubioside, their isolation, identification and determinations as well as the proximate composition of kokum.

2.4 Value added products from Kokum

Kokum is a rich source of beneficial compounds like HCA (hydroxyl citric acid), garcinol, citric acid, malic acid, anthocyanin pigments and ascorbic acid (Mishra *et al.*, 2006). The proximate composition of kokum rind contains moisture (30%), protein (1.92%), crude fiber (14.28%), pectin (5.71 %), crude fat (10%), hydroxyl citric acid (22.80%) and carbohydrate (36.40%) (Krishanamurthy *et al.*, 1982).

Kokum Amsal: This can be prepared by repeated soaking and drying of kokum rind in juice of the kokum pulp and sun drying. It is also called as unsalted kokum in commerce (Sampathu and Krishnamurthy, 1982). Kokum colour application in the area of fruit processed products, alcoholic and non-alcoholic beverages, preservatives and instant foods (Krishnamurthy *et al.*, 1982). Wine red syrup extracted from the rind of the ripe fruit with the help of sugar is stored in households for making cool drinks in summer (Anon., 1996).

Dried green kokum rind: It is a product prepared from the mature green kokum fruits. Fruits are cut into four longitudinal pieces and keep it in 2500 ppm solution of potassium meta-bisulphate for 2 hours and then dried at 50-55ºC temperature either in drier or under sun drying (Subaschandra, 1996).

Amrith kokum: Kshirsagar *et al.* (2001) reported that Amrith kokum can be prepared from the fresh ripe kokum rind by adding cane sugar at 1: 2 proportions. The mixture is kept in sunlight after packing in glass containers for 8 days by occasional stirring. Then the mixture was strained through muslin cloth and the syrup was stored in food grade plastic container or in glass bottles with addition of sodium benzoate at 610 mg/kg of syrup. This product is required to be diluted 5-6 times with water before consumption as sharabat. Pinch of salt and cumin powder is added for taste if required.

Kokum butter: It is extracted by crushing the kernels, boiling in water and skimming of the fat from the top, by churning the crushed kernels with water. Oil can be obtained by solvent extraction. The yield of oil is about 25 per cent (Nandkarni, 1954 and Anon., 1996). Jagadeesha (2006) studied on processing of kokum fruits and developed several natural health foods from Kokum *viz.*, kokum squash, bale blended kokum squash, grape blended kokum squash, kokum nectar, grape blended kokum nectar, lime blended kokum nectar, kokum jam, kokum pickle and kokum wine.

2.5 Fermentation

Fermentation is the "slow decomposition process of organic substances induced by microorganisms, or induced by complex nitrogenous substances (enzymes) of plant or animal origin" (Walker, 1998).

Fermentation is regarded as a desirable effect of microbial activity in foods. In general, the desirable effects of microbial activity may be caused by the biochemical activity of the microorganisms. Microbial enzymes breaking down carbohydrates, lipids, proteins, and other food components, can improve food digestion in the human gastrointestinal tract and thus increase nutrient uptake (Adams and Nout, 2001).

2.6 Lactic Acid Bacteria

The lactic acid bacteria (LAB) are a group of gram-positive bacteria, non-spore forming, cocci or rods, which produce lactic acid as the major end product of the fermentation of carbohydrates. They are the most important bacteria in desirable food fermentations, being responsible for the fermentation of most "pickled" (fermented) vegetables.

The Lactic acid bacteria are a dissimilar group of organisms with diverse metabolic capacity. This diversity makes them very adaptable to a range of conditions and is largely responsible for their success in acid food fermentations (Axelsson, 1998).

Lactic acid bacteria carry out their reactions by the conversion of carbohydrate to lactic acid plus carbon dioxide and other organic acids without the need for oxygen. *Lactobacillus acidophilus, L. bulgaricus, L. plantarum, L. caret, L. pentoaceticus, L. brevis* and *L. thermophilus* are examples of lactic acid-producing bacteria involved in food fermentations.

2.7 Probiotic culture of lactic acid bacteria

Probiotics are live microorganisms that are similar to beneficial microorganisms found in the human gut. They are also called "friendly bacteria" or "good bacteria." Probiotics are available to consumers mainly in the form of dietary supplements and foods. They can be used as complementary and alternative medicine (Prado *et al.*, 2008).

World Health Organization and the Food and Agriculture Organization of the United Nations defined that probiotics are "live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host".

Probiotics are not the same thing as prebiotics. Prebiotics are non digestible food ingredients that selectively stimulate the growth and/or activity of beneficial microorganisms already in people's colons. When probiotics and prebiotics are mixed together, they form a symbiotic.

Probiotics are available in foods and dietary supplements (for example, capsules, tablets and powders) and in some other forms as well. Examples of foods containing probiotics are yogurt, fermented and unfermented milk, miso, tempeh and some juices and soy beverages. In probiotic foods and supplements, the bacteria may have been present originally or added during preparation (Prado *et al.*, 2008).

Probiotic foods and beverages are manufactured by either method: (a) by adding the probiotic strains simultaneously with the standard cultures in the fermentation tank; (b) by adding the probiotic culture directly into non-fermented final products. Generally, species of Lactobacillus and Bifidobacterium are used in most of the probiotic applications. However, due to some drawbacks related to dairy products, there are emerging interests in using non-dairy ingredients as substrates for delivering probiotics to wider group of consumers (Prado *et al*., 2008).

2.7.1 *Lactobacillus acidophilus*

Lactobacillus acidophilus strains were characterized as lactic acid bacteria with strictly homo fermentative metabolism (> 85% lactic acid). The hexoses are preferentially fermented via Embden-Meyerhof-Parnas (EMP), (as the strains produce aldolase and phosphoketolase) and only then the pentoses and gluconate are fermented. *Lactobacillus acidophilus* group isolated from the human faces or intestine are thought to have beneficial effects on health being thus considered to be probiotic bacteria (Saito, 2004).

2.7.2 *Lactobacillus plantarum*

Lactobacillus plantarum is a beneficial bacterium that can be used for improved health. It is the largest genus of the lactic acid bacteria group over 50 *Lactobacillus* species. One of the most versatile probiotics found in plant material and the gastrointestinal tract of animals, including humans. It is used in the fermentation of foods like sauerkraut, kimchi, pickles and sourdough bread. It has been used for this purpose for hundreds of years. It has the ability to destroy pathogens and to preserve critical nutrients, vitamin and antioxidants (Taylor *et al*., 2007).

2.7.3 *Lactobacillus delbrueckii*

Lactobacillus delbrueckii have been traditionally used as starters for milk fermentation in yoghurt production. *L. delbrueckii* contributes to fast lactic acid development in yoghurt and also to flavour and textural properties (Curry and Crow, 2003).

2.8 Probiotic beverages from cereals and legumes

Gomes and Malcata (1999) reported that strains of *Lactobacillus* have been recognized as complex microorganisms that require fermentable carbohydrates, amino acids, B vitamins, nucleic acids and minerals to grow and therefore fermentation of cereals may represent a cheap way to obtain a rich substrate that sustains the growth of beneficial microorganisms. A multitude of non-dairy fermented cereal products has been created throughout history for human nutrition, recently reported on probiotic characteristics of microorganisms involved in traditional fermented cereal foods.

Blandino *et al*. (2003) reported that Mahewu (amahewu) is a sour beverage made from the maize porridge. The sorghum, millet malt, or wheat flour is mixed with water left to ferment under ambient temperature. The spontaneous fermentation process is carried out by the natural flora of the malt at the ambient temperature. The predominant microorganism found in African Mahewu is *Lactococcus lactis* subsp. *lactis*. It is consumed in Africa and some Arabian Gulf countries.

Muianja *et al*. (2003) reported that Bushera is a traditional beverage prepared in the Western highlands of Uganda, consumed by both the young children and the adults. Bushera is a fermented product of germinated millet sorghum flour. The germinated sorghum flour is mixed with boiling water and fermented by lactic acid bacteria for 6 days. The lactic acid bacteria isolated from Bushera comprised of five genera, Lactobacillus, Lactococcus, Leuconostoc, Enterococcus and Streptococcus. *Lactobacillus brevis* was more frequently isolated than other species.

2.9 Fermented fruit and vegetable food products using probiotic lactic acid bacteria

The wide varieties of fruits and vegetables and the large number of LAB strains provide new challenges and opportunities for the development and commercialization of value added non-dairy fermented probiotic beverages. The survival of probiotic strains depend on factors, such as, nutrients, pH, temperature and the presence of inhibitors. Several tropical fruit juices widely used as substrates for the fermentation by different strains of LAB.

Most of the probiotics are bacteria similar to those naturally found in human gut, especially in those of breastfed infants (who have natural protection against many diseases). Most often, the bacteria come from two groups, Lactobacillus or Bifidobacterium. Within each group, there are different species (for example, *Lactobacillus acidophilus* and *Bifidobacterium bifidus*) and within each species, different strains (or varieties). A few common probiotics were *Saccharomyces boulardii* (yeast) and Lactic acid bacteria like *Lactobacillus acidophilus* and *Lactobacillus plantarum*.

Lactic acid fermentation is an important bio-preservation process, which may enhance or maintain nutritive and sensory value of the product. Bacteria used most frequently by researchers to run lactic acid fermentation include strains of *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus delbruekii* and *Lactobacillus paracasei*. Experiments showed that the above mentioned probiotic strains are capable of surviving in a medium such as juice produced from pomegranate fruits (Mousavi *et al*., 2011).

Tuorila and Cardello (2002) revealed that fruit juices have been suggested as an ideal medium for the functional health ingredients because they inherently contain beneficial nutrients and taste profiles that are pleasing to all the age groups, and perceived as healthy and refreshing.

Fruit juices have several nutritional benefits and are regularly consumed with daily morning meals in several countries. It has been suggested that fruit juices could serve as suitable media for cultivating probiotic bacteria (Mattila-Sandholm *et al.*, 2002). Probiotic bacteria are added to fruit juices for added benefits. The methods for integrating the strains with the fruit juices vary according to the requirement of the manufacturers. This is due to the fact that the probiotic cultures can be added either during processing of the juice or at the time of packaging. Both these methods need different technologies in place at the manufacturing plant.

Kim *et al*. (1998) isolated lactic acid bacterial strains from kimchi, *viz. Lactobacillus acidophilus, Lactobacillus plantarum*, *Leuconostoc mesenteroides,* with or without *Saccharomyces cerevisiae* and were used as inoculants in fruit-vegetable juice fermentation.

Yoon *et al*. (2004) determined the suitability of the tomato juice as a raw material for the production of probiotic juice by *Lactobacillus acidophilus* LA 39, *Lactobacillus plantarum* C3, L *Lactobacillus casie* A4 and *Lactobacillus delbrueckii* D7. The tomato juice was inoculated with a 24 hr. old culture and incubated at 30°C. The lactic acid cultures reduced the pH to 4.1 and the viable cell counts reached nearly

(1.0-9.0) x 10^9 cfu/ml after 72 hr. of fermentation. The viable cell counts of the four lactic acid bacteria in the fermented tomato juice ranged from 10^6 to 10^8 cfu/ml after 4 weeks of cold storage at 4°C.

Lucknow and Delahunty (2004) reported that fruits and vegetables are rich in the functional food components such as minerals, vitamins, dietary fibers, antioxidants, and do not contain any dairy allergens that might prevent usage by certain segments of the population.

Heenan *et al.* (2004) reported that current industrial probiotic foods are basically dairy products, which may represent inconveniences due to their lactose and cholesterol content.

Yoon *et al.* (2005) isolated four lactic acid bacterial species (*Lactobacillus acidophilus, Lactobacillus caesi, Lactobacillus delbrueckii* and *Lactobacillus plantarum*) and evaluated for red beets as a potential substrate for the production of probiotic beet juice. All the lactic cultures were found capable of rapidly utilizing beet juice for cell synthesis and lactic acid production.

Yoon *et al*. (2006) developed a probiotic cabbage juice using lactic acid bacteria. Cabbage juice was inoculated with a 24 hr. old lactic culture and incubated at 30ºC. The cultures (*Lactobacillus plantarum* C3, *Lactobacillus casei* A4 and *Lactobacillus delbrueckii* D7) grew well on cabbage juice and reached about 1 x 10^9 cfu/ml after 48 hr. of fermentation. *Lactobacillus casei* produced a lower amount of titratable acidity, expressed as lactic acid, than *Lactobacillus delbrueckii*, or *Lactobacillus plantarum*. After 4 weeks of the cold storage at 4ºC, the viable cell counts of *Lactobacillus plantarum* and *Lactobacillus delbrueckii* were 4.1 x 10^7 and 4.5 x 10^5 cfu/ ml, respectively. *Lactobacillus casei* did not survive at low pH and lost cell viability completely after 2 weeks of the cold storage. The authors concluded that the fermented cabbage juice could serve as a healthy beverage for vegetarians and lactose-allergic consumers.

Zlatica *et al.* (2006) studied the suitability of various kinds of vegetables (cabbage, tomatoes, pumpkin and courgette) for the preparation of vegetable juices processed by lactic acid fermentation. Authors reported that all tested vegetable juices have proven to be suitable substrates for lactic acid fermentation.

US patent 4855147 – Beverages by lactic acid fermentation and method of production was reported by Calvo *et al.* (2007). The US patent described the production of lactic acid fermented tomato beverage from tomato juice with the sugar content of 5.4% and pH of 5.2 heated to 110 °C for sterilization and then cooled to 35 °C.

Moraru *et al.* (2007) studied on probiotic vegetable juices of celery and beetroot juices by inoculation of *Bifidobacterium* fermentation for 48 hours. Authors reported that the fermented beet root juice has a pleasant taste, where as the celery juice has a pronounced sour taste.

El-Nawawy *et al*. (2009) investigated on the fermentation of permeate beverage consisting of fresh permeate and 10% sucrose with single or mixed probiotic cultures (*Lactobacillus acidophilus* and *Lactobacillus rhamnosus)* then fresh fruit component (Lemon juice, guava and mango pulps). Antioxidant contents for all fermented permeate with different fruit juice (ranged from 28.1 to 45.8 mg/100g) were higher as compared with permeates (11.2mg/100g). Vitamin C and phenolic compounds were highest in fermented permeate with lemon juice (32 mg/100g) followed by guava (188mg/100g). At the end of the storage the viability of all the probiotic culture used was higher than 1×10^{6} cfu/ml.

Dushyantha *et al*. (2010) reported the fermented beverages from kokum fruit juice using native LAB isolates along with reference strain *Lactobacillus acidophilus*. Better result with biochemical and organoleptic properties were obtained by beverages prepared using reference strain *Lactobacillus acidophillus* and native lactic acid bacterial isolates (KL1).

Fermentation of pomegranate juice by lactic acid bacteria were conducted by Mousavi *et al.* (2011) by using four strains of lactic acid bacteria: *Lactobacillus plantarum, L. delbruekii, L. paracasei, L. acidophilus.* The results indicated that *L. plantrum* and *L. delbruekii* increased the pH sharply at the initial stages of fermentation and the sugar consumption was also higher in comparison with other strains, better microbial growth was also observed for these two strains during fermentation.

Optimization of the fermentation of cantaloupe juice by *Lactobacillus casei* NRRL B-442 was studied by Fonteles *et al.* (2011). The cell viability was 8.3 log cfu/ml at the end of the fermentation. The consistent growth and viability of the probiotic microorganism in cantaloupe juice during fermentation and storage suggested that melon is a suitable vehicle for *L. casei* delivering.

Pereira *et al.* (2011) optimised the conditions of *Lactobacillus casei* NRRL B-442 cultivation in cashew apple juice, fermentation time and inoculums amount. The initial pH, fermentation temperature, inoculums level and fermentation time for juice production were 6.4, 30°C, 7.48 log cfu/ml and 16 hr. respectively. Viable cell counts were higher than 8 log cfu /ml throughout the storage period (42 days). The values of lightness, yellowness and total colour change increased and the values of redness reduced along the fermentation and storage period.

Othman *et al.* (2012) evaluated the effect of supplementation of jackfruit puree on probiotic (*Lactobacillus acidophilus* FTDC 1295) in terms of cell count, viability and nutritional value of dadih. Four samples of dadih were prepared in this investigation; Control, jackfruit dadih, probiotic dadih and jackfruit probiotic dadih. Results revealed that dadih supplemented with jackfruit puree directly improved the probiotic cell counts which are significantly higher than the dadih without jackfruit puree. The chemical compositions (moisture, total solids, fat, protein, mineral, organic acid, and pH) showed variations in its pattern due to the differential in formulations and the incorporations of probiotic bacteria. In addition, the total phenolic content and the antioxidant capacity were reported to be the highest in dadih supplemented with jackfruit puree and probiotic.

Adou *et al.* (2012) studied the changes in physico-chemical properties of the juice of two cashew apples varieties (yellow and red). Study revealed the tremendous nutritional potential of fruit in terms of vitamin C, sugar, organic acids, dry matter and ash. Juices showed a significant variation in all measured compounds related to colour and maturity. The cashew apple juice can be a good alternative to daily supplementation of vitamin C for children and adults. The cashew apple is rich in nutritional composition but is ignored because of its astringency.

Mousavi *et al.* (2013) reported that fructose and glucose content of the pomegranate juice were significantly consumed by *Lactobacillus plantarum* and *Lactobacillus acidophilus* during fermentation. Authors reported that *Lactobacillus plantarum* utilized more sugars in comparison with *Lactobacillus acidophilus.*

Vasudha and Mishra (2013) reviewed on non dairy probiotic beverages and authors highlighted the research done on probiotic beverages from non dairy sources. Non dairy probiotic beverages can serve as a healthy alternative for dairy probiotics and also favour consumption by lactose intolerant consumers.

Costa *et al.* (2013) evaluated the use of sonicated pineapple juice as substrate for producing a probiotic beverage by *Lactobacillus casei* NRRL B442. Maximal microbial viability was found by cultivating *Lactobacillus casei* at 31°C and pH 5.8 (optimised condition). After fermentation samples of sweetened and non sweetened juices were stored. After 42 days of refrigeration (4°C) the microbial viability was 6.03 log cfu/ml in the non sweetened juice and 4.77 log cfu/ml in the sweetened sample. The pH of the both the juices decreased due to lactic acid production. The characteristic colour was maintained throughout the shelf life and no browning was observed.

Filannino *et al.* (2013) studied the fermentation of pomegranate juice using by two strains, which were previously isolated from tomatoes and carrots (*Lactobacillus plantarum* LP09) and another commercial strain *Lactobacillus plantarum* (LP09). Unstarted pomegranate juice was used as control. After fermentation all starters grew to 9.0 log cfu/ ml. The concentration of total polyphenolics and antioxidant activity were highest for started pomegranate juice. Compared to unstarted juice colour and browning indexes of fermented pomegranate juices were preferable.

Randazzo *et al.* (2013) compared the survival of six probiotic wild strains of *Lactobacillus rhamnosus* with that of a type strain during 78 days of storage at 25°C and 5°C in peach synthetic medium (PSM) and commercial peach jam (PJ). All strains exhibited better performances in PJ than in PSM, showing count values higher than 7 log cfu/g up to 78 days of storage at 5 °C. Almost all wild strains remained above the critical value of 6 log cfu/ g in samples stored at 25 °C up to 45 days, while the *Lactobacillus rhamnosus* GG type strain, used as control, was not able to survive later than 15 days.

Shukla *et al*. (2013) optimized the probiotic beverage using whey, pineapple juice and *Lactobacillus acidophilus* on the basis of sensory quality evaluation. Fermentation time using 1 per cent inoculum of *L. acidophilus* was optimized on the basis of sensory quality evaluation, growth and activity in terms of pH and acidity. The 65:35 blend ratio of whey and pineapple juice fermented for 5 hr. gave desirable results with highest sensory scores for overall acceptability and a total viable count of more than 10^6 cfu/ml.

Study conducted by Pakbin *et al*. (2014) revealed that *Lactobacillus delbrueckii* grew well in peach juice, reached nearly 10×10^9 cfu/ml, after 48 hours of fermentation at 30°C and was capable of more sugar consumption, pH inclination and production of lactic acid during fermentation. After four weeks of cold storage at 4°C, the viable cell counts of *L. delbrueckii* were 1.72×10^7 cfu/ml, in fermented peach juice.

Kumar *et al*. (2015) studied on probiotication of mango and sapota juices using *Lactobacillus plantarum* NCDC LP. Superior antioxidant activity was reported in both mango and sapota fruit juices. *Lactobacillus plantarum* decreased the pH (4.3 to 3.2) and increased titrable acidity (0.49 to 0.66%) within 72hr. Higher total phenolic content was observed in sapota juice (145 mg/ 100 ml) than mango juice (60.24 mg/100 ml) after fermentation.

Kalita *et al*. (2015) examined the three strains of lactic acid bacteria: *Lactobacillus plantarum*, *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* for the production of fermented probiotic litchi juice. Fermentation was carried out at 37 °C for 72 hr. under microaerophilic conditions. The results indicated that *L. plantarum* and *L. rhamnosus* lowered the pH at the initial stages of fermentation along with greater sugar consumption than *L. acidophilus* and showed better microbial growth during fermentation. *L. plantarum* and *L. Rhamnosus* showed higher viability during the storage time. Lactobacillus increased the antioxidant activity with *L. acidophilus* exhibiting highest antioxidant capacity.

The probiotification of mango juice was performed at 30ºC for 72 hr. under micro-aerophilic conditions using lactic acid bacteria (Reddy *et al*., 2015). *Lactobacillus acidophilus* (MTCC10307), *Lactobacillus delbrueckii* (MTCC911), *Lactobacillus plantarum* (MTCC9511) and *Lactobacillus casei* were used for fermentation. The lactic acid bacteria reduced the pH to as low as 3.2 from 4.5 after fermentation. The substrate concentration was reduced to 5.8% (w/v) from 12% (w/v). *Lactobacillus plantarum* exhibited the fastest utilization of sugar and reduction of pH in the mango juice when compared to the other strains used. The viability of the cells was maintained at 1.0×10^7 cfu/ml throughout the storage period.

Peerajan *et al*. (2016) investigated the fermentation of *Phyllanthus emblica* fruit by *Lactobacillus paracasei* HII01 with respect to carbon sources, polyphenols and antioxidant properties. The current study revealed that beverage produced by *L. paracasei* mediated fermentation of *P. emblica* fruit is a good dietary product with high polyphenolic content and antioxidant properties.

Martins *et al.* (2016) evaluated the viability of this *Lactobacillus rhamnosus* HN001 in fruit salads and the phsyico-chemical, microbiological and sensory properties of this food. The viability of *Lactobacillus rhamnosus* in fruit salads was 8.49 log cfu/g after 120 hours. SEM (Scanning electron microscope) images showed that fruit tissue provided protection for probiotic bacteria. Adhesion sites were observed in higher quantity in banana, apple and guava. Fruit salads containing *Lactobacillus rhamnosus* showed counts of psychotrophic microorganisms of at least 2 log cfu/g lower than control salad after 120 hr. of refrigerated storage. The fruit salad was well accepted by consumers.

Priya and Vasudevan (2016) determined the suitability of papaya juice for production of probiotic juice by lactic acid bacteria. The optimum conditions for production of papaya juice are at 48hr. of fermentation time and 3% of inoculum size. At 48 hr. maximum number of viable cells survived. *L. plantarum* (5.3 to 3.2) showed a more rapid drop in pH than *L. acidophilus*.

Tayo and Akpeji (2016) studied the physicochemical properties, probiotic viability and sensory evaluation of stored probioticated pineapple juice using lactic acid bacteria (*Pediococcus pentosaceus* LaG1, *Lactobacillus rhamnosus* GG, *Pediococcus* *pentosaceus* LBF2) as a single and mixed starter. Increase in the lactic acid production and reduction in pH and colour during storage were observed. The pineapple juice supported the viability, lactic acid production, vitamin C development and the antagonistic potential of the probiotic candidate.

Meera *et al.* (2016) conducted study on development probiotic papaya juice powder using freeze drying. 6% and 8% inoculums of *Lactobacillus acidophilus* were used for the fermentation of fruit juice. After fermentation for 48hr. at 37°C, drying was carried out using freeze-drying technology. Freeze drying conditions were fixed in terms of ºBrix, acidity, vacuum, freezing condenser temperature and temperature of drying to get desirable results with a total viable count of more than 10^7cfu/ml and with highest sensory quality. The product obtained was rich in natural antioxidants like β-carotene and ascorbic acid. Physio-chemical and microbiological studies revealed that bacteria and other functional components were stable during storage at various temperatures of 5ºC, 25 ºC and 37ºC for 60 days.

Ozcan *et al*. (2015) studied the fruit based (apple and bluberry) fermented dairy beverages prepared with *Lactobacillus acidophilus* and *Lactobacillus rhamnosus.* The type of fruit and probiotic bacteria used were significantly effective on microbiological and sensory properties of fermented beverage ($p<0.01$). The growth proportion index (GPI) of *Lactobacillus rhamnosus* was significantly higher than *Lactobacillus acidophilus* in all samples during storage. In this study, both *Lactobacillus* strains showed good probiotic viability (>7 log cfu / g) and remain at this satisfactory viability levels even after 28 days of storage. All the products obtained with higher sensory scores.

According to Reddy *et al*. (2015) four lactic acid bacteria such as *Lactobacillus* casei, *Lactobacillus delbrueckii*, *Lactobacillus plantarum* and *Lactobacillus acidophilus* were found capable of rapidly utilizing mango juice for cell synthesis and lactic acid production. They reduced the pH to as low as 3.2 and increased the acidity to as high as 1.72% and the viable cell counts reached to 1.0×10^9cfu/ml after fermentation of 72 hr. at 30ºC. All the four lactic acid bacteria survived under the low pH and high acidity conditions during 4 weeks of cold storage at 4ºC.

2.9.1 Blended fermented beverages from fruits and vegetables prepared using probiotic lactic acid bacteria

The effect of fermentation on polyphenolic content and antioxidant capacity of cabbage juice was studied by Jaiswal and Abu-Ghannam (2013). Lactic acid (LA) was the major end product of the fermented cabbage juice attaining the concentrations of 6.97, 9.69 and 12.2 g/l LA for *Lactobacillus plantarum, L. rhamnosus* and *L. brevis*, respectively. Fermentation retains more than 75% of total phenolic content (TPC) and total flavonoid content (TFC) of the initial raw material and similar set of results were observed for antioxidant capacity. During refrigerated storage (4°C), all the probiotic cultures met the criterion of maintaining counts greater than 8 log cfu /ml; in addition to maintaining bioactive components and antioxidant capacity.

Giang *et al*. (2013) reported that the juice from ripe cashew apple after removal of tannin and pasteurization at 90°C for 10 min. Sucrose (11% w/v) can be added as growth substance of *Lactobacillus acidophilus*. The optimum conditions of *Lactobacillus acidophilus* growth in cashew apple juice are: the initial pH 4.0-4.5, the temperature 37 °C. Cell biomass of *L. acidophilus* after being fermented at 37 °C for 48 hr. was greater than10^9 cfu/ml.

The production of a probiotic mixture of black cherry and barberry juice by lactic acid bacteria (LAB) has been reported by Shahram *et al.* (2014). Authors revealed that a mixture with 0.2% whey powder could be considered as a suitable matrix for the growth of probiotic bacteria and functional beverage production.

Sivudu *et al.* (2014) conducted a comparative study on the suitability of watermelon and tomato juice as a raw material for production of probiotic mixed juice by using *Lactobacillus fermentum* and *Lactobacillus casei.* After four weeks of cold storage at 4 ºC, *L. fermentum* grown at lower temperature (30°C) and *L. casei* grown at higher temperature (37°C) survived better. The addition of sucrose at the beginning of fermentation increased the amount of titrable acidity by at least two times (>1.8% lactic acid).

Fermentation of milk permeate with 2% of mixed starter culture (1:1:1) containing *Lactobacillus delbruekii subsp bulgaricus, Streotococcus thermophillus* and *bifidobacterium longum* with the addition of sucrose was reported by Atallah (2015b). Equal volume of carrot or mango pulp is added, stored for 30 days at 4°C. Results revealed that total solids, ash, fat, protein, fibre contents and acidity were slightly increased, while the total carbohydrate, antioxidant activity, total phenols, vitamin C and pH value were decreased during cold storage of prepared beverages.

Development of probiotics beverages of sweet lime and sugarcane juices and its physiochemical, microbiological and shelf- life studies was conducted by Khatoon and Gupta (2015). Suitability of culture growth was also checked in various combinations which were prepared with herbs of Ashwagandha (*Withania somnifera*) and Green tea extract (*Camellia sinensis*), wheat grass juice, whey and oats. *Lactobacillus acidophilus* grew well in all these combinations and reached nearly up to 10^8 cfu/ml after 24 hr. of fermentation at 37 ºC. After 3 weeks of storage at 4ºC, the viable cell counts of *Lactobacillus acidophilus* in the juices of sugarcane control, sugarcane juice with wheat grass juice and *Withnia somnifera,* sugarcane juice with green tea and whey were 4.0×10^8, 2.0×10^8 and 5.5×10^8 cfu/ml, respectively.

Atallah (2015a) studied on fermentation of milk permeate with 2% of mixed starter culture (1:1:1) containing *Lactobacillus delbruekii subsp bulgaricus, Streotococcus thermophillus* and *bifidobacterium longum* with the addition of sucrose. Equal volume of papaya or guava pulp is added, stored for 30 days at 4°C. Unheated fermented permeate retained better bacterial counts (higher than 6 log cfu/ g) than that of heated permeate. Results showed that total solids, ash, fat, protein, fibre contents and acidity were slightly increased, while the total carbohydrate, antioxidant activity, total phenols, vitamin C and pH value were decreased during cold storage of prepared beverages.

According to Hashemiravan *et al.* (2015) the optimum temperature and time for fermentation of mixture of malt extract and red fruit juices (apple, red grape, pomegranate, craneberry, blackberry and blackcurrent) by the bacteria "*Lactobacillus casei* 1608" was 37 ˚C for 48 hours. The results revealed that 5% red fruit juices, 4% malt extract and 1.5 $x10^7$ cfu/ml of *Lactobacillus casei* was considered as the best treatment. This sample had the maximum rates of cell viability during 4 weeks of cold storage at 4˚C.

Askin and Atik (2016) conducted study on effect of storage temperature and time on colour and antioxidant properties of fermented grape beverage. After having been exposed to lactic acid fermentation, it was bottled and then stored at 4°C and 20°C for 60 days. The results of colour parameters obtained showed the highest proportion of red color in the samples at the beginning (dA % = 94.87%). As expected, the brown color increased with storage time and the highest value was determined at 60 days depending on the storage temperature. A high content of total phenolic (1743 ± 8.67 mg GAE/l) and antioxidant activity value (8.53 mM Trolox m/l) was present in the fresh beverage.

Production of low-alcohol fruit beverages through fermentation of pomegranate and orange juices with kefir grains was studied by Kazakos *et al*. (2016). Addition of orange juice improved the ability of kefir grains to ferment pomegranate juice and increased the survival rates of lactic acid bacteria (LAB) contained in kefir grains during storage. Lactic acid formation was observed in all products, especially in the mixed substrate (1.3-1.9 g/l). The results show the possibility to produce low-alcoholic nutritious fruit beverages with potential antioxidant (due to pomegranate constituents) and probiotic properties (due to the probiotic species present in kefir grains). In addition sensorial tests that were conducted showed the consumers acceptance for all the fermented juices.

Mauro *et al.* (2016) evaluated the blueberry and carrot juice blend as a fermentable substrate for *Lactobacillus reuteri* LR92, in order to develop a fermented non-dairy functional beverage. After 48 hr. of fermentation, the *L. reuteri* population reached to 10.26 ± 0.23 log cfu/ml and after 28 days of storage at 4ºC the bacterial population maintained elevated numbers of viable cell (8.96 ± 0.08 log cfu/ml) with increased in the antioxidant capacity of the fermented blend during storage period of 28 days at 4ºC.

2.10 Prebiotics

Goderska *et al.* (2007) studied the effect of prebiotics (oat gruel and homogenised banana fruit) on fermentation of carrot juice with *Lactobacillus acidophilus* DSM 20079 and *Bifidobacterium bifidum* DSM 20215 bacteria. The best survivability of the *Lactobacillus acidophilus* DSM 20079 bacteria up to the 28th day of refrigerated storage was obtained in the carrot juice with the addition of oat gruel. The highest sensory rating was obtained for the carrot juice with *Lactobacillus acidophilus* DSM 20079 and 10% of homogenised banana.

Kaur *et al.* (2016) studied tomato juice as substrate for lactic acid bacteria (*Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Lactobacillus casei*) through utilization of sugar (inulin as a prebiotic). After fermentation, decreased pH and sugar but increased acidity was observed. Increased viable count of lactic acid bacteria was observed when inulin was added as prebiotic and it was noticed that on the addition of prebiotic the generation time of probiotic decreased.

2.10.1 Honey

Honey is a sweet liquid produced by honey bee from nectar of flowering plants that require insect pollination by honey bee (*Apis mellifera*). In the general the direct consumption of honey is very less. The consumption of these natural antioxidants could be increased when they are used in the form of beverages. Incorporating honey in beverages can improve the taste, aroma, palatability, and nutritive value (Singh *et al.*, 2014).

Honey is composed primarily of fructose and glucose but also contains fructo-oligosaccharides (Chow, 2002) and many amino acids, vitamins, minerals and enzymes (White, 1979).

Bansal *et al.* (2005) reported that honey contains proteins (proline the maximum), vitamins, minerals, good quantities of antioxidants, varying concentrations of catalase, flavonoids, ascorbic acid and alkaloids are present.

According to El-Arab *et al.* (2006) the principal carbohydrate constituents of honey are fructose (32.56 to 38.2%) and glucose (28.54 to 31.3 %), which represents 85–95% of total sugars that are readily absorbed in the gastrointestinal tract.

Sanz *et al*. (2005) reported that honey could enhance the growth of two probiotic bacteria viz. Lactobacillus and Bifidobacteria that is essential for better intestinal health.

The study was undertaken to determine the suitability of tomato juice as a raw material for production of fermented juice by lactic acid bacteria (*Lactobacillus plantarum* MTCC 6161) and yeast (*Saccharomyces cereviceae* UCD 522) by Priya *et al.* (2012). Tomato juice blended with 5 per cent honey was inoculated with a 24 hr. old culture and incubated at 26-28ºC. The results indicated that the blended tomato juice fermented by lactic acid bacterial culture reduced the pH to 3.92 or below and enhanced the acidity (0.44 %), vitamin C (5.54 mg/100ml), lycopene (0.31mg/100ml) and alcohol (3.53%) content.

Rotar *et al.* (2015) evaluated how goji berries (Wolfberries, Solanaceae family) and honey affect the sensorial quality of yoghurt, the chemical properties, the viability of lactic acid bacteria (LAB) and the concurrent micro flora development. Two types of yoghurts (yoghurt with goji berries and yoghurt with honey and goji berries) were developed. The addition of honey affected the entire yoghurt microflora including LAB, manifesting bactericidal effect. The addition of goji berries and honey maintained the viability of LAB at probiotic levels (10^6-10^7 log cfu/ml) during 21 days of storage.

The effect of honey (5-10g/100ml) and freeze-dried vegetables pulp (1-3g/100ml) on probiotic stability during fermented vegetable juice (beetroot, carrot and celery) storage in refrigeration conditions was studied by Profir *et al.* (2015). During storage, samples were taken at 7, 14 and 21 days and probiotics viability was determined. A good cell survival was obtained by all samples at the end of the storage period, higher than 1×10^7 cfu/ml. Sensory analysis was performed on the 7th day after inoculation and the samples were accepted by all panelists. The addition of honey in vegetables juice before fermentation was concluded to be a possible way for the development of a probiotic fermented beverage.

Adhikari *et al*. (2011) determined the effect of prebiotic (fructo oligosaccharide) or a symbiotic ingradient (fructo oligosaccharide + *Lactobacillus acidophilus)* on the sensory properties and consumer acceptability of peach flavoured drinkable yoghurts. Both the descriptive and consumer data indicated that the differences among the samples were either because of the fat content or the presence of symbiotics and prebiotics. Honey also increased and supported the growth of bifidobacterium, which is mainly due to the presence of a variety of oligosaccharides (Chow, 2002; Bansal *et al*., 2005; El- Arab, 2006).

2.10.2 Lactose

Lactose is the natural carbohydrate source and prebiotic compound found in the milk of mammals (Harju, 2001). Lactose is a valuable asset as a basic nutrient and the main substrate in fermentative processes that led to the production of fermented milk products (Adam *et al*, 2010).

According to the U.S. Code of Federal Regulations (2009), lactose is the carbohydrate normally obtained from whey. The 99% lactose is having protein content of 0.1%, Ash 0.1%-0.3% and moisture 4.5%-5.5%.

Marhamatizadeh *et al.* (2012) studied on development of probiotic drinks with apple and orange concentrates was assessed (brix 11 and 15) by using milk glucose, maltose and lactose as growth supplements. Glucose and lactose had positive effects on increasing shelf life period. It is conceived that more acidity in brix 15 than 11 was due to the influence of orange concentrate on acidity in comparison with apple concentrate with no effects.

Barbosa *et al.* (2016) investigated the effect of different sugars (Fructose, lactose and glucose) on growth of lactic acid bacteria in dried orange juice (powder) during drying and subsequent storage under different conditions of temperature, light exposure and water activity. High survival observed for cells grown in the presence of lactose, followed by glucose and fructose. The survival of dried bacteria was enhanced at 4°C, water activity of 0.03 and absence of daylight. Authors concluded that, it is possible to improve the survival rate of lactic acid bacteria and produce an orange juice powder with probiotic characteristics with shelf life of at least 12 months.

2.11 Chemical composition of lactic acid bacteria fermented products from fruits and vegetables

2.11.1 pH

Yoon *et al.* (2004) revealed that the tomato juice fermentation by lactic acid bacteria results in reduction of pH from 6.5 to 4.1.

Yoon *et al.* (2005) evaluated red beets as a potential substrate for the production of probiotic beet juice *Lactobacillus acidophilus* and *Lactobacillus plantarum* that produced greater amount of lactic acid than other cultures and reduced the pH of fermented beet juice from an initial value of 6.3 to below 4.5 after 48 hr. of fermentation at 30ºC.

Zlatica *et al.* (2006) reported that vegetable juices fermented by lactic acid fermentation showed sufficiently low pH values (3.6) after 72 or 48 hr. of fermentation, also had a pleasantly sour taste.

Buruleanu *et al.* (2009) reported that pH value of the carrot juices was decreased from an initial value by 6.45 to below 4.3 after 48 hours of lactic acid fermentation with *Bifidobacterium* sp. and to 3.84 after 24 hours when inoculated with *Lactobacillus acidophilus*.

Dushyanth *et al.* (2010) reported that the pH of fermented kokum juice varies from 3.06 to 3.26 at the end of fermentation by inoculating the *Lactobacillus acidophilus*.

Nosrati *et al.* (2014) reported that the pH of the vegetable juice mixture (tomato and carrot) reached from an initial value of 4.14 to the minimum value 3.78 after 28 days of cold storage at 4°C.

2.11.2 Total Soluble Solids (TSS)

Dushyantha *et al.* (2010) studied the kokum juice fermented by lactic acid bacteria and reported that TSS of fermented kokum juice varies from 7.16 to 8.50 ºbrix at the end of fermentation by inoculating the *Lactobacillus acidophilus*.

Priya and Munishamanna (2013) reported that the TSS content of fermented tomato juice by yeast and lactic acid bacteria contains 7 ºbrix with good sensory characters for consumption.

Fermentation of papaya juice by *Lactobacillus acidophilus* and *Lactobacillus plantarum* resulted in the reduction of initial pH of 5.3 to as low as 3.2 after 72 hr. fermentation (Priya and Vasudevan, 2016).

2.11.3 Titrable acidity

Yoon *et al.* (2004) studied probiotication of tomato juice by lactic acid bacteria and reported that probiotic fermentation of tomato juice using *Lactobacillus casei* and *Lactobacillus plantarum* decreased the pH and increased the acidity to 0.65 per cent.

Yoon *et al.* (2005) reported that fermentation of cabbage juice by lactic acid bacteria increased the acidity in the range of 0.74 to 0.97 per cent in the form of lactic acid.

Yoon *et al.* (2006) reported that probiotic cabbage juice containing *Lactobacillus plantarum* and *Lactobacillus delbrueckii* produced nearly 1% titrable acidity expressed as lactic acid after 72 hr. of fermentation. Under same growth conditions *Lactobacillus casei* produced 0.74% lactic acid.

Moraru *et al.* (2007) reported that the level of acidity in the *Bifidobacterium* sp. fermented juices of celery and beet juice varies with 4.2 per cent in celery juice and 2.7 per cent beet juice. This variation in acidity of two different juices were due to different types of acids present in the two juice types.

Buruleanu *et al*. (2009) reported that the fermented carrot juices containing 0.7 per cent acids as lactic acid.

Dushyantha *et al.* (2010) studied the titrable acidity of fermented kokum beverage varies from 1.62 to 1.76 per cent at the end of fermentation by inoculating the *Lactobacillus acidophilus*.

Lactic acid level reached its highest level of 6.1 g/l and 4.9 g/l by *Lactobacillus acidophilus* and *Lactobacillus plantarum,* respectively, after the fermentation of pomegranate juice. In addition, the amount of lactic acid produced by *Lactobacillu acidophilus* was significantly higher than that synthesized by *Lactobacillus plantarum* (Mousavi *et al.*, 2013).

Sivudu *et al.* (2014) studied the fermentation of watermelon and tomato mixed juice by *Lactobacillus fermentum* with 14% and 7% sucrose resulted in the increased acidity from 0.26±0.03% to 1.41±0.00% and 1.20±0.05% respectively.

Fermentation of sugar cane and sweet lime juices by *Lactobacillus acidophilus* resulted in production of 0.77% and 0.91% of lactic acid respectively (Khatoon *et al.*, 2015).

Tayo and Akpeji (2016) reported that after one week of cold storage, the lactic acid production ranged from 53.14–91.88 mg/ml in probioticated pineapple juice.

2.11.4 Ascorbic acid (Vitamin C)

Rakin *et al.* (2004) found the ascorbic acid content of 91.6 mg/100ml in fermented mixture of carrot and beet root juice, 103 mg/100ml in fermented beet root juice and 82.5±10 mg/100ml in fermented carrot juice.

El-Nawawy *et al.* (2009) reported that after fermentation by *Lactobacillus rhamnosus* in combination with the *Bifidobacterium longum* culture, the ascorbic acid content of permeate with guava blend increased from 25mg/100g to 188mg/100g.

Adou *et al.* (2012) reported that the ascorbic acid content in the fermented juice of cashew apple with different maturity stages with yellow and red colour types were varied from 370 to 480 mg/100g.

Atallah, (2015b) reported that during storage period of 30 days at 4°C ascorbic acid content of fermented unheated milk permeate with mango and carrot pulp decreased from 14.10 and 4.05 mg/100g to 13.21 and 3.20 mg/100g in mango and carrot blended beverage respectively. But ascorbic acid content was not detected in heated or unheated milk permeate without mango and carrot blend.

2.11.5 Total Sugars

Yoon *et al*. (2005) reported that fermentation of cabbage juice by *Lactobacillus casei*, *Lactobacillus plantarum* and *Lactobacillus delbrueckii* reduced the total sugar after 72 hr. fermentation from 45.6 to 36.5, 35.08 to 19.33 and 35.08 to 28.45 mg/ml respectively.

Latha (2012) reported that the initial value of total sugar in the kokum juice was 15.45%. After fermentation by different Lactic acid bacteria strains, the changes in total sugar ranged from 6.4 to 15.0 %.

Study conducted by Kavya (2013) revealed that, the initial total sugar content of the tomato juices of different varieties varied between 14.27 to 15.7%. After 6 days of fermentation, the changes in total sugar ranged from 3.68 to 7.08% between the tomato juices of different varieties influenced by both yeast and LAB fermentation.

2.11.6 Reducing sugar

Highly significant differences were observed between kokum lactic acid bacterial isolates with respect to reducing sugar. The highest reducing sugars were recorded in KL3 (2.38 %) followed by KL2 (2.32 %), KL1 (2.25 %) and RL (1.90 %) (Dushyantha *et al.*, 2010).

Influence of lactic acid bacteria (LAB) on reducing sugar (%) of fermented kokum juice was evaluated by Latha (2012). After fermentation, reducing sugar (%) in samples with *Streptococcus thermophillus*, *Lactobacillus brevis*, *Lactobacillus plantarum* and LAB isolate from kokum were 3.30%, 6.50%, 6.50%, and 4.90% respectively.

Reducing sugar content for different varieties of tomato juice after fermentation by *Lactobacillus acidophilus* were 2.48% for Local variety, 2.28% for Premium, 1.83% for Rupali, 1.25% for Roma and 1.05% for Regular variety as reported by Kavya (2013).

2.11.7 Antioxidant activity

El-Nawawy *et al.* (2009) reported that the antioxidant activity of fermented permeate with natural fruit juices (Guava, mango and lemon juice) was higher when compared to fermented permeate without fruit juices.

Statistical analysis revealed that the fermented pomegranate juice by *Lactobacillus plantarum* had a significantly higher antioxidant activity compared to fermented pomegranate juice by *Lactobacillus acidophilus* (Mousavi *et al*., 2013).

Filannino *et al.,* (2013) reported that larger decrease in the free radical scavenging capacity of started (with *Lactobacillus plantarum*) and unstarted organic pomegranate juices were detected with lowest antioxidant activity being in unstarted pomegranate juice during the storage period of 30 days at 4°C.

Atallah (2015b) reported that antioxidant activity of unheated milk permeate with mango pulp after fermentation by probiotic bacteria was found to be 34.23 mg/100g and along the storage period of 30 at 4°C antioxidant activity decreased to 33.00mg/100g.

The DPPH free radical scavenging capacity of the unfermented fresh sweet lime and sugarcane juices was 18% and 21% respectively. After the fermentation with *Lactobacillus acidophilus,* DPPH radical scavenging activity of fermented sweet lime and fermented sugarcane was 12 % and 16 % respectively, on the 7^{th} day of storage as reported by Khatoon and Gupta (2015).

Askin and Atik (2016) reported that the antioxidant activity of fermented grape juice was 8.53 µMol of trolox/ml at the beginning of the storage. At the end of the storage (4°C), fermented grape juice showed 7.45 µMol of trolox/ml of antioxidant activity and at 20°C showed 7.22 µMol of trolox/ml of antioxidant activity.

Mauro *et al*. (2016) studied that the fermentation of blueberry and carrot blend resulted in decreased antioxidant activity during storage up to 28 days from 9.47 to 8.20 µMol of trolox/ ml.

Phyllanthus emblica juice fermented by *Lactobacillus paracasei* containing cane sugar and honey showed highest antioxidant capacity (26.55±0.58 and 26±1.3 mg of ascorbic acid equivalent antioxidant capacity respectively). High antioxidant properties were observed on the 15th day of the storage of fermented juice (Peerajan *et al*., 2016).

2.11.8 Total phenol content

Khatoon and Gupta (2015) reported that total polyphenol content of fermented sweet lime juice fortified with 10 % liquid whey, 0.2% green tea extract and 5% oats was 5.78 GAE (µg)/1 mg and fermented sugarcane juice fortified with 10% liquid whey and 0.2% green tea extract was 6.73 GAE (µg)/1 mg.

Total phenolic content (TPC) of unheated milk permeate with mango pulp after fermentation by probiotic bacteria was found to be 35.23 mg/100g. During storage period of 30 days at 4°C TPC decreased to 33.42 mg/100g. Total phenolic content was not detected in heated or unheated milk permeate without mango blend (Atallah, 2015b).

Peerajan *et al*. (2016) reported that increased amount of polyphenol content (10.32 ± 0.28 mg gallic acid equivalent/ml and 10.385 ± 0.23 mg pyrogallol equivalent/ml) was recorded at the 15th day after fermentation of crushed *Phyllanthus emblica* fruit with cane sugar and 10% (w/w) of *Lactobacillus paracasei* as starter culture.

The polyphenolic content of the fermented *Phyllanthus emblica* juice was kinetically analyzed using both gallic acid and pyrogallol as standards by Peerajan *et al*. (2016). Total polyphenol level was gradually increased until 15th day after fermentation process with 10.32 ± 0.28 mg gallic acid equivalent/ml and 10.385 ± 0.23 mg pyrogallol equivalent/ml on 15th day.

Mauro *et al*. (2016) evaluated that the fermentation of blueberry and carrot blend resulted in increased total phenolic content during storage up to 28 days from 112.27 to 120.98 mg GAE/100ml.

The total phenolic content of fermented grape juice was determined as 1743 mg GAE/l at the beginning of storage. At the end of the storage period, total phenolic content of 1370 mg GAE/l (4°C) and 1324 mg GAE/l (20°C) was deteacted (Askin and Atik *et al*., 2016).

2.11.9 Protein content

El-Nawawy *et al*. (2009) reported that protein content of the fermented permeate with natural fruit juices (Guava, mango and lemon juice) ranged from 5.6 to 7.3%, only slight decrease was observed after 60 days of storage.

Mauro *et al*. (2016) reported that the fermented beverage of blueberry and carrot blend by *Lactobacillus reuteri* composed of 0.17% of protein.

Protein content of fermented milk permeate with guava blend and papaya blend with and without heat treatment ranged from 0.32 to 0.43 g/100g and increased along the storage period. Heat treated fermented milk permeate with guava blend showed highest protein content of 0.43 g/100g than any other treatments which was reported by Atallah (2015a).

Atallah (2015b) reported that highest protein content was observed in heated fermented beverage of milk permeate with carrot pulp and protein content increased during the storage period which was ranged from 0.54 to 0.56 g/100g. Protein content in carrot blended beverage was observed to be higher than the heated or unheated mango blended milk permeate beverage and fermented milk permeate.

Rotar *et al*. (2015) reported that 3.66 to 4.23 % protein content was detected in the yoghurt with different concentration of goji berries and honey. Highest protein content was observed in the yoghurt with honey and 7% goji berries (4.23%) during 14th day of storage.

Khatoon and Gupta (2015) reported that protein content of fermented sweet lime juice fortified with 10 % liquid whey, 0.2% green tea extract and 5% oats was 2.74g/100g and fermented sugarcane juice fortified with 10% liquid whey and 0.2% green tea extract was 0.94g/100g.

2.11.10 Microbial population

Yoon *et al.* (2006) conducted study on probiotic cabbage juice inoculated with 24hr. old lactic culture and incubated at 30°C. Changes in the pH, acidity, sugar content and viable cell counts during fermentation were monitored. *Lactobacillus casei, Lactobacillus plantarum and Lactobacillus delbruekii* grew well in the juice and reached nearly 10×10^{8} cfu/ ml after fermentation. After storage at 4°C viable cell counts of *Lactobacillus plantarum* and *Lactobacillus delbrueckii* were 4.1×10^{7} and $4.7\ \times10^{5}$ cfu/ ml respectively.

El-Nawawy *et al.* (2009) reported that the viability of Bifidobacterial count was found to be higher in the fermented permeate with lemon blend than in guava and mango blend. Also viability was higher than 1×10^{6} cfu/ml along the storage period.

According to Nosrati *et al.* (2014) the results of bacterial growth in the vegetable juice with 35% *Lactobacillus casei* + 65% *Lactobacillus plantarum* with 2% brix, had the highest growth during fermentation and the maximum viable cell counts during 4 weeks cold storage at 4°C. During 14th day of cold storage at 4°C, bacteria population increased in all of the treatments, and it was decreased during the third and fourth weeks of cold storage at 4°C in all treatments.

Pakbin *et al.* (2014) reported that *Lactobacillus plantarum* and *Lactobacillus delbrueckii* were capable of surviving the conditions of low pH and high acidity in fermented peach juice during cold storage (four weeks) at 4°C. But *Lactobacillus casei* lost cell viability completely after only one week of cold storage.

Atallah (2015b) reported that addition of mango and carrot pulp to fermented milk permeate increased the counts of *bifidobacterium longum* (higher than 10^{6} cfu/ml) as compared to the fermented milk permeate alone. Coliforms, yeast and moulds were not detected when fresh or during storage in all the beverage samples.

According to Atallah (2015a) lactic acid bacterial (LAB) counts were detected in unheated fermented permeate with fruit juice blend (Guava and papaya). LAB counts varied from 6.51 to 6.15 log cfu/ml in guava and 6.59 to 6.14 log cfu/ml in papaya during initial and 30 days of storage respectively. No LAB counts were detected in heated fermented permeate beverages of guava and papaya.

According to Khatoon and Gupta (2015) *Lactobacillus acidophilus* grew well and capable of utilizing pasteurized sweet lime and sugar cane juice for their growth without any nutritional supplementation. 18×10^8 cfu/ml of viable cells were observed in sweet lime juice and were not detected from second week of cold storage (4°C). Viable cell counts of 4.0×10^8 cfu/ml was detected in sugar cane juice even after third week of storage.

Kalita *et al*. (2015) reported that all the three species of lactic acid bacteria *viz. Lactobacillus plantarum, Lactobacillus rhamnosus* and *Lactobacillus acidophilus* were found to be capable of growing well on sterilized litchi juice without nutrient supplementation. *Lactobacillus plantarum* grew rapidly on litchi juice and reached nearly 8.2 $x10^8$ cfu/ml after 72 hr. of fermentation at 37°C. The viability of the counts of all the three strains was higher than 10^6 cfu/ml even after 4 weeks of cold storage (4°C). Even though the viable count *Lactobacillus plantarum, Lactobacillus rhamnosus* and *Lactobacillus acidophilus* decreased slightly during cold storage, cell viability remained between 4.2 x 10^7 and 3.5 x 10^7 after 4 weeks of cold storage at 4°C respectively.

After fermentation of fig juice, *Lactobacillus delbrueckii* reached nearly 9 log cfu/ml after 48 h of fermentation at 30 °C. During four weeks of cold storage at 4°C, the viable cell counts of *Lactobacillus delbrueckii* and *Lactobacillus plantarum* were still 6 and 5 log cfu/ml, respectively. *Lactobacillus casei* were survived until second week of cold storage time, reduced from 9 to 3 log cfu/ml. The results of the sensory evaluation showed that fermented fig juice samples were significantly different ($P<0.05$) from the control sample in taste, odor, consistency and overall acceptability (Khezri *et al*., 2016).

2.11.11 Organoleptic characterization of fermented beverage from fruits and vegetables

A fermented beverage known as Koji was prepared by natural as well as controlled fermentation (*Lactobacillus plantarum*). The bottled beverages prepared by natural fermentations were rated as the best during storage for six months at room temperature (Berry *et al*., 1989).

El-Nawawy *et al.* (2009) reported that organoleptic evaluation of fermented permeate with natural fruit juices (Guava, mango and lemon juice) scored highest ratings for the fermented permeate with lemon followed by guava when fresh and along the storage period.

In the fermented kokum juice, the highest score was obtained by the treatment reference strain (*Lactobacillus acidophilus*) (14.75 out of 20.00) followed by KL1 and KL2 (14.0 and 13.50 out of 20.00 respectively). The lowest score was recorded in treatment KL3 (13.00 out of 20.00) (Dushyantha *et al*., 2010).

Priya *et al.* (2012) studied the microbial fermentation of tomato juice blending with honey for nutritional improvement and reported that the developed fermented beverage from tomato juice with good sensory attributes.

Atallah (2015a) reported that score for over all acceptability of fermented permeate with guava blend was highest during 30 days of storage period (86.5 to 88 out of 100) than the papaya blend (84.5 to 86 out of 100) and fermented permeate without fruit blend (64.8 to 68).

Atallah (2015b) reported that fresh beverage of fermented mango with milk permeate beverage scored 92 (score out of 100) where as fermented carrot with milk permeate beverage gained 88 point (score out of 100).

2.12.12 Colour

Filannino *et al.* (2013) reported that the colour intensity of organic pomegranate juice fermented by different strains of *Lactobacillus plantarum* and unstarted pomegranate juice was found to be increased during the storage.

Costa *et al.*, (2013) reported that characteristic colour was maintained during initial storage period for fermented and unfermented pineapple juice. After 7 days of storage total colour difference (ΔE^*) increased from 0.54 to 5.95 and 2.37 to 6.31 for fermented and unfermented pineapple juice respectively. Fermented pineapple juice showed more saturated yellow colour compared to unfermented juice.

According to Khatoon and Gupta (2015) colour of the fermented sugarcane juice was slightly darkened, but still attractive to the eye. The juice with 5% v/v whey had a slight milky taste and its transparency was lesser.

Kalita *et al.* (2015) reported that the colour difference between the control and fermented litchi juice by probiotic lactic acid bacteria increased up to72 hr. of fermentation and initial period of fermentation and stabilized there up to end the storage period (4 weeks).

The study conducted by Askin and Atik (2016) showed that the red colour components, especially ACNs (malvidin-3-O-glucoside) of fermented grape juice, were significantly lower at the end of the storage (after 60 days) and there was a higher content of browning components. Mean colour intensity of fermented juice at the initial period and after 60 days of storage at 4°C was recorded as 4.18 and 1.32 respectively. Mean colour intensity of fermented juice at the initial period and after 60 days of storage at 20°C was recorded as 4.18 and 1.35 respectively.

Colour of the *Phyllanthus emblica* fermentation mixture with cane sugar was dark brown. *Phyllanthus emblica* fermentation mixture with honey was pale yellow in colour and turned dark yellow in color after 15 days (Peerajan *et al.*, 2016).

3. MATERIAL AND METHODS

The experiments related to fermentation of pomegranate juice were conducted in the Department of Post Harvest Technology, College of Horticulture, Bagalkot, Karnataka during the year 2016-17. The details of the material used and the methods followed during the investigation were presented in this chapter.

3.1 Geographical location and climate

Bagalkot is situated in the Northern Dry Zone (Zone-3) of Karnataka. The centre is located at 75° 42' East longitude and 16° 10' North latitude with an altitude of 542.00 m above Mean Sea Level (MSL). The district is grouped under arid and semi-arid region with mean annual rainfall of 517.3 mm and mean temperature of 32.6°C.

3.2 Collection of pomegranate fruits and kokum dried rind for preparation of juice for experiments

Pomegranate fruits of variety 'Bhagwa' were collected from a pomegranate orchard, Kaladagi, Bagalkot District. Kokum dried rind samples were collected from Sirsi taluk, Uttara kannada district for the experimentation. The kokum rind was processed into kokum juice and the same was used for the experiment.

3.3 Method of extraction of juices

3.3.1 Pomegranate juice extraction

Fresh and disease free pomegranate fruits were washed thoroughly in the running water and cut into halves with the help of clean stainless steel knife. Half cut fruits were placed in the hand operated juice extractor and pressed out to obtain juice. Extracted juice is allowed to settle in refrigerator for an hour. Subsequently juice was filtered through muslin cloth to get a clear juice extract. Per cent yield of juice and waste index were calculated after extraction of juice.

3.3.2 Kokum juice extraction (Wasker, 2000)

Healthy kokum rind samples were washed in water. The washed rind (300 g) was placed in water (6 liters) at rate of 6 per cent rind and heat processed to 50-55°C for 5-10 minutes and allowed for overnight for juice extraction from rind. The extracted rind is removed by filtration.

3.4 Lactic acid bacterial strains used for the fermentation studies

Authenticated lactic acid bacterial cultures were procured from NCIM (National collection of industrially important microorganism, Pune) in the form of lyophilized cultures. These strains were used in the fermentation studies. The details of strains obtained from NCIM used in the study are given in Appendix IV.

3.4.1 Preparation of LAB starter culture

Purified and authenticated different lactic acid bacterial strains were streaked on the solidified MRS (DeMann, Rogosa and Sharpe) agar in petri plates and incubated at 37°C for two days. Isolated colonies were taken from plates and cultured in slants containing solidified MRS agar media and incubated at 37°C for 2-3 days. Loop full inoculums of lactic acid bacterial strains were transferred to conical flasks containing 100 ml of MRS broth. The inoculated flasks were incubated for 2 to 3 days at 37°C. The broth cultures of LAB containing cell load of 10^8 cfu/ml were used for fermentation of juice. The broth culture of LAB were taken at 5 per cent of juice volume (v/v), centrifuged at 4000 rpm for 10 min. and the cell pellet obtained was added to juice for preparation of fermented beverage (Plate 2 and 3).

3.5 Experiment details

3.5.1 Experiments-I: Development of fermented pomegranate beverage with and without kokum juice using probiotic lactic acid bacteria

The design of the experiment was completely randomised design with thirteen treatments and three replications.

Treatment details

T_1 - Uninoulated Pomegranate juice (UPJ)

T_2 - 100 % Pomegranate juice + *Lactobacillus acidophilus*

T_3 - 100 % Pomegranate juice + *Lactobacillus plantarum*

T_4 - 100 % Pomegranate juice + *Lactobacillus delbrueckii*

T_5 - 85 % Pomegranate juice + 15% Kokum juice + *Lactobacillus acidophilus*

T_6 - 85 % Pomegranate juice +15% Kokum juice + *Lactobacillus plantarum*

T_7 - 85 % Pomegranate juice +15% Kokum juice + *Lactobacillus delbrueckii*

T_8 - 75 % Pomegranate juice + 25% Kokum juice + *Lactobacillus acidophilus*

T_9 - 75 % Pomegranate juice + 25% Kokum juice + *Lactobacillus plantarum*

T_{10} - 75 % Pomegranate juice + 25% Kokum juice + *Lactobacillus delbrueckii*

T_{11} - 65 % Pomegranate juice + 35% Kokum juice + *Lactobacillus acidophilus*

T_{12} - 65 % Pomegranate juice + 35% Kokum juice + *Lactobacillus plantarum*

T_{13} - 65 % Pomegranate juice + 35% Kokum juice + *Lactobacillus delbrueckii*

3.5.1.1 Methodology for preparation of fermented beverage

The extracted pomegranate and kokum fruit juices were blended wherever needed in the treatments. TSS (Total soluble solids) was adjusted to 18° brix by adding cane sugar using digital refractometer. Juice was pasteurised at 70°C for 5 min. and cooled. All the treatments (except T_1) were inoculated with lactic acid bacterial culture (5% v/v) as per the treatment details. Inoculated treatments were incubated at 37°C for 72 hr. After three days of fermentation the fermented juices was filtered through muslin cloth and the filtrate was filled in sterilized glass bottles. All the treatments were stored in refrigerator (4°C) (Plate 4).

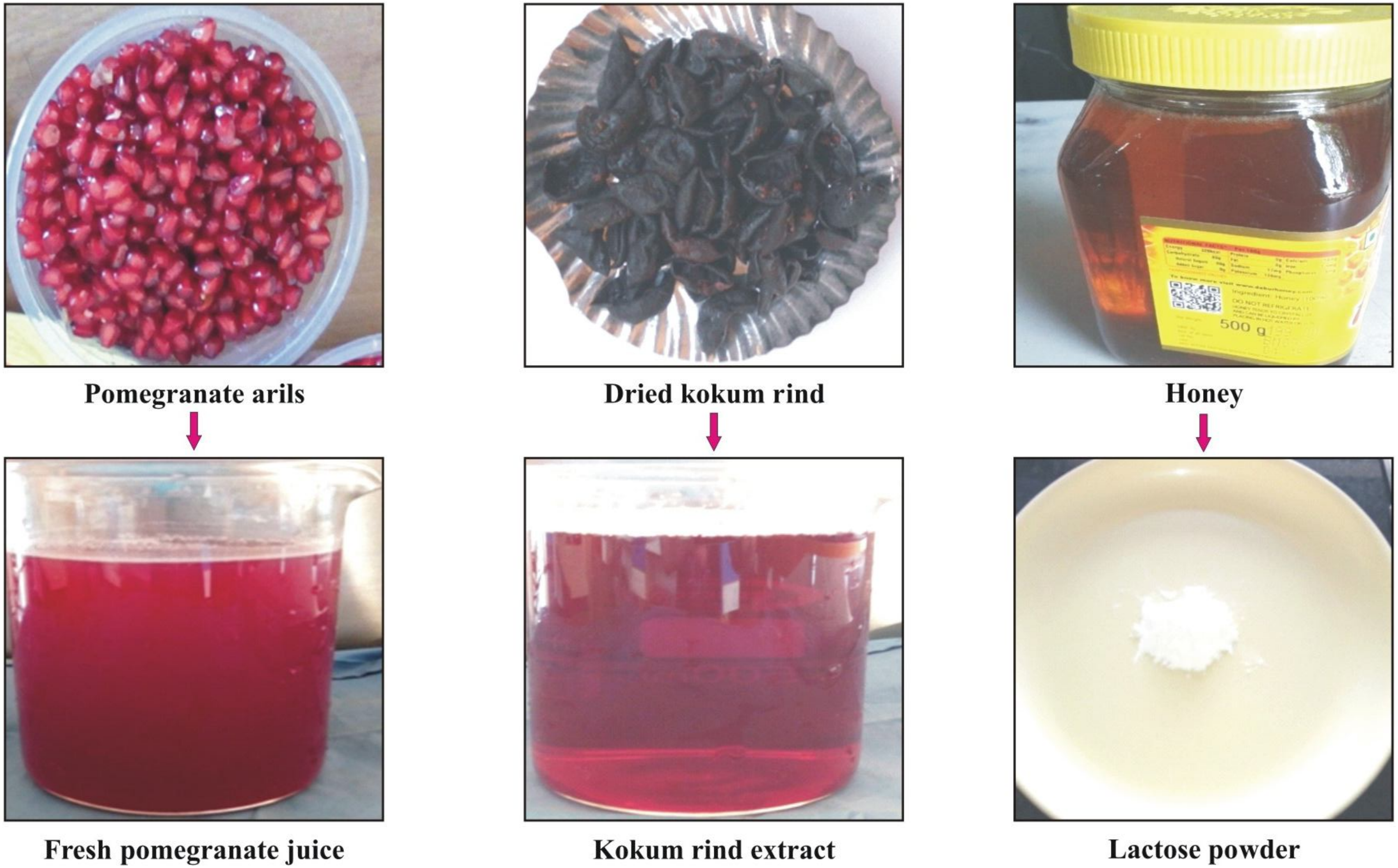
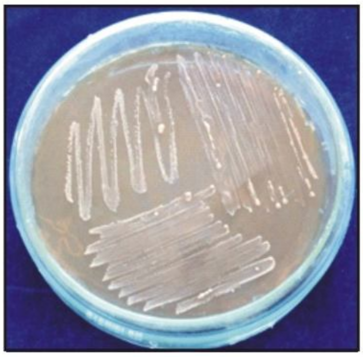
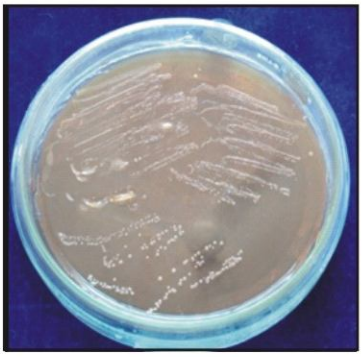


Plate 1. Pomegranate juice, kokum rind extract, honey and lactose used in the present study

Lactobacillus acidophilus
(NCIM 2903)



Lactobacillus plantarum
(NCIM 2373)



Lactobacillus delbrueckii
(NCIM 2025)

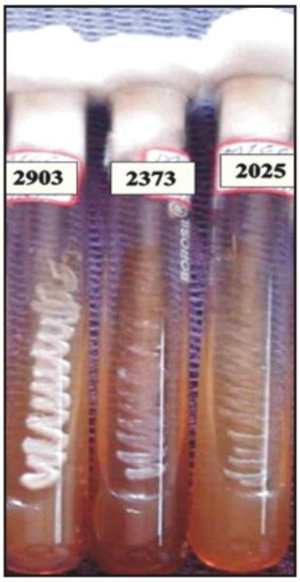


Plate 2. Pure cultures of lactic acid bacterial strains used in the experiment

Plate 3. DeMann, Rogosa and Sharpe MRS broth cultures of lactic acid bacteria

3.5.1.2 Sensory evaluation

Sensory evaluation of fermented beverage was carried out by 15 semi trained panel consisting of Teacher and Post graduate students of college of horticulture, Bagalkot with the help of nine point hedonic rating scale (1=dislike extremely, 2= dislike very much, 3= dislike moderately, 4= dislike slightly, 5=neither like nor dislike, 6= like slightly, 7= like moderately, 8= like very much and 9 = like extremely). The products along with the control were coded and served randomly to the panellist for sensory evaluation immediately after fermentation. Score card used for sensory evaluation is given in Appendix I.

The best seven treatments and control from sensory evaluation was taken to experiment II.

3.5.2 Experiment-II: Biochemical properties and sensory evaluation of fermented pomegranate beverage with and without kokum juice during storage

The best seven treatments and control beverages of experiment-I were prepared for storage studies. The design of the experiment was factorial completely randomised design with eight treatments and three replications. The beverages were subjected for biochemical, sensory and microbiological analysis by following standard procedures.

Treatment details

Factor-I: Treatments

T_1 - Uninoulated Pomegranate juice (Control)

T_3 - 100 % Pomegranate juice + *Lactobacillus plantarum*

T_5 - 85 % Pomegranate juice + 15% Kokum juice + *Lactobacillus acidophilus*

T_6 - 85 % Pomegranate juice + 15% Kokum juice + *Lactobacillus plantarum*

T_7 - 85 % Pomegranate juice + 15% Kokum juice + *Lactobacillus delbrueckii*

T_8- 75 % Pomegranate juice + 25% Kokum juice + *Lactobacillus acidophilus*

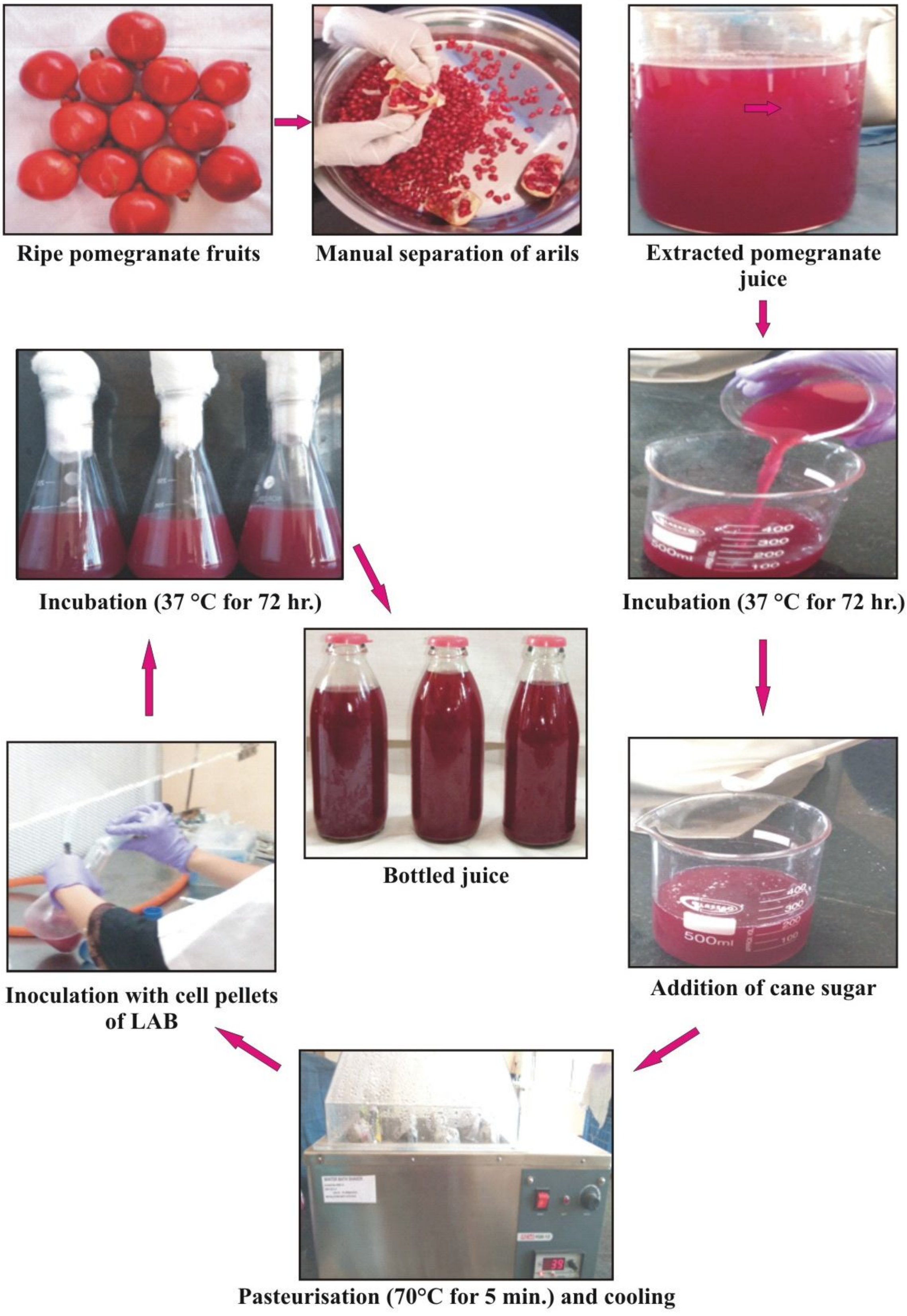


Plate 4. Preparation of LAB fermented pomegranate beverage blended with kokum juice

Plate 5. Lactic acid bacteria fermented pomegranate beverage with and without kokum juice

Plate 6. Sensory evaluation of LAB fermented pomegranate beverage with and without kokum juice

T_{10}- 75 % Pomegranate juice + 25% Kokum juice + *Lactobacillus delbrueckii*

T_{11} - 65 % Pomegranate juice + 35% Kokum juice + *Lactobacillus acidophilus*

Factor-II: Storage period (45 days)

S_1 - Initial

S_2 - 15 days

S_3 - 30 days

S_4 - 45 days

3.5.3 Experiment-III: Effect of prebiotics on fermentation of pomegranate juice with kokum blend using probiotic lactic acid bacteria during storage

The best one treatment from experiment-II was selected based on biochemical, sensory and microbiological properties. The design of the experiment was factorial completely randomised design with eight treatments and three replications. The beverages were subjected for biochemical, sensory and microbiological analysis by following standard procedures.

3.5.3.1 Methodology for preparation of fermented beverage

The best treatment was selected from experiment-II *i.e.*, 85 per cent Pomegranate juice + 15 per cent Kokum juice + *Lactobacillus plantarum* (T_6) as it recorded superior biochemical, sensory and microbial properties. Instead of cane sugar, juices were added with prebiotics (honey and lactose) as mentioned in the treatment details. Treatment without addition of prebiotics was taken as control (T_1). Juices were heat processed at 70°C for 5 min. and cooled. All the treatments were inoculated with lactic acid bacterial culture *i.e.*, *Lactobacillus plantarum* at 5% v/v. Inoculated treatments were incubated at 37°C for 72 hr. After three days of fermentation, juices were filled in sterilized glass bottles. All the treatments were stored in refrigerator (4 °C).

Plate 7. Lactic acid bacteria fermented pomegranate beverage with and without kokum juice

T_1 - 100% UPJ
T_3 - 100% PJ + *Lp*
T_5 - 85% PJ + 15% KJ + *La*
T_6 - 85% PJ + 15% KJ + *Lp*
T_7 - 85% PJ + 15% KJ + *Ld*
T_8 - 75% PJ + 25% KJ + *La*
T_{10} - 75% PJ + 25% KJ + *Ld*
T_{11} - 65% PJ + 35% KJ + *La*

Plate 8. Sensory evaluation of LAB fermented pomegranate beverage with and without kokum juice

Plate 9. Fermented pomegranate beverage blended with kokum and prebiotics

T_1 - 85% PJ + 15% KJ + *Lp*

T_2 - 85% PJ + 15% KJ + *Lp* + 2% H

T_3 - 85% PJ + 15% KJ + *Lp* + 3% H

T_4 - 85% PJ + 15% KJ + *Lp* + 5% H

T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L

T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L

T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L

T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H + 2%L

Plate 10. Sensory evaluation of fermented pomegranate beverage blended with kokum and prebiotics

Treatment details

Factor-I: Treatments

T_1 - 85% Pomegranate juice + 15% Kokum juice

T_2 - 85% Pomegranate juice + 15% Kokum juice + 2 % honey

T_3 - 85% Pomegranate juice + 15% Kokum juice + 3 % honey

T_4 - 85% Pomegranate juice + 15% Kokum juice + 5 % honey

T_5 - 85% Pomegranate juice + 15% Kokum juice + 2 % lactose

T_6 - 85% Pomegranate juice + 15% Kokum juice + 3 % lactose

T_7 - 85% Pomegranate juice + 15% Kokum juice + 5 % lactose

T_8 - 85% Pomegranate juice + 15% Kokum juice + 2% honey + 2 % lactose

Factor-II: Storage period (45 days)

S_1 - Initial

S_2 - 15 days

S_3 - 30 days

S_4 - 45 days

3.6 Observation recorded

3.6.1 Yield of juice (%)

A known quantity of pomegranate was taken and juice was extracted. Juice weight was recorded with the help of weighing balance. Percentage yield of juice in pomegranate was estimated by using the formula.

$$\text{Juice yield (\%)} \quad \frac{\text{Weight juice (g) x 100}}{\text{Weight of fruit (g)}}$$

3.6.2 Waste index (%)

Waste index in pomegranate was estimated using the formula:

$$\text{Waste index in pomegranate (\%)} = \frac{\text{Weight of fruit (g)} - \text{Weight of juice (g)} \times 100}{\text{Weight of fruit (g)}}$$

3.6.3 Total soluble solids (%)

The total soluble solids (TSS) in samples were measured by using digital refractometer and expressed as ° brix.

3.6.4 Reducing sugar (%)

Reducing sugar in samples was estimated as per the Di-nitrosalicylic acid method (Miller, 1972). The values obtained were expressed as per cent.

3.6.5 Total sugars (%)

The total sugar content present in the product were estimated by the same method as in case of reducing sugar after inversion of the non-reducing sugar using dilute hydrochloric acid (Anon., 1984). One ml of evaporated extract was taken and kept in boiling water till the alcohol completely evaporated and allowed it to cool. Then phenolphthalein indicator was added followed by 1 N sodium hydroxide till the solution turned to pink. Again 0.1 N Hydrochloric acid was added to discolour the solution. Then Dinitro-salicylic acid was added to discolour the solution. Then Dinitro-Salicylic acid (DNSA) method for reducing sugar was followed. The results were expressed in terms of percentage.

3.6.6 pH

pH of the samples were measured using digital pH meter. Standard buffer solutions of pH 4.0 and 7.0 were used to calibrate the instrument (Jackson, 1973).

3.6.7 Citric acid and lactic acid (%)

A known volume of sample (2ml) was taken and filtered through muslin cloth and volume was made up to 100 ml with distilled water. From this, five ml of aliquot was taken and titrated against standard NaOH (0.1N) using phenolphthalein indicator.

The appearance of light pink colour indicated the end point. The values were expressed in terms of citric acid and lactic acid as per cent titrable acidity of beverages (Anon., 1984).

$$TA\ (\%) = \frac{TV \times \text{Normality of NaoH} \times \text{Equivalent weight of acid} \times \text{Volume made up} \times 100}{\text{Volume of sample taken} \times \text{Weight of sample} \times 1000}$$

Where, TV is Titre value

3.6.8 Ascorbic acid (mg/100 ml)

Ascorbic acid (Vitamin-C) was estimated titrimetrically using 2, 6-dichlorophenol indophenols dye as per the modified procedure of AOAC (Anon., 1984). Five ml of sample was taken and diluted to a known volume with four per cent oxalic acid. This was filtered through a muslin cloth to get a clear juice then volume was made to 100 ml using four per cent oxalic acid. Five ml of aliquot was titrated against 2, 6 dichlorophenol indophenol dye till the pink colour appeared. The results were expressed as mg of ascorbic acid per 100 ml of beverages.

$$\text{Ascorbic acid (mg/100 ml)} = \frac{\text{Ascorbic acid (mg) in standard} \times TV2 \times \text{Volume made up} \times 100}{TV1 \times \text{Weight of sample} \times \text{Aliquot taken}}$$

Where,

TV1 = Titre value of standard and TV2 = Titre value of sample

3.6.9 Antioxidant activity (%)

The percentage of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity of the samples was determined by a method described by Kathiravan *et al.* (2014). The hydrogen atom or electron donation abilities of the juice were measured from the bleaching of a purple-coloured methanol solution of stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH). A known volume of sample (0.1 ml) or 0.1 ml of methanol (control) mixed with 2.9 ml of 0.004 % DPPH solution (10 mg in 250 ml of methanol prepared freshly) and methanol used as a blank. The mixture was vortexed thoroughly for 1 min and left at 37°C temperature for 30 minutes in darkness and then the spectrophotometer absorbance was read against blank at 517 nm (Model: UV Spectrophotometer, Spectronic ^{R} Genesys ^{TM} 2 Instruments, USA). DPPH free radical scavenging ability (%) was calculated using the formula:

(A 517 nm of control – A 517 nm of sample / A 517 nm of control) × 100

3.6.10 Total phenol (mg GAE/ 100 ml)

Total phenol content of samples was estimated by Folin Ciocalteu reagent (FCR) method (Sadasivam and Manickam, 2005). A sample of 0.5 ml was taken and 10 ml of ethanol was added and filtered the solution using filter paper from which one ml filtered solution was taken in a test tube and boiled at 100°C till the solution was evaporated. One ml of distilled water was added to the test tube and from this 0.5 ml solution was taken into another test tube to which 2.5 ml of distilled water, 1 ml of FCR reagent and 2 ml of sodium carbonate was added and boiled in water bath for 10 minutes. Then the contents of the test tubes were cooled and the absorbance was measured at 650 nm by using spectrophotometer. Total phenol content was calculated with the help of standard graph and expressed in milligram gallic acid equivalents per hundred grams.

3.6.11 Protein content (%)

Protein in samples was estimated as per the Lowry's method. A sample of 0.1 ml was taken and added with distilled water to make it up to 1 ml each. 5 ml of alkaline copper reagent was added to the test tube, mixed thoroughly and allowed to react for 10 min. Then it was added with 0.5 ml of Folin-Ciocalteu reagent (FCR), mixed and kept in dark for 30 minutes. The absorbance values of standard and the sample was recorded against blank which was set at 660 nm. The milligram of protein per gram of sample was calculated from the standard graph using Bovine Serum Albumin (BSA) fraction V. The values obtained are expressed as per cent (Satyanarayana and Chakrapani, 2006).

3.6.12 Colour (*L* a* b**)

Sample colour was measured with a Colour Flex EZ (Model CFEZ 1919, Hunter Associates Laboratory, Inc., Reston) with a 45 mm (diameter) measuring tube using a white tile background. *L**, *a** and *b** values denote lightness (white-black), red-green and yellow-blue scales, respectively. Three colour readings per beverage sample were made. There were three replicate beverage samples for each treatment.

3.6.13 Sensory evaluation

Sensory evaluation of samples was carried out as per the method given in 3.5.1.2. Fermented beverages were served immediately after fermentation (Initial) along with the freshly prepared pomegranate juice and thereafter at an interval of 15 days up to 45 days of storage.

3.6.14 Microbial analysis

3.6.14.1 Microbial count

After fermentation, the samples were subjected for microbiological analysis for lactic acid bacterial counts by employing standard dilution plate count method (Hoben and Somasegaran, 1982).

Dilution

A serial dilution technique was carried out to estimate the lactic acid bacterial (LAB) load in the fermented beverages. One milliliter of the sample was transferred to the test tube containing nine millilitre of distilled water. The test tube was vortexed with the help of spinix cyclomixer. Dilutions up to 10^{-6} were prepared for LAB counts.

The MRS (deMann, Rogosa and Sharpe) agar media was used to enumerate LAB count in fermented beverage.

Enumeration

The media was sterilised in the autoclave at 121°C for 20 minutes. In each sterilised petri dish, 1 ml of respective sample was transferred; 25 ml of media was poured in duplicate plates. The plates were rotated both clock and anti clock wise direction for uniform mixing of the sample and media. After solidification the plates were kept upside down position incubated at 35-37°C for three days.

Counting

The colonies were counted and the total counts were expressed as colony forming unit (cfu) per millilitre of fermented beverages

3.7 Statistical analysis

The data on the sensory evaluation of experiment I was analysed according to completely randomised design (CRD). The data on the physico-chemical parameters and sensory evaluation of experiment II and III were analysed according to factorial completely randomised design (FCRD). Statistical analysis was performed using Web Agri Stat Package (WASP) Version 2 (Jangam and Thali, 2010). The level of significance used in ‘F’ and ‘t’ test was $p=0.01$. Critical difference values were calculated whenever F test was significant.

4. EXPERIMENTAL RESULT

The investigation entitled “Studies on fermentation of pomegranate juice by lactic acid bacteria” was conducted in the Department of Post-Harvest Technology, College of Horticulture, Bagalkot during the year 2016-2017. The results obtained in the present study were being presented in this chapter.

The data pertaining to biochemical properties of various juice combinations *viz.*, 100% PJ, 85% PJ + 15% KJ, 75% PJ+ 25% KJ and 65% PJ + 35% KJ were presented in appendix II. Biochemical properties with respect to citric acid, lactic acid, pH, TSS, total sugar, reducing sugar, antioxidant activity, total phenol and ascorbic acid content were evaluated before LAB inoculation.

4.1 Development of fermented pomegranate beverage with and without kokum juice using probiotic lactic acid bacteria

Sensory evaluation of fermented pomegranate beverage with and without kokum juice was performed by semi trained panellists (Plate 5 and 6). The data pertaining to colour, flavour, taste, consistency and overall acceptability of fermented pomegranate beverage with and without kokum juice as influenced by the treatments is presented in Table 1.

4.1.1 Colour (Score out of 9)

The mean scores of colour of treatments ranged from 7.46 to 7.83. All the treatments were on par with each other. However, numerically highest score were recorded in T_3 (7.83) followed by T_6 (7.81), T_1 and T_{11} (7.75 each). Among all the treatments least score for colour was obtained in T_4 (7.46).

4.1.2 Flavour (Score out of 9)

The mean scores of flavour for different treatments varied between 6.49 and 7.14. All the treatments were on par with each other for flavour. However, numerically highest score were recorded in T_3 (7.14) followed by T_5 (7.05), T_1 (6.99) and the lowest in T_4 (6.49).

4.1.3 Taste (Score out of 9)

The mean scores of taste of treatments varied between 7.12 and 8.30. There was a significant difference among the treatments for taste. The highest score was noticed in the treatment T_6 (8.30) which was on par with T_3 (8.24), T_5 (8.16) and T_7 (8.12). The least score was observed in T_{12} (7.12).

4.1.4 Consistency (Score out of 9)

The mean scores of consistency of treatments varied from 5.62 to 7.16. Numerically highest score for consistency was observed in treatment T_3 (7.16) followed by T_5 (7.11), T_1 (7.00) and lowest in T_{13} (5.62).

4.1.5 Overall acceptability (Score out of 9)

The mean scores of overall acceptability of treatments varied from 6.12 to 7.30. There was a significant difference among the treatments for overall acceptability. The highest score for overall acceptability was observed in the treatment T_6 (7.30) which was on par with T_3 (7.24), T_5 (7.23), T_7 (7.16), T_1 (7.12), T_8 (7.09), T_{11} (7.06) and T_{10} (7.04). The least score was observed in the treatment T_{13} (6.12).

4.1.6 Rank

Ranking for different treatments of fermented pomegranate beverage with and without kokum juice was given by taking consideration of average values of sensory evaluation *viz.*, colour, flavour, taste, consistency and overall acceptability. Among the treatments T_6 (85% Pomegrante juice + 15% Kokum juice + *Lactobacillus plantarum*) ranks 1st with average score of 7.51 followed by T_5 (85% Pomegrante juice + 15% Kokum juice + *Lactobacillus acidophilus*; 7.46).

Table 1: Sensory evaluation of lactic acid bacteria fermented pomegranate beverage with and without kokum juice

Treatments	Colour*	Flavour*	Taste*	Consistency*	Overall acceptability*	Average	Rank
T_1 - 100% UPJ	7.75	6.88	8.07	7.00	7.12	7.36	5
T_2 - 100% PJ + *La*	7.50	6.65	7.46	6.08	6.46	6.83	11
T_3 - 100% PJ + *Lp*	7.83	7.14	8.24	6.71	7.24	7.43	3
T_4 - 100% PJ + *Ld*	7.46	6.49	7.90	6.39	6.33	6.91	10
T_5 - 85% PJ + 15% KJ + *La*	7.73	7.05	8.16	7.11	7.23	7.45	2
T_6 - 85% PJ + 15% KJ + *Lp*	7.81	6.99	8.30	7.16	7.3	7.51	1
T_7 - 85% PJ + 15% KJ + *Ld*	7.67	6.96	8.12	6.82	7.16	7.34	6
T_8 - 75% PJ + 25% KJ + *La*	7.73	6.90	8.09	7.06	7.09	7.37	4
T_9 - 75% PJ + 25% KJ + *Lp*	7.62	6.51	7.66	6.58	6.76	7.02	9
T_{10} - 75% PJ + 25% KJ + *Ld*	7.66	6.89	8.10	6.64	7.04	7.26	7
T_{11} - 65% PJ + 35% KJ + *La*	7.75	6.68	8.06	6.69	7.06	7.24	8
T_{12} - 65% PJ + 35% KJ + *Lp*	7.60	6.51	7.13	6.44	6.13	6.75	12
T_{13} - 65% PJ + 35% KJ + *Ld*	7.51	6.53	7.12	5.62	6.12	6.58	13
S.Em±	**0.12**	**0.16**	**0.05**	**0.30**	**0.04**		
CD (1%)	**NS**	**NS**	**0.20**	**NS**	**0.11**		

UPJ - Uninoculated pomegranate juice
PJ - Pomegranate juice
KJ - Kokum juice

***La* -** *Lactobacillus acidophilus* (2903)
***Lp* -** *Lactobacillus plantarum* (2373)
***Ld* -** *Lactobacillus delbrueckii* (2025)

NS – Non significant
*Score out of 9

4.2 Biochemical quality and sensory evaluation of fermented pomegranate beverage with and without kokum juice during storage

4.2.1 Citric acid (%)

The data with respect to citric acid (%) content of fermented pomegranate beverage with and without kokum juice of different treatments stored up to 45 days is presented in Table 2. The citric acid per cent of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

The treatments mean values of citric acid per cent over different storage period ranged from 0.33 to 1.64 per cent. The highest citric acid was recorded in T_{11} (1.64%) which was on par with T_8 (1.63%) and the lowest in T_1 (0.33%).

Citric acid content of fermented beverage increased up to 30 days of storage and afterwards found decreased up to 45 days. However, in uninoculated beverage (control) citric acid percentage followed decreasing trend as the storage period advanced. Significantly, the highest citric acid content was observed at 30 DAS (0.99%) and the least at initial period (0.89%).

The interaction between the treatments and storage period were found to be significantly different. The maximum citric acid content was noted in $T_{11}S_3$ (1.71%) which was on par with T_8S_3 (1.70%) and $T_{11}S_4$ (1.69%).The least was observed in T_1S_4 (0.26%).

4.2.2 Lactic acid (%)

The data with respect to lactic acid (%) content of fermented pomegranate beverage with and without kokum juice of different treatments stored up to 45 days is presented in Table 2. The lactic acid content of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

The mean lactic acid content of the treatments varied between 0.06 and 2.35 per cent. The highest lactic acid was recorded in T_{11} (2.35%) followed by T_8 (2.23%), T_{10} (1.90%), T_6 (1.24%) and the lowest in T_1 (0.06%).

Table 2: Changes in citric acid (%) and lactic acid (%) content of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period

Treatments	Citric acid (%)				Mean	Lactic acid (%)				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)		S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 100% UPJ	0.42	0.33	0.30	0.26	**0.33**	0.07	0.06	0.06	0.05	**0.06**
T_3 - 100% PJ + *Lp*	0.69	0.79	0.81	0.70	**0.75**	0.97	1.19	1.24	1.22	**1.15**
T_5 - 85% PJ + 15% KJ + *La*	0.71	0.76	0.78	0.75	**0.75**	0.98	1.22	1.31	1.29	**1.20**
T_6 - 85% PJ + 15% KJ + *Lp*	0.73	0.78	0.80	0.76	**0.77**	1.03	1.25	1.35	1.33	**1.24**
T_7 - 85% PJ + 15% KJ + *Ld*	0.55	0.57	0.59	0.55	**0.57**	0.78	0.91	0.94	0.92	**0.89**
T_8 - 75% PJ + 25% KJ + *La*	1.51	1.63	1.70	1.68	**1.63**	2.13	2.23	2.29	2.28	**2.23**
T_{10} - 75% PJ + 25% KJ + *Ld*	1.01	1.16	1.27	1.05	**1.31**	1.77	1.91	1.98	1.97	**1.90**
T_{11} - 65% PJ + 35% KJ + *La*	1.53	1.65	1.71	1.69	**1.64**	2.24	2.34	2.42	2.40	**2.35**
Mean	**0.89**	**0.96**	**0.99**	**0.93**		**1.24**	**1.39**	**1.45**	**1.43**	

	S.Em± (Citric acid)	CD (1%) (Citric acid)	S.Em± (Lactic acid)	CD (1%) (Lactic acid)
Treatments	**0.007**	**0.02**	**0.01**	**0.07**
Storage period	**0.005**	**0.02**	**0.01**	**0.04**
Interaction (T×S)	**0.01**	**0.05**	**0.03**	**0.14**

UPJ - Uninoculated pomegranate juice
PJ - Pomegranate juice
KJ - Kokum juice
***La* -** *Lactobacillus acidophilus* (2903)
***Lp* -** *Lactobacillus plantarum* (2373)
***Ld* -** *Lactobacillus delbrueckii* (2025)
DAS – Days after storage

Lactic acid content of all the treatments except T_1 (control) increased up to 30 days of storage and after 30 days of storage slight decrease in lactic acid content was observed. Significantly, the highest lactic acid content was recorded at 30 DAS (1.45%) and the least at initial period (1.24%).

The interaction between the treatments and storage period were found to be significantly different. The maximum lactic acid content was recorded in $T_{11}S_3$ (2.42%) which was on par with $T_{11}S_2$ (2.34%) and T_8S_3 (2.29%).

4.2.3 pH

The data with respect to pH of fermented pomegranate beverage with and without kokum juice of different treatments stored up to 45 days is presented in Table 3. The pH of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

Irrespective of storage periods, the mean pH of the treatments ranged from 2.48 to 3.54. The lowest pH was recorded in T_{11} (2.48) followed by T_8 (2.55), T_5 (2.56), T_6 (2.92) and the highest in T_1 (3.54).

pH of fermented beverage decreased up to 30 days of storage and afterwards increased up to 45 days. However, pH of uninoculated beverage (control) followed increasing trend as the storage period advanced. The lowest pH was recorded at 30 DAS (2.85) followed by 15 DAS and 45 DAS (2.92 each) and the highest at initial period (2.99).

The interaction between the treatments and storage period were found to be significantly different. The minimum pH was observed in $T_{11}S_3$ (2.40) which were on par with $T_{11}S_2$, $T_{11}S_4$, T_8S_3 and T_5S_3 (2.48 each).

4.2.4 TSS (° brix)

The data with respect to TSS of fermented pomegranate beverage with and without kokum juice of different treatments stored up to 45 days is presented in Table 3. The TSS of beverage varied significantly among the storage periods, treatments and with their interactions.

Table 3: Changes in pH and TSS (° brix) of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period

Treatments	pH				Mean	Total soluble solids (° brix)				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)		S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 100% UPJ	3.41	3.48	3.60	3.67	**3.54**	18.08	18.39	18.6	18.62	**18.42**
T_3 - 100% PJ + *Lp*	3.25	3.16	3.05	3.15	**3.15**	12.22	11.97	11.51	11.44	**11.78**
T_5 - 85% PJ + 15% KJ + *La*	2.66	2.58	2.48	2.53	**2.56**	11.63	11.22	10.97	10.90	**11.18**
T_6 - 85% PJ + 15% KJ + *Lp*	3.04	2.94	2.84	2.88	**2.92**	11.58	11.14	10.88	10.77	**11.09**
T_7 - 85% PJ + 15% KJ + *Ld*	3.21	3.12	3.00	3.09	**3.11**	11.84	11.56	11.34	11.31	**11.51**
T_8 - 75% PJ + 25% KJ + *La*	2.61	2.55	2.48	2.55	**2.55**	11.42	11.16	10.76	10.58	**10.98**
T_{10} - 75% PJ + 25% KJ + *Ld*	3.17	3.04	2.98	3.01	**3.05**	11.94	11.78	11.68	11.66	**11.77**
T_{11} - 65% PJ + 35% KJ + *La*	2.56	2.48	2.40	2.48	**2.48**	10.81	10.55	10.39	10.30	**10.51**
MEAN	**2.99**	**2.92**	**2.85**	**2.92**		**12.44**	**12.22**	**12.01**	**11.95**	

	pH S.Em±	pH CD (1%)	TSS S.Em±	TSS CD (1%)
Treatments	**0.01**	**0.04**	**0.01**	**0.03**
Storage period	**0.007**	**0.02**	**0.007**	**0.02**
Interaction (T×S)	**0.02**	**0.08**	**0.02**	**0.07**

UPJ - Uninoculated pomegranate juice
PJ - Pomegranate juice
KJ - Kokum juice

***La* -** *Lactobacillus acidophilus* (2903)
***Lp* -** *Lactobacillus plantarum* (2373)
***Ld* -** *Lactobacillus delbrueckii* (2025)

DAS – Days after storage

Irrespective of storage periods, the mean TSS of the treatments varied between 10.51 and 18.42° brix. The lowest TSS was observed in T_{11} (10.51° brix) followed by T_8 (10.98° brix), T_6 (11.09° brix). The highest TSS was observed in T_1 (18.42° brix) followed by T_3 (11.78° brix), T_{10} (11.77° brix).

TSS of all treatments decreased as the storage period advanced except in T_1 (control) where increasing trend was observed. Significantly, the lowest TSS was recorded at 45 DAS (11.95° brix) and highest TSS was observed during initial period (12.44° brix).

The interaction between the treatments and storage period showed minimum TSS content in $T_{11}S_4$ (10.30° brix) and maximum TSS content in T_1S_4 (18.62° brix) which was on par with T_1S_3 (18.60° brix).

4.2.5 Total sugar (%)

The data with pertaining to total sugar content of fermented beverage with and without kokum juice of different treatments stored up to 45 days is presented in Table 4. The total sugar content of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

The mean total sugar content of the treatments ranged from 10.60 to 15.67 per cent. Significantly, the lowest total sugar content was recorded in T_{11} (10.60%) which was on par with T_8 (10.79%) followed by T_6 (11.03) and the highest total sugar content was recorded in T_1 (15.67%).

Significant difference was observed with respect to total sugar content of fermented beverage during stored period of 45 days. Lowest score for total sugar content was recorded at 30 DAS (11.53%) which was on par with 15 DAS (11.66%) and 45 DAS (11.61%)**.** Maximum total sugar content was noticed at initial period (11.91%) which was significantly different from all the storage periods.

In case of interaction effect between treatments and storage period, there was a gradual decrease in the total sugar content in all the treatments up to 30 DAS was observed except in unfermented beverage where total sugar content increased during the

Table 4: Changes in total sugar (%) and reducing sugar (%) content of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period

Treatments	Total sugar (%)				Mean	Reducing sugar (%)				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)		S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 100% UPJ	14.96	15.2	15.73	16.81	**15.67**	10.21	10.30	10.34	10.38	**10.30**
T_3 - 100% PJ + *Lp*	11.51	11.30	10.98	10.90	**11.17**	3.66	3.58	3.51	3.45	**3.55**
T_5 - 85% PJ + 15% KJ + *La*	12.22	11.20	10.90	10.78	**11.28**	3.74	3.67	3.52	3.43	**3.59**
T_6 - 85% PJ + 15% KJ + *Lp*	11.09	10.73	10.70	10.65	**11.03**	3.50	3.45	3.33	3.23	**3.38**
T_7 - 85% PJ + 15% KJ + *Ld*	11.66	11.47	11.22	11.15	**11.37**	4.11	3.95	3.91	3.88	**3.96**
T_8 - 75% PJ + 25% KJ + *La*	11.32	11.12	10.86	10.81	**10.79**	3.43	3.35	3.27	3.20	**3.31**
T_{10} - 75% PJ + 25% KJ + *Ld*	11.77	11.62	11.35	11.31	**11.51**	4.30	4.18	4.02	3.92	**4.10**
T_{11} - 65% PJ + 35% KJ + *La*	10.79	10.67	10.48	10.45	**10.60**	3.22	2.93	2.75	2.66	**2.89**
Mean	**11.91**	**11.66**	**11.53**	**11.61**		**4.52**	**4.42**	**4.33**	**4.27**	

	Total sugar S.Em±	Total sugar CD (1%)	Reducing sugar S.Em±	Reducing sugar CD (1%)
Treatments	**0.06**	**0.24**	**0.02**	**0.11**
Storage period	**0.04**	**0.17**	**0.02**	**0.07**
Interaction (T×S)	**0.14**	**0.49**	**0.05**	**0.22**

UPJ - Uninoculated pomegranate juice
PJ - Pomegranate juice
KJ - Kokum juice

***La* -** *Lactobacillus acidophilus* (2903)
***Lp* -** *Lactobacillus plantarum* (2373)
***Ld* -** *Lactobacillus delbrueckii* (2025)

DAS – Days after storage

storage period of 45 days. The lowest total sugar content was recorded in $T_{11}S_4$ (10.45%) and highest was observed in T_1S_4 (16.81%).

4.2.6 Reducing sugar content (%)

The data pertaining to reducing sugar content of fermented beverage with and without kokum juice of different treatments stored up to 45 days is presented in Table 4. The reducing sugar content of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

The mean reducing sugar content of the treatments ranged from 2.89 to 10.30 per cent. Significantly, the lowest reducing sugar content was recorded in T_{11} (2.89%) followed by T_8 (3.31%) and the highest reducing sugar content was recorded in T_1 (10.30%).

Significant difference was observed with respect to reducing sugar content of fermented beverage during storage period of 45 days. The minimum reducing sugar content was recorded during 45 DAS (4.27%) which was on par with 30 DAS (4.33%). Maximum reducing sugar content was noticed at initial period (4.52%) which was significantly different from all the storage periods.

In case of interaction effect between treatments and storage period, there was a gradual decrease in the reducing sugar content in all the treatments except in unfermented juice was observed where as reducing sugar content increased during the storage period of 45 days. The lowest reducing sugar content was recorded in $T_{11}S_4$ (2.66%) and highest was observed in T_1S_4 (10.30%).

4.2.7 Antioxidant activity (%)

The data with respect to antioxidant activity of fermented pomegranate beverage with and without kokum juice of different treatments stored up to 45 days is presented in Table 5. The antioxidant activity of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

Irrespective of storage periods, the mean antioxidant activity of the treatments ranged from 59.05 to 77.07 per cent. The highest antioxidant activity was observed in

Table 5: Changes in antioxidant activity (%) and total phenol content (mg GAE/100 ml) of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period

Treatments	Antioxidant activity (%)				Mean	Total phenol (mg GAE/100 ml)				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)		S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 100% UPJ	63.94	61.26	57.53	53.48	**59.05**	233.01	230.43	224.85	220.64	**227.23**
T_3 - 100% PJ + *Lp*	82.02	78.22	75.41	68.17	**75.96**	254.25	251.79	247.81	244.87	**249.68**
T_5 - 85% PJ + 15% KJ + *La*	83.16	78.87	73.41	63.41	**74.71**	253.16	250.04	248.46	244.03	**248.92**
T_6 85% PJ + 15% KJ + *Lp*	84.6	79.51	74.92	69.27	**77.07**	256.74	252.58	250.63	248.07	**252.00**
T_7 - 85% PJ + 15% KJ + *Ld*	75.79	73.27	69.14	65.55	**70.94**	252.61	249.93	247.22	244.54	**248.57**
T_8 - 75% PJ + 25% KJ + *La*	81.72	76.16	72.03	64.45	**73.59**	246.76	242.27	236.26	230.82	**239.03**
T_{10} - 75% PJ + 25% KJ + *Ld*	74.1	72.17	68.03	63.9	**69.55**	243.23	240.61	238.97	237.45	**240.06**
T_{11} - 65% PJ + 35% KJ + *La*	75.52	72.61	69.41	61.29	**69.71**	246.81	243.89	241.88	240.73	**243.32**
Mean	**77.6**	**74.01**	**69.98**	**63.69**		**248.32**	**245.19**	**242.01**	**238.89**	

	S.Em±	**CD (1%)**	**S.Em±**	**CD (1%)**
Treatments	**0.43**	**1.62**	**0.41**	**1.54**
Storage period	**0.30**	**1.14**	**0.29**	**1.09**
Interaction (T×S)	**0.86**	**3.24**	**0.82**	**3.08**

UPJ - Uninoculated pomegranate juice
PJ - Pomegranate juice
KJ - Kokum juice

***La* -** *Lactobacillus acidophilus* (2903)
***Lp* -** *Lactobacillus plantarum* (2373)
***Ld* -** *Lactobacillus delbrueckii* (2025)

DAS – Days after storage
GAE – Gallic acid equivalent

T_6 (77.07%) which was on par with T_3 (75.96%) and the lowest was noted in T_1 (59.05%).

Antioxidant activity of the fermented beverage decreased as the storage period advanced. Significantly, the highest antioxidant activity was recorded at initial period (77.60%) and the least at 45 DAS (63.69%).

The interaction between the treatments and storage period were found to be significantly different. The maximum antioxidant activity was recorded in T_6S_1 (84.60%) which was on par with T_5S_1 (83.16%), T_3S_1 (82.02%) and T_8S_1 (81.72%).

4.2.8 Total phenol content (mg GAE/100 ml)

The data with pertaining to total phenol content of fermented pomegranate beverage with and without kokum juice of different treatments stored up to 45 days is presented in Table 5. The total phenol content of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

The mean total phenol content of the treatments ranged from 227.23 to 252.00 mg GAE/100 ml. Significantly, the highest total phenol content was recorded in T_6 (252.00 mg GAE/100 ml) followed by T_3 (249.68 mg GAE/100 ml), T_5 (248.92 mg GAE/100 ml), T_5 (248.57 mg GAE/100 ml) and the lowest total phenol content was recorded in T_1 (227.23 mg GAE/100 ml).

Significant difference was observed with respect to total phenol content of fermented beverage during storage period of 45 days. Maximum score was recorded at initial period (248.32 mg GAE/100 ml) which was significantly different from all the storage periods. Significantly, lowest score was recorded at 45 DAS (238.89 mg GAE/100 ml).

In case of interaction effect between treatments and storage period, there was a gradual decrease in the total phenol content in all the treatments during the storage period of 45 days was observed. The highest total phenol content was recorded in T_6S_1 (256.74 mg GAE/100 ml) which was on par with T_3S_1 (254.25 mg GAE/100 ml). The least was observed in T_1S_4 (220.64 mg GAE/100 ml).

4.2.9 Ascorbic acid (Vitamin C) (mg/100 ml)

The data with respect to ascorbic acid content of fermented pomegranate beverage with and without kokum juice of different treatments stored up to 45 days is presented in Table 6. The acsorbic acid content of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

Irrespective of storage periods, the mean ascorbic acid content of the treatments ranged from 2.97 to 10.05 mg/100 ml. The highest acsorbic acid content was recorded in T_6 (10.05 mg/100 ml) followed by T_3 (9.31 mg/100 ml), T_5 (8.62 mg/100 ml) and the lowest in T_1 (2.97 mg/100 ml).

Ascorbic acid content of beverage decreased as the storage period advanced. Significantly, the highest ascorbic acid was observed at initial period (8.87 mg/100 ml) and the least at 45 DAS (5.60 mg/100 ml).

The interaction between the treatments and storage period were found to be significantly different. The maximum ascorbic acid was observed in T_6S_1 (13.58 mg/100 ml) followed by T_3S_1 (11.68 mg/100 ml), T_5S_1 (10.44 mg/100 ml). The least score was observed in T_1S_4 (2.50 mg/100 ml).

4.2.10 Instrumental colour analysis (*L* a* b** values)

The data on the *L**, *a**and *b** colour values of fermented pomegranate beverage with and without kokum juice of different treatments stored up to 45 days is presented in Table 7 and 8. *L** value varied significantly among the storage period but not with the treatments and interaction between the treatments and storage period. *a** and *b** values of beverage varied significantly among the treatments and storage periods but not with their interactions.

Among different treatments, minimum and maximum score for lightness (*L** value) was observed in T_3 (10.47) and T_1 (11.79) respectively. Highest score for *a** and *b** values were noticed in T_{11} (28.14 and 12.15 respectively).

The effect of storage period on *L**, *a**, *b** colour values of fermented beverage found to be significant. Highest score for *L** and *b** values were observed at initial

Table 6: Changes in ascorbic acid (mg/100 ml) content of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period

Treatments	Ascorbic acid (mg/100 ml)				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 100% UPJ	3.67	3.07	2.66	2.50	**2.97**
T_3 - 100% PJ + *Lp*	11.68	9.26	8.54	7.75	**9.31**
T_5 - 85% PJ + 15% KJ + *La*	10.44	8.96	8.10	6.99	**8.62**
T_6 - 85% PJ + 15% KJ + *Lp*	13.58	10.28	8.54	7.81	**10.05**
T_7 - 85% PJ + 15% KJ + *Ld*	9.53	7.20	6.61	5.91	**7.31**
T_8 - 75% PJ + 25% KJ + *La*	8.88	7.03	6.60	5.99	**7.12**
T_{10} - 75% PJ + 25% KJ + *Ld*	6.95	5.12	4.75	3.85	**5.17**
T_{11} - 65% PJ + 35% KJ + *La*	6.22	5.31	4.77	4.03	**5.08**
Mean	**8.87**	**7.03**	**6.32**	**5.60**	

	S.Em±	CD (1%)
Treatment	**0.07**	**0.27**
Storage period	**0.05**	**0.19**
Interaction (T× S)	**0.14**	**0.54**

UPJ - Uninoculated pomegranate juice
PJ - Pomegranate juice
KJ - Kokum juice

***La* -** *Lactobacillus acidophilus* (2903)
***Lp* -** *Lactobacillus plantarum* (2373)
***Ld* -** *Lactobacillus delbrueckii* (2025)

DAS – Days after storage
GAE – Gallic acid equivalent

Table 7: Changes in colour (*L and *a**) value fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period**

Treatments	*L**				Mean	*a**				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)		S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 100% UPJ	16.15	12.01	10.57	8.44	**11.79**	9.46	10.42	11.68	12.92	**11.12**
T_3 - 100% PJ + *Lp*	15.33	11.38	11.23	8.54	**11.62**	12.43	13.67	14.87	16.52	**14.37**
T_5 - 85% PJ + 15% KJ + *La*	13.67	12.19	10.52	8.57	**11.23**	14.53	15.82	16.99	18.92	**16.56**
T_6 - 85% PJ + 15% KJ + *Lp*	14.58	11.46	10.28	7.81	**11.03**	14.89	16.18	16.96	18.56	**16.65**
T_7 - 85% PJ + 15% KJ + *Ld*	14.32	11.6	10.55	8.90	**11.34**	14.16	15.56	17.2	18.57	**16.37**
T_8 - 75% PJ + 25% KJ + *La*	13.23	11.31	10.28	8.62	**10.86**	20.42	21.99	23.72	26.24	**23.09**
T_{10} - 75% PJ + 25% KJ + *Ld*	13.44	11.35	10.17	7.96	**10.73**	18.95	20.93	22.49	23.92	**21.57**
T_{11} - 65% PJ + 35% KJ + *La*	12.68	11.04	10.05	8.12	**10.47**	24.46	26.69	29.42	32	**28.14**
Mean	**14.17**	**11.54**	**10.46**	**8.37**		**16.16**	**17.66**	**19.16**	**20.95**	

	*L** S.Em±	*L** CD (1%)	*a** S.Em±	*a** CD (1%)
Treatments	**0.27**	**NS**	**0.20**	**0.78**
Storage period	**0.19**	**0.72**	**0.14**	**0.55**
Interaction (T×S)	**0.54**	**NS**	**0.41**	**NS**

UPJ - Uninoculated pomegranate juice
PJ - Pomegranate juice
KJ - Kokum juice

***La* -** *Lactobacillus acidophilus* (2903)
***Lp* -** *Lactobacillus plantarum* (2373)
***Ld* -** *Lactobacillus delbrueckii* (2025)

DAS – Days after storage
NS – Non-signifiant

period (14.17 and 13.49 respectively). Least score for $L*$ and $b*$ values were noted 45 DAS (8.37 and 6.96 respectively). The $L*$ and $b*$ values decreased as the storage period advanced where as $a*$ value of beverage increased during storage.

The interaction between the treatments and storage period on $L*$ $a*$ and $b*$ values were not found to be significant. However, maximum score for $L*$ $a*$ and $b*$ values was observed in T_1S_1 (16.15), $T_{11}S_4$ (32.00), and $T_{11}S_1$ (16.41) respectively.

4.2.11 Lactic acid bacterial population (cfu/ml)

The probiotic lactic acid bacterial population in the fermented beverage with and without kokum juice of different treatments during initial period and after 30 days of storage is presented in Table 9. The lactic acid bacterial population of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

The mean bacterial population of the treatments varied between 0 and 2.91×10^6 cfu/ml. The results showed that population varied significantly. The highest lactic acid bacterial population were obtained in T_6 (2.91×10^6 cfu/ml) which was on par with T_8 (2.58×10^6 cfu/ml) followed by T_5 and T_{11} (2.33×10^6 cfu/ml each). Lactic acid bacterial population was not detected in uninoculated pomegranate juice.

Lactic acid bacterial population were reduced drastically as the storage period advanced. Significantly, the highest population was observed at initial period (2.79×10^6 cfu/ml) and the least at 30 DAS (0.58×10^6 cfu/ml).

The interaction between the treatments and storage period were found to be significantly different. Among interactions, the highest population was observed in T_5S_1 (4.50×10^6 cfu/ml) which was on par with T_8S_1 (3.83×10^6 cfu/ml). However, after 30 days of storage, highest population was observed in T_6S_2 (2×10^6 cfu/ml) followed by T_8S_2 (1×10^6 cfu/ml). Among fermented beverages, the lowest population was recorded in $T_{10}S_2$ (0.06×10^6 cfu/ml).

Table 8: Changes in colour (*b) value fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period**

Treatments			*b**				Mean
			S1 (Initial)	S2 (15DAS)	S3 (30DAS)	S4 (45DAS)	
T_1	-	100% UPJ	12.34	8.84	7.03	5.70	**8.48**
T_3	-	100% PJ + *Lp*	11.88	10.74	9.33	7.29	**9.81**
T_5	-	85% PJ + 15% KJ + *La*	13.04	10.83	9.05	6.92	**9.96**
T_6	-	85% PJ + 15% KJ + *Lp*	12.91	11.53	8.80	7.26	**10.12**
T_7	-	85% PJ + 15% KJ + *Ld*	12.92	11.02	9.87	7.65	**10.36**
T_8	-	75% PJ + 25% KJ + *La*	14.67	11.40	9.10	6.73	**10.47**
T_{10}	-	75% PJ + 25% KJ + *Ld*	13.77	11.86	9.76	6.81	**10.55**
T_{11}	-	65% PJ + 35% KJ + *La*	16.41	14.84	9.98	7.37	**12.15**
Mean			**13.49**	**11.38**	**9.11**	**6.96**	

	S.Em±	CD (1%)
Treatments	**0.27**	**1.05**
Storage period	**0.19**	**0.74**
Interaction (T×S)	**0.54**	**NS**

UPJ - Uninoculated pomegranate juice
PJ - Pomegranate juice
KJ - Kokum juice
***La* -** *Lactobacillus acidophilus* (2903)
***Lp* -** *Lactobacillus plantarum* (2373)
***Ld* -** *Lactobacillus delbrueckii* (2025)
DAS – Days after storage
NS – Non-signifiant

Table 9: Changes in lactic acid bacterial population (10^6 cfu/ml) in fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period

Treatments	Lactic acid bacterial population (10^6 cfu/ml)		Mean
	S_1 (Initial)	S_2 (30DAS)	
T_1 - 100% UPJ	ND	ND	**ND**
T_3 - 100% PJ + *Lp*	3.66	0.83	**2.25**
T_5 - 85% PJ + 15% KJ + *La*	4.50	0.16	**2.33**
T_6 - 85% PJ + 15% KJ + *Lp*	3.83	2.00	**2.91**
T_7 - 85% PJ + 15% KJ + *Ld*	1.50	0.10	**0.80**
T_8 - 75% PJ + 25% KJ + *La*	4.16	1.00	**2.58**
T_{10} - 75% PJ + 25% KJ + *Ld*	0.50	0.06	**0.28**
T_{11} - 65% PJ + 35% KJ + *La*	4.16	0.50	**2.33**
Mean	**2.79**	**0.58**	
	S.Em±	**CD (1%)**	
Treatments	**0.17**	**0.53**	
Storage period	**0.08**	**0.26**	
Interaction (T×S)	**0.24**	**0.75**	

UPJ - Uninoculated pomegranate juice
PJ - Pomegranate juice
KJ - Kokum juice

***La* -** *Lactobacillus acidophilus* (2903)
***Lp* -** *Lactobacillus plantarum* (2373)
***Ld* -** *Lactobacillus delbrueckii* (2025)

DAS – Days after storage
Cfu – Colony forming unit
ND – Not detected

4.2.12 Sensory evaluation

4.2.12.1 Colour

The data on the sensory scores for colour of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period is presented in Table 10. The colour scores of beverage was not found be significant among the treatments. However, significant differences were observed among the storage period and in interaction between treatments and storage period.

Irrespective of storage periods, the mean colour score of the treatments ranged between 7.67 and 7.83. Numerically highest score was recorded in T_6 (7.83) and the least score in T_{10} (7.67).

The effect of storage period on the colour of beverage was found to be significant. Maximum score was recorded at initial period (7.90) and lowest score was noticed at 45 DAS.

The interaction between the treatments and storage period were found to be significantly different. The maximum score for colour was recorded in T_1S_1, T_1S_2 and T_5S_1 (7.95 each). However, minimum score was observed in T_1S_4 (7.46).

4.2.12.2 Flavour

The data on the sensory scores for flavour of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period is presented in Table 10. The flavour scores of beverage was found be significant among storage period and interaction between treatments and storage period. However, it was not found to be significant among treatments.

Irrespective of storage periods, the mean flavour score of the treatments varied from 7.58 to 7.77. Numerically, highest score was noticed in T_6 (7.77) and the least score in T_{10} (7.58).

The effect of storage period on the flavour of beverage was found to be significant. Maximum score for flavour was recorded at initial period (7.84) followed by 45 DAS (7.63).

Table 10: Organoleptic evaluation for colour and flavour of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period

Treatments	Colour*				Mean	Flavour*				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)		S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 100% UPJ	7.95	7.95	7.50	7.46	**7.71**	7.95	7.93	7.48	7.45	**7.70**
T_3 - 100% PJ + *Lp*	7.91	7.80	7.83	7.73	**7.82**	7.91	7.65	7.76	7.70	**7.75**
T_5 - 85% PJ + 15% KJ + *La*	7.95	7.81	7.83	7.68	**7.82**	7.86	7.70	7.73	7.68	**7.74**
T_6 - 85% PJ + 15% KJ + *Lp*	7.93	7.80	7.88	7.71	**7.83**	7.91	7.78	7.70	7.71	**7.77**
T_7 - 85% PJ + 15% KJ + *Ld*	7.93	7.80	7.83	7.63	**7.80**	7.81	7.68	7.77	7.63	**7.72**
T_8 - 75% PJ + 25% KJ + *La*	7.91	7.66	7.83	7.71	**7.78**	7.75	7.61	7.83	7.71	**7.72**
T_{10} - 75% PJ + 25% KJ + *Ld*	7.90	7.53	7.53	7.71	**7.67**	7.64	7.73	7.51	7.43	**7.58**
T_{11} - 65% PJ + 35% KJ + *La*	7.75	7.81	7.71	7.65	**7.73**	7.90	7.53	7.53	7.71	**7.67**
Mean	**7.90**	**7.77**	**7.74**	**7.66**		7.84	7.70	**7.66**	**7.63**	

	Colour S.Em±	Colour CD (1%)	Flavour S.Em±	Flavour CD (1%)
Treatments	**0.03**	**NS**	**0.037**	**NS**
Storage period	**0.02**	**0.09**	**0.026**	**0.10**
Interaction (T×S)	**0.07**	**0.28**	**0.075**	**0.28**

UPJ - Uninoculated pomegranate juice
PJ - Pomegranate juice
KJ - Kokum juice

***La* -** *Lactobacillus acidophilus* (2903)
***Lp* -** *Lactobacillus plantarum* (2373)
***Ld* -** *Lactobacillus delbrueckii* (2025)

DAS – Days after storage
NS - Non signifiant
* - Score out of 9

In the interaction between the treatments and storage period, the highest score for flavour was recorded in T_1S_1 (7.95) which was on par with T_1S_2 (7.93), T_3S_1 and T_6S_1 (7.91 each). The lowest score for flavour was observed in $T_{10}S_4$ (7.43) which was on par with T_1S_4 (7.45).

4.2.12.3 Taste

The data on the sensory scores for taste of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period is presented in Table 11. The taste scores of beverage were found to be significant among the treatments, storage period and their interaction.

Irrespective of storage periods, the mean taste score of the treatments ranged between 7.82 and 7.14. Highest score was recorded in T_6 (7.82) and the least score in T_{10} (7.14).

The effect of storage period on the taste of beverage was found to be significant. Maximum score was recorded at initial period (7.65) which was on par with 15 DAS (7.64).

In the interaction between the treatments and storage period, the highest score for taste was recorded in and T_6S_2 (8.08 each). The lowest score was observed in $T_{10}S_4$ (6.75).

4.2.12.4 Consistency

The data on the sensory scores for consistency of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period is presented in Table 11. The consistency scores of beverage was not found be significant among the treatments and in interaction between treatments and storage period. However, significant differences were observed among the storage period.

Irrespective of storage periods, the mean consistency score of the treatments ranged between 7.60 and 7.97. Highest score was recorded in T_6 (7.97) and the least score in T_{10} (7.53).

Table 11: Organoleptic evaluation for taste and consistency of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period

Treatments	Taste*				Mean	Consistency*				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)		S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 100% UPJ	7.56	7.36	6.98	6.85	**7.19**	7.70	8.03	7.40	7.26	**7.60**
T_3 - 100% PJ + *Lp*	7.96	7.43	7.11	6.88	**7.35**	7.86	7.63	8.23	7.56	**7.82**
T_5 - 85% PJ + 15% KJ + *La*	7.85	7.93	7.55	7.08	**7.60**	8.13	7.76	7.66	7.33	**7.72**
T_6 - 85% PJ + 15% KJ + *Lp*	7.95	8.08	7.76	7.51	**7.82**	8.43	8.10	7.93	7.43	**7.97**
T_7 - 85% PJ + 15% KJ + *Ld*	7.35	7.43	7.15	6.80	**7.18**	8.10	7.50	7.23	7.46	**7.57**
T_8 - 75% PJ + 25% KJ + *La*	7.75	7.91	7.65	7.35	**7.66**	8.00	7.66	7.55	7.20	**7.60**
T_{10} - 75% PJ + 25% KJ + *Ld*	7.30	7.43	7.08	6.75	**7.14**	8.06	7.60	7.56	6.90	**7.53**
T_{11} - 65% PJ + 35% KJ + *La*	7.50	7.56	7.16	6.77	**7.24**	8.10	7.60	7.46	7.26	**7.60**
Mean	**7.65**	**7.64**	**7.30**	**7.00**		**8.05**	**7.73**	**7.63**	**7.30**	

	Taste S.Em±	Taste CD (1%)	Consistency S.Em±	Consistency CD (1%)
Treatments	**0.01**	**0.07**	**0.09**	**NS**
Storage period	**0.01**	**0.05**	**0.06**	**0.23**
Interaction (T×S)	**0.03**	**0.14**	**0.18**	**NS**

UPJ - Uninoculated pomegranate juice
PJ - Pomegranate juice
KJ - Kokum juice

***La* -** *Lactobacillus acidophilus* (2903)
***Lp* -** *Lactobacillus plantarum* (2373)
***Ld* -** *Lactobacillus delbrueckii* (2025)

DAS – Days after storage
NS - Non signifiant
* - Score out of 9

The effect of storage period on the consistency of beverage was found to be significant. Maximum score was recorded initial period (8.05) followed by 15 DAS (7.73). Minimum score was observed at 45 DAS (7.30).

In the interaction between the treatments and storage period, the highest score for consistency was recorded in T_6S_1 (8.43) However, lowest score was observed in $T_{10}S_4$ (6.90).

4.2.12.5 Overall acceptability

The data on the sensory scores for overall acceptability of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period is presented in Table 12, Plate 7 and 8) The overall acceptability scores of beverage was found to be significant among the treatments, storage period. However it was not found to be significant with their interaction.

Irrespective of storage periods, the mean overall acceptability score of the treatments ranged between 7.48 and 7.85. Highest score was recorded in T_6 (7.85) and the least score in T_{10} (7.48).

The effect of storage period on the overall acceptability of beverage was found to be significant. Maximum score was recorded initial period (7.85) and least score was observed at 45 DAS (7.40).

In the interaction between the treatments and storage period, the highest score for overall acceptability was recorded in T_6S_1 (8.09). The lowest score was observed in $T_{10}S_4$ (7.20).

4.3 Effect of different prebiotics on fermentation of pomegranate juice with kokum blend using probiotic lactic acid bacteria during storage

4.3.1 Citric acid (%)

The data pertaining to citric acid content (%) of fermented beverage of blended pomegranate with prebiotics as influenced by treatments and storage period up to 45

Table 12: Organoleptic evaluation for overall acceptability of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period

Treatments	Overall acceptability*				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 100% UPJ	7.74	7.87	7.33	7.26	**7.55**
T_3 - 100% PJ + *Lp*	7.79	7.76	7.73	7.47	**7.69**
T_5 - 85% PJ + 15% KJ + *La*	7.93	7.78	7.69	7.44	**7.71**
T_6 - 85% PJ + 15% KJ + *Lp*	8.09	7.9	7.82	7.59	**7.85**
T_7 - 85% PJ + 15% KJ + *Ld*	7.82	7.58	7.49	7.38	**7.57**
T_8 - 75% PJ + 25% KJ + *La*	7.89	7.67	7.71	7.49	**7.69**
T_{10} - 75% PJ + 25% KJ + *Ld*	7.76	7.54	7.42	7.2	**7.48**
T_{11} - 65% PJ + 35% KJ + *La*	7.82	7.61	7.46	7.35	**7.56**
Mean	**7.85**	**7.71**	**7.58**	**7.40**	
	S.Em±		**CD (1%)**		
Treatments	**0.02**		**0.11**		
Storage period	**0.02**		**0.07**		
Interaction (T×S)	**0.05**		**NS**		

UPJ - Uninoculated pomegranate juice
PJ - Pomegranate juice
KJ - Kokum juice

***La* -** *Lactobacillus acidophilus* (2903)
***Lp* -** *Lactobacillus plantarum* (2373)
***Ld* -** *Lactobacillus delbrueckii* (2025)

DAS – Days after storage
NS - Non signifiant
* - Score out of 9

days are presented in Table 13. The citric acid per cent of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

The mean citric acid per cent over different storage period ranged from 0.74 to 0.84 per cent. The highest citric acid was recorded in T_7 (0.84%) followed by T_6 (0.82%) and the lowest in T_1 (0.74 %).

Citric acid content of fermented beverage increased as the storage period advanced. Significantly, the highest citric acid content was observed 45 DAS (0.82 %) and the least at initial period (0.76%).

The interaction between the treatments and storage period were found to be significantly different. The maximum citric acid content was noted in T_7S_4 (0.87%) which was on par with T_7S_3 (0.85%). The least score was observed in T_1S_1 (0.71%).

4.3.2 Lactic acid (%)

The data with respect to lactic acid content (%) of fermented beverage of blended pomegranate with prebiotics as influenced by treatments stored up to 45 days is presented in Table 13. The lactic acid percent of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

The mean lactic acid content of the treatments varied between 0.99 and 1.32 per cent. The highest lactic acid was recorded in T_7 (1.32%) followed by T_4 (1.16%), T_5 and T_6 (1.15% each) and the lowest in T_1 (0.99%).

Lactic acid content of all the treatments increased as the storage period advanced. Significantly, the highest lactic acid content was recorded at 45 DAS (1.20%) and the least at initial period (1.07%).

The interaction between the treatments and storage period were found to be significantly different. The maximum lactic acid content was recorded in T_7S_4 (1.48%) and lowest in T_1S_1 (0.92%).

Table 13: Effect of prebiotics on citric acid (%) and lactic acid (%) content of fermented pomegranate beverage blended with kokum juice as influenced by treatments and storage period

Treatments	Citric acid (%)				Mean	Lactic acid (%)				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)		S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 85% PJ + 15% KJ + *Lp*	0.71	0.72	0.75	0.77	**0.74**	0.92	0.95	1.02	1.08	**0.99**
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H	0.75	0.75	0.77	0.78	**0.76**	1.05	1.10	1.14	1.16	**1.11**
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H	0.73	0.75	0.79	0.83	**0.78**	1.06	1.12	1.18	1.17	**1.13**
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H	0.78	0.80	0.81	0.83	**0.81**	1.09	1.15	1.19	1.21	**1.16**
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L	0.78	0.79	0.81	0.83	**0.80**	1.10	1.13	1.15	1.20	**1.15**
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L	0.78	0.81	0.83	0.84	**0.82**	1.13	1.15	1.15	1.19	**1.15**
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L	0.80	0.83	0.85	0.87	**0.84**	1.14	1.30	1.37	1.48	**1.32**
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H +2% L	0.74	0.77	0.77	0.79	**0.77**	1.04	1.09	1.10	1.12	**1.09**
Mean	**0.76**	**0.78**	**0.80**	**0.82**		**1.07**	**1.12**	**1.16**	**1.20**	

	Citric acid S.Em±	Citric acid CD (1%)	Lactic acid S.Em±	Lactic acid CD (1%)
Treatments	0.003	0.01	0.006	0.02
Storage period	0.002	0.008	0.004	0.01
Interaction (T×S)	0.006	0.02	0.013	0.04

PJ - Pomegranate juice
KJ - Kokum juice
***Lp* -** *Lactobacillus plantarum* (2373)
***H* -** Honey
L - Lactose
NS - Non signifiant

4.3.3 pH

The data with respect to pH of fermented beverage of blended pomegranate with prebiotics as influenced by treatments stored up to 45 days is presented in Table 14. The pH of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

Irrespective of storage periods, the mean pH of the treatments ranged from 2.79 to 3.18. The lowest pH was recorded in T_7 (2.79) followed by T_6 (2.88), T_4 (2.93) and the highest in T_1 (3.18).

pH of fermented beverage decreased as the storage period advanced up to 45 days. The lowest pH was recorded at 45 DAS (2.78) and the highest at initial period (3.29).

The interaction between the treatments and storage period were not found to be significant. Numerically lowest pH was observed in T_7S_4 (2.60) and maximum pH in T_8S_1 (3.38) followed by T_1S_1 (3.37).

4.3.4 TSS (° brix)

The data with respect to total soluble solid content (° brix) of fermented beverage of blended pomegranate with prebiotics as influenced by treatments stored up to 45 days is presented in Table 14. The TSS of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

Irrespective of storage periods, the mean TSS of the treatments varied between 10.79 and 11.88 (° brix). The lowest TSS was observed in T_7 (10.79° brix) followed by T_6 (10.96° brix). The highest TSS was observed in T_2 (11.88° brix) followed by T_3 and T_8 (11.83° brix each) and T_4 (11.76° brix).

TSS of all treatments decreased as the storage period advanced except in T_1 (control) where increasing trend was observed. Significantly, the lowest TSS content was observed at 45 DAS (11.22° brix) and the highest was recorded during initial period (11.58° brix).

Table 14: Effect of prebiotics on pH and TSS (° brix) content of fermented pomegranate beverage blended with kokum juice as influenced by treatments and storage period

Treatments	pH				Mean	TSS (° brix)				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)		S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 85% PJ + 15% KJ + *Lp*	3.37	3.24	3.16	2.96	**3.18**	11.11	11.01	10.94	10.89	**10.99**
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H	3.45	3.24	2.98	2.87	**3.13**	12.20	11.91	11.77	11.65	**11.88**
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H	3.37	3.10	2.84	2.76	**3.02**	12.07	11.89	11.74	11.61	**11.83**
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H	3.25	2.98	2.79	2.71	**2.93**	12.03	11.76	11.65	11.61	**11.76**
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L	3.29	3.08	2.90	2.81	**3.01**	11.13	11.01	10.95	10.88	**10.99**
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L	3.17	2.97	2.75	2.65	**2.88**	11.10	11.00	10.89	10.84	**10.96**
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L	3.06	2.79	2.71	2.60	**2.79**	10.91	10.80	10.75	10.71	**10.79**
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H +2% L	3.38	3.23	3.02	2.87	**3.12**	12.10	11.90	11.70	11.61	**11.83**
Mean	**3.29**	**3.08**	**2.89**	**2.78**		**11.58**	**11.41**	**11.3**	**11.22**	
	S.Em±		**CD (1%)**			**S.Em±**		**CD (1%)**		
Treatments	0.01		0.06			0.009		0.03		
Storage period	0.01		0.04			0.006		0.02		
Interaction(T×S)	0.03		**NS**			0.01		0.06		

PJ - Pomegranate juice
KJ - Kokum juice
***Lp* -** *Lactobacillus plantarum* (2373)
***H* -** Honey
L - Lactose
DAS – Days after storage
NS - Non signifiant

Among the interaction between the treatments and storage period, lowest TSS content was observed in T_7S_4 (10.71° brix) and maximum TSS content in T_2S_1 (12.20° brix).

4.3.5 Total sugar (%)

The data on the total sugar content of fermented beverage of blended pomegranate with prebiotics as influenced by treatments stored up to 45 days is presented in Table 15. The total sugar content of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

Irrespective of storage periods, the mean total sugar content of the treatments ranged from 10.74 to 11.86 per cent. The lowest total sugar content was recorded in T_7 (10.74%) followed by T_1 (10.79%), T_4 (10.81%) and the highest in T_2 (11.86%) on par with T_8 (11.87%).

Total sugar content of fermented beverage decreased as the storage period advanced. The lowest total sugar content was recorded at 45 DAS (11.17%) and the highest at initial period (11.49%).

The interaction between the treatments and storage period were found to be significantly different. The minimum total sugar content was observed in T_7S_4 (10.53%) which were on par with T_7S_3 (10.60%).

4.3.6 Reducing sugar (%)

The data on the reducing sugar content of fermented beverage of blended pomegranate with prebiotics as influenced by treatments stored up to 45 days is presented in Table 15. The reducing sugar content of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

Irrespective of storage periods, the mean reducing sugar content of the treatments ranged from 3.51 to 3.86 per cent. The lowest reducing sugar content was recorded in T_7 (3.51%) followed by T_5 and T_6 (3.58%) and the highest in T_1 (3.86%).

Table 15: Effect of prebiotics on total sugar (%) and reducing sugar (%) of fermented pomegranate beverage blended with kokum juice as influenced by treatments and storage period

Treatments	Total sugar (%)				Mean	Reducing sugar (%)				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)		S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 85% PJ + 15% KJ + *Lp*	11.00	10.81	10.72	10.65	**10.79**	4.05	3.87	3.79	3.73	**3.86**
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H	12.15	11.87	11.76	11.67	**11.86**	3.92	3.86	3.77	3.72	**3.82**
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H	11.99	11.90	11.8	11.69	**11.84**	3.87	3.79	3.71	3.59	**3.74**
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H	11.96	11.87	11.75	11.68	**11.81**	3.80	3.69	3.60	3.48	**3.64**
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L	11.00	10.86	10.77	10.68	**10.82**	3.73	3.64	3.55	3.41	**3.58**
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L	10.99	10.84	10.72	10.66	**10.80**	3.67	3.62	3.55	3.47	**3.58**
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L	10.94	10.88	10.6	10.53	**10.74**	3.63	3.55	3.47	3.40	**3.51**
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H +2% L	11.94	11.89	11.84	11.80	**11.87**	3.93	3.88	3.82	3.77	**3.85**
Mean	**11.49**	**11.36**	**11.24**	**11.17**		**3.82**	**3.74**	**3.66**	**3.57**	

	Total sugar S.Em±	Total sugar CD (1%)	Reducing sugar S.Em±	Reducing sugar CD (1%)
Treatments	**0.01**	**0.04**	**0.005**	**0.02**
Storage period	**0.007**	**0.03**	**0.004**	**0.01**
Interaction(T×S)	**0.02**	**0.08**	**0.01**	**0.04**

PJ - Pomegranate juice
KJ - Kokum juice

***Lp* -** *Lactobacillus plantarum* (2373)
***H* -** Honey
L - Lactose

DAS – Days after storage

Reducing sugar content of fermented beverage decreased as the storage period advanced. The lowest reducing sugar content was noticed 45 DAS (3.57%) and the highest at initial period (3.82%).

The interaction between the treatments and storage period were found to be significantly different. The minimum reducing sugar content was observed in T_7S_4 (3.40%) which was on par with T_5S_4 (3.41%) and maximum reducing sugar content was noticed in T_1S_1 (4.05%).

4.3.7 Antioxidant activity (%)

The data with respect to antioxidant activity of fermented beverage of blended pomegranate with prebiotics as influenced by treatments and storage period up to 45 days are presented in Table 16. The antioxidant activity of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

Irrespective of storage periods, the mean antioxidant activity of the treatments ranged from 76.51 to 80.40 per cent. The highest antioxidant activity was observed in T_4 (80.40%) followed by T_3 (78.95%), T_2 (78.57%) and the lowest was recorded in T_1 (76.51%) which was on par with T_5 (76.88%).

Antioxidant activity of the fermented beverage increased up to 15th days of storage and decreasing trend was observed after 15 days in all the treatments. Significantly, the highest antioxidant activity was recorded at 15 DAS (83.43%) and the least at 45 DAS (70.58%).

The interaction between the treatments and storage period were found to be significantly different. The maximum antioxidant activity was recorded in T_4S_2 (85.81%) and lowest in T_1S_4 and T_5S_4 (68.72% each).

4.3.8 Total phenol (mg GAE/100 ml)

The data with pertaining to total phenol content fermented beverage blended pomegranate with prebiotics as influenced by treatments and storage period up to 45 days are presented in Table 16. The total phenol content of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

Table 16: Effect of prebiotics on antioxidant activity (%) and total phenol (mg GAE/100 ml) content of fermented pomegranate beverage blended with kokum juice as influenced by treatments and storage period

Treatments	Antioxidant activity (%)				Mean	Total phenol (mg GAE/100 ml)				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)		S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 85% PJ + 15% KJ + *Lp*	79.88	82.36	75.06	68.72	**76.51**	242.39	246.08	241.97	238.48	**242.23**
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H	82.22	83.46	76.85	71.76	**78.57**	249.21	251.78	246.46	244.45	**247.97**
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H	82.50	84.15	76.99	72.17	**78.95**	254.25	257.99	254.58	250.73	**254.38**
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H	84.01	85.81	78.23	73.55	**80.40**	257.78	261.00	257.52	254.61	**257.73**
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L	80.85	83.05	74.92	68.72	**76.88**	244.45	247.78	243.91	238.54	**243.67**
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L	81.26	82.64	75.75	70.38	**77.5**	245.48	248.53	244.79	240.49	**244.82**
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L	82.09	83.60	75.61	70.24	**77.88**	247.50	249.03	243.57	239.59	**244.92**
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H +2%L	80.02	82.36	75.33	69.14	**76.71**	249.46	251.23	247.02	240.41	**247.03**
Mean	**81.60**	**83.43**	**76.09**	**70.58**		**248.81**	**251.68**	**247.48**	**243.41**	

	S.Em±	CD (1%)	S.Em±	CD (1%)
Treatments	**0.43**	**1.62**	**0.33**	**1.25**
Storage period	**0.30**	**1.14**	**0.23**	**0.88**
Interaction (T×S)	**0.86**	**3.24**	**0.66**	**2.51**

PJ - Pomegranate juice
KJ - Kokum juice

***Lp* -** *Lactobacillus plantarum* (2373)
***H* -** Honey
L - Lactose

DAS – Days after storage
GAE – Gallic acid equivalent

The mean total phenol content of the treatments ranged between 242.23 and 257.73 mg GAE/100ml. Significantly, the highest total phenol content was recorded in T_4 (257.73 mg GAE/100 ml) followed by T_3 (254.38 mg GAE/100 ml), T_2 (247.97 mg GAE/100 ml), T_8 (247.03 mg GAE/100 ml) and the lowest total phenol content was recorded in T_6 (242.23 mg GAE/100 ml).

Significant difference was observed with respect to total phenol content of fermented beverage during stored period of 45 days. Maximum score was recorded at 30 DAS (251.68 mg GAE/100 ml) which was significantly different from all the storage periods. Significantly, lowest score was recorded at 45 DAS (243.41mg GAE/100 ml).

In case of interaction effect between treatments and storage period, increase in the total phenol content in all the treatments up to 15^{th} days of storage was noticed but decreasing trend was observed after 15 days of storage in all the treatments. The highest total phenol content was recorded in T_4S_2 (261.00 mg GAE/100 ml) and lowest score was observed in T_1S_4 (238.48 mg GAE/100 ml).

4.3.9 Ascorbic acid (mg/100 ml) (Vitamin C)

The data with respect to ascorbic acid content of fermented beverage of blended pomegranate with prebiotics as influenced by treatments stored up to 45 days are presented in Table 17. The vitamin C content of fermented beverage varied significantly among the storage periods, treatments and with their interactions.

Irrespective of storage periods, the mean ascorbic acid of the treatments varied from 6.72 to 10.52 mg/100 ml. The highest ascorbic acid content was recorded in T_4 (10.52 mg/100 ml) followed by T_3 (10.12 mg/100 ml), T_2 (9.69 mg/100 ml) and the lowest in T_1 (6.72 mg/100 ml).

Ascorbic acid content of fermented beverage decreased as the storage period advanced. Significantly, the highest vitamin C was observed at initial period (9.42 mg/100ml) and the least at 45 DAS (7.12 mg/100 ml).

The interaction between the treatments and storage period were found to be significantly different. The maximum ascorbic acid was observed in T_4S_1 (12.10

Table 17: Effect of prebiotics on ascorbic acid (mg/ 100 ml) and protein (%) content of fermented pomegranate beverage blended with kokum juice as influenced by treatments and storage period

Treatments	Ascorbic acid (mg/ 100 ml)				Mean	Protein (%)				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)		S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 85% PJ + 15% KJ + *Lp*	8.10	6.99	6.19	5.61	**6.72**	0.20	0.19	0.16	0.11	**0.17**
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H	11.88	10.28	8.76	7.87	**9.69**	1.65	1.61	1.57	1.03	**1.46**
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H	12.09	10.61	9.02	8.79	**10.12**	1.85	1.83	1.81	1.60	**1.77**
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H	12.1	11.22	9.76	8.99	**10.52**	2.21	2.00	1.83	1.17	**1.93**
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L	7.86	7.18	6.79	6.37	**7.05**	0.31	0.21	0.19	0.16	**0.22**
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L	7.81	7.19	6.87	6.50	**7.09**	0.32	0.26	0.21	0.16	**0.24**
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L	7.85	7.29	6.82	6.57	**7.13**	0.33	0.29	0.25	0.19	**0.26**
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H +2%L	7.70	7.24	6.83	6.23	**7.00**	0.32	0.23	0.18	0.13	**0.21**
Mean	**9.42**	**8.50**	**7.63**	**7.12**		**0.90**	**0.83**	**0.77**	**0.63**	

	Ascorbic acid S.Em±	Ascorbic acid CD (1%)	Protein S.Em±	Protein CD (1%)
Treatments	**0.03**	**0.14**	**0.01**	**0.06**
Storage period	**0.02**	**0.10**	**0.01**	**0.04**
Interaction (T×S)	**0.07**	**0.28**	**0.03**	**0.13**

PJ - Pomegranate juice
KJ - Kokum juice
***Lp* -** *Lactobacillus plantarum* (2373)
***H* -** Honey
L - Lactose
DAS – Days after storage
GAE – Gallic acid equivalent

mg/100ml) which was on par with T_3S_1 (12.09 mg/100 ml). The least score was observed in T_1S_4 (5.61 mg/100 ml).

4.3.10 Protein content (%)

The data with respect to protein content of fermented beverage of blended pomegranate with prebiotics as influenced by treatments and storage period up to 45 days are presented in Table 17. The protein content of fermented beverage varied significantly among the storage periods, treatments and with their interactions

Irrespective of storage periods, the mean protein content of the treatments ranged from 0.17 to 1.93%. The highest protein content was recorded in T_4 (1.93%) followed by T_3 (1.77%), T_2 (1.46%) and the lowest in T_1 (0.17%).

Protein content of beverage decreased as the storage period advanced. Significantly, the highest protein content was observed at initial period (0.90%) and the least at 45 DAS (0.63%).

The interaction between the treatments and storage period were found to be significantly different. The maximum protein content was observed in T_4S_1 (2.21%) and minimum was observed in T_1S_4 (0.11%).

4.3.11 Instrumental colour analysis (*L* a* b** values)

The data on the L^*, a^*, b^* colour values of fermented beverage of blended pomegranate with prebiotics as influenced by treatments and storage period up to 45 days are presented in Table 18 and 19. L^*, a^*, b^* values varied significantly among the treatments and storage period but not with their interaction.

Among different treatments, the lowest score for lightness (L^* value) was observed in T_1 (11.56) and highest in T_7 (14.43). Highest score for a^* and b^* values were noted in T_2 (17.85) and T_4 (12.83) respectively.

The effect of storage period on L^*, a^*, b^* values of fermented beverage was found to be significant. Highest score for L^* and b^* values were observed at initial period (17.45 and 14.11 respectively). Least score for L^* and b^* values were noted at 45 DAS (9.55 and 7.25 respectively). The L^* and b^* values decreased as the storage period

Table 18: Effect of prebiotics on colour (*L and *a**) value of fermented pomegranate beverage blended with kokum juice as influenced by treatments and storage period**

Treatments	*L**				Mean	*a**				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)		S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 85% PJ + 15% KJ + *Lp*	15.48	13.78	9.73	7.26	**11.56**	13.01	14.89	15.64	18.4	**15.48**
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H	16.41	15.19	10.84	8.703	**12.79**	15.85	16.42	17.89	21.24	**17.85**
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H	17.88	14.82	11.11	8.58	**13.10**	13.83	14.29	17.16	19.62	**16.22**
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H	16.80	15.72	11.25	9.40	**13.29**	13.44	14.79	16.47	19.25	**15.99**
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L	17.77	15.25	13.29	11.14	**14.36**	12.96	13.89	15.08	16.33	**14.56**
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L	18.87	15.28	12.67	10.68	**14.37**	12.85	13.41	14.7	16.49	**14.36**
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L	18.76	15.79	12.28	10.88	**14.43**	11.30	12.58	14.42	18.2	**14.12**
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H +2% L	17.63	14.49	12.30	9.75	**13.54**	12.25	13.53	14.59	16.68	**14.26**
Mean	**17.45**	**15.04**	**11.68**	**9.55**		**13.18**	**14.22**	**15.74**	**18.27**	

	*L** S.Em±	*L** CD (1%)	*a** S.Em±	*a** CD (1%)
Treatments	**0.20**	**0.75**	**0.20**	**0.77**
Storage period	**0.14**	**0.53**	**0.14**	**0.54**
Interaction(T×S)	**0.40**	**NS**	**0.41**	**NS**

PJ - Pomegranate juice
KJ - Kokum juice

***Lp* -** *Lactobacillus plantarum* (2373)
***H* -** Honey
L - Lactose

DAS – Days after storage
NS – Non significant

advanced where as redness ($a*$ value) of beverage increased during storage with highest score at 45 DAS (18.27) and lowest at initial period (13.18).

The interaction between the treatments and storage period on $L*$, $a*$ and $b*$ values was not found to be significant. However, highest score for $L*$ $a*$ and $b*$ values was observed in T_6S_1 (18.87), T_2S_4 (21.24) and T_4S_1 (16.36) respectively.

4.3.12 Lactic acid bacterial population (cfu/ml)

The probiotic lactic acid bacterial population in the fermented beverage of blended pomegranate with prebiotics as influenced by treatments and storage period up to 45 days are presented in Table 20. The probiotic lactic acid bacterial population in fermented beverage varied significantly among the storage periods, treatments and with their interactions.

The mean bacterial population of the treatments varied between 1.79 and 5.79×10^6 cfu/ml. The results showed that population varied significantly. The highest probiotic lactic acid bacterial population was observed in T_7 (5.79×10^6 cfu/ml) and lowest was in T_1 (1.79×10^6 cfu/ml).

Lactic acid bacteria population reduced drastically as the storage period advanced. Significantly, the highest population was observed at initial period (7.86×10^6 cfu/ml) and bacterial population was not detected at the end of the storage period.

The interaction between the treatments and storage period were found to be significantly different. Initially, the highest population was observed in T_7S_1 (10.83×10^6 cfu/ml) and after 30 days of storage highest population was obtained in T_6S_3 and T_7S_3 (4.17×10^6 cfu/ml each) which were on par with T_5S_3 (4.16×10^6 cfu/ml) and T_4S_3 (3.80×10^6 cfu/ml) bacterial population was not detected in any of the treatment at 45 DAS.

4.3.13 Sensory evaluation

4.3.13.1 Colour

The data on the sensory scores for colour of fermented beverage of blended pomegranate with prebiotics as influenced by treatments and storage period up to 45

Table 19: Effect of prebiotics on colour (*b) value of fermented pomegranate beverage blended with kokum juice as influenced by treatments and storage period**

Treatments	*b**				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 85% PJ + 15% KJ + *Lp*	13.49	10.47	8.38	6.32	**9.66**
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H	13.98	12.49	10.24	7.74	**11.11**
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H	15.26	13.00	10.57	7.11	**11.48**
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H	16.36	14.13	12.43	8.40	**12.83**
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L	13.68	11.24	9.42	7.42	**10.44**
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L	13.28	11.73	9.51	6.27	**10.19**
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L	13.71	12.05	10.15	7.74	**10.91**
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H +2% L	13.14	11.52	9.54	6.98	**10.29**
Mean	**14.11**	**12.08**	**10.03**	**7.25**	

	S.Em±	CD (1%)
Treatments	**0.16**	**0.60**
Storage period	**0.11**	**0.42**
Interaction (T×S)	**0.32**	**NS**

PJ - Pomegranate juice
KJ - Kokum juice
***Lp* -** *Lactobacillus plantarum* (2373)
***H* -** Honey
L - Lactose
DAS – Days after storage
NS – Non significant

Table 20: Effect of prebiotics on lactic acid bacterial population (10^6 cfu/ml) of fermented pomegranate beverage blended with kokum juice as influenced by treatments and storage period

Treatments	Lactic acid bacterial population (10^6 cfu/ml)				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 85% PJ + 15% KJ + *Lp*	3.50	2.33	1.33	ND	**1.79**
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H	7.66	5.33	2.83	ND	**3.95**
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H	7.83	5.33	3.16	ND	**4.08**
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H	7.83	5.98	3.80	ND	**4.40**
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L	8.00	7.06	4.16	ND	**4.80**
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L	9.50	7.50	4.17	ND	**5.29**
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L	10.83	8.16	4.17	ND	**5.79**
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H +2%L	7.75	5.50	3.13	ND	**4.09**
Mean	**7.86**	**5.90**	**3.40**	**0.00**	
	S.Em±		**CD (1%)**		
Treatments	**0.10**		**0.37**		
Storage period	**0.07**		**0.26**		
Interaction (T×S)	**0.20**		**0.75**		

PJ - Pomegranate juice
KJ - Kokum juice
***Lp* -** *Lactobacillus plantarum* (2373)
***H* -** Honey
L - Lactose
DAS – Days after storage
ND – Not detected

days are presented in Table 21. The colour scores of beverage was not fount be significant among the treatments and in interaction between treatments and storage period. However, significant differences were observed among the storage period.

Irrespective of storage periods, the mean colour score of the treatments ranged between 7.66 and 7.80. Numerically highest score was recorded in T_3 (7.80) and the least score in T_7 (7.66).The effect of storage period on the colour of beverage was found to be significant. Maximum score was noticed at initial period (7.92) and minimum at 45 DAS (7.62).

The interaction between the treatments and storage period showed highest score for colour in T_6S_1 (8.11) and lowest score was recorded in T_6S_4 (7.31).

4.3.13.2 Flavour

The data on the sensory scores for flavour of fermented beverage of pomegranate blend with prebiotics as influenced by treatments and storage period up to 45 days are presented in Table 21. The flavour scores of beverage differed significantly among treatments, storage period and with their interaction.

Irrespective of storage periods, the mean flavour score of the treatments varied from 6.92 to 7.41. Highest score was noted in T_4 (7.41) which was on par with T_3 (7.38) and the least score in T_7 (6.92).

The effect of storage period on the flavour of beverage was found to be significant. Maximum score for flavour was recorded in S_1 (7.60) and minimum score in S_4 (6.67).

In the interaction between the treatments and storage period, the highest score for flavour was recorded in T_4S_1 (7.86) which were found statistically similar with T_3S_1 and T_4S_2 (7.78 each). The lowest score for flavour was observed in T_7S_4 (6.41) which were on par with T_6S_4 (6.45).

4.3.13.3 Taste

The data on the sensory scores for taste of fermented beverage of blended pomegranate with prebiotics as influenced by treatments and storage period up to 45

Table 21: Effect of prebiotics on colour and flavour (organoleptic evaluation) of fermented pomegranate beverage blended with kokum juice as influenced by treatments and storage period

Treatments	Colour*				Mean	Flavour*				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)		S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 85% PJ + 15% KJ + *Lp*	7.95	7.85	7.43	7.71	**7.72**	7.65	7.58	7.16	6.75	**7.28**
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H	7.93	7.83	7.76	7.63	**7.79**	7.70	7.63	7.25	6.78	**7.34**
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H	7.90	7.80	7.81	7.68	**7.80**	7.78	7.68	7.28	6.78	**7.38**
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H	7.98	7.80	7.80	7.56	**7.78**	7.86	7.78	7.15	6.85	**7.41**
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L	7.91	7.83	7.66	7.71	**7.78**	7.45	7.31	6.95	6.75	**7.11**
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L	8.11	7.50	7.96	7.31	**7.73**	7.48	7.35	6.98	6.45	**7.06**
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L	7.90	7.53	7.50	7.71	**7.66**	7.38	7.25	6.63	6.41	**6.92**
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H +2% L	7.81	7.71	7.71	7.65	**7.72**	7.55	7.45	7.01	6.61	**7.15**
Mean	**7.92**	**7.73**	**7.72**	**7.62**		**7.60**	**7.5**	**7.05**	**6.67**	

	Colour S.Em±	Colour CD (1%)	Flavour S.Em±	Flavour CD (1%)
Treatments	**0.04**	**NS**	**0.01**	**0.06**
Storage period	**0.03**	**0.12**	**0.01**	**0.04**
Interaction (T×S)	**0.9**	**NS**	**0.03**	**0.13**

PJ - Pomegranate juice
KJ - Kokum juice
***Lp* -** *Lactobacillus plantarum* (2373)
***H* -** Honey
L – Lactose
DAS – Days after storage
NS – Non significant
* - Score out of 9

days are presented in Table 22. The taste scores of beverage was fount be significant among the treatments, storage period and their interaction.

Irrespective of storage periods, the mean taste score of the treatments ranged between 6.71 and 7.73. Highest score was recorded in T_4 (7.73) and the least score in T_8 (6.71) which was found statistically similar with T_7 (6.76).

The effect of storage period on the taste of beverage was found to be significant. Maximum score was recorded at initial period (7.43) and lowest at 45 DAS (6.72).

In the interaction between the treatments and storage period, the highest score for taste was recorded in T_4S_1 (7.97) which were on par with T_3S_1 (7.93). The lowest score was observed in T_8S_4 (6.38) which were found statistically similar with T_7S_4 (6.45) and T_6S_4 (6.46).

4.3.13.4 Consistency

The data on the sensory scores for consistency of fermented beverage of blended pomegranate with prebiotics as influenced by treatments and storage period up to 45 days are presented in Table 22. The consistency scores of beverage was not fount be significant among the treatments and in interaction between treatments and storage period. However, significant differences were observed among the storage period.

Irrespective of storage periods, the mean consistency score of the treatments ranged between 7.71 and 7.83. Highest score was recorded in T_4 (7.83) and the least score in T_6 (7.71).

The effect of storage period on the consistency of beverage was found to be significant. Maximum score was recorded at initial period (7.91) and minimum at 45 DAS (7.67).

In the interaction between the treatments and storage period, the highest score for consistency was recorded in T_3S (7.95) and lowest score was observed in T_8S_4 (7.60).

Table 22: Effect of prebiotics on taste and consistency (organoleptic evaluation) of fermented pomegranate beverage blended with kokum juice as influenced by treatments and storage period

Treatments	Taste*				Mean	Consistency*				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)		S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 85% PJ + 15% KJ + *Lp*	7.38	7.21	6.96	6.55	**7.02**	7.91	7.80	7.75	7.63	**7.77**
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H	7.86	7.76	7.45	6.95	**7.5**	7.90	7.88	7.80	7.71	**7.82**
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H	7.93	7.80	7.63	7.13	**7.62**	7.95	7.83	7.81	7.68	**7.82**
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H	7.97	7.85	7.74	7.35	**7.73**	7.93	7.88	7.80	7.71	**7.83**
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L	7.10	7.08	6.78	6.54	**6.87**	7.91	7.83	7.80	7.70	**7.81**
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L	7.11	6.96	6.75	6.46	**6.82**	7.90	7.50	7.73	7.71	**7.71**
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L	7.06	6.90	6.64	6.45	**6.76**	7.90	7.83	7.70	7.63	**7.76**
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H +2% L	7.00	6.88	6.59	6.38	**6.71**	7.88	7.79	7.81	7.60	**7.77**
Mean	**7.43**	**7.30**	**7.06**	**6.72**		**7.91**	**7.79**	**7.77**	**7.67**	

	Taste S.Em±	Taste CD (1%)	Consistency S.Em±	Consistency CD (1%)
Treatments	**0.01**	**0.05**	**0.02**	**NS**
Storage period	**0.01**	**0.03**	**0.01**	**0.06**
Interaction (T×S)	**0.02**	**0.11**	**0.05**	**NS**

PJ - Pomegranate juice
KJ - Kokum juice
***Lp* -** *Lactobacillus plantarum* (2373)
***H* -** Honey
L – Lactose
DAS – Days after storage
NS – Non significant
* - Score out of 9

4.3.13.5 Overall acceptability

The data on the sensory scores for overall acceptability of fermented beverage of blended pomegranate with prebiotics as influenced by treatments and storage period up to 45 days are presented in Table 23, Plate 9 and 10). The overall acceptability scores of beverage was fount be significant among the treatments and storage period, but it was not found to be significant with their interaction.

Irrespective of storage periods, the mean overall acceptability score of the treatments ranged between 7.27 and 7.70. Highest score was recorded in T_4 (7.70) and the least score was observed in T_7 (7.27).

The effect of storage period on the overall acceptability of beverage was found to be significant. Maximum score was recorded at initial period (7.71) and least score was observed at 45 DAS (7.14).

In the interaction between the treatments and storage period, the highest score for overall acceptability was recorded in T_4S_1 (7.96) and lowest score was observed in T_7S_4 (6.97).

Table 23: Effect of prebiotics on overall acceptability (organoleptic evaluation) of fermented pomegranate beverage blended with kokum juice as influenced by treatments and storage period

Treatments	Overall acceptability				Mean
	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	
T_1 - 85% PJ + 15% KJ + *Lp*	7.66	7.45	7.34	7.10	**7.39**
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H	7.84	7.76	7.57	7.27	**7.61**
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H	7.89	7.78	7.61	7.30	**7.64**
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H	7.96	7.87	7.62	7.36	**7.70**
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L	7.60	7.42	7.31	7.13	**7.37**
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L	7.57	7.48	7.28	7.01	**7.33**
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L	7.58	7.37	7.15	6.97	**7.27**
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H +2% L	7.56	7.51	7.32	7.03	**7.36**
Mean	**7.71**	**7.58**	**7.40**	**7.14**	
	S.Em±		**CD (1%)**		
Treatment	**0.01**		**0.05**		
Storage period	**0.01**		**0.04**		
Interaction (T×S)	**0.03**		**NS**		

PJ - Pomegranate juice
KJ - Kokum juice

***Lp* -** *Lactobacillus plantarum* (2373)
***H* -** Honey
L – Lactose

DAS – Days after storage
NS – Non significant
* - Score out of 9

5. DISCUSSION

In recent days, fruit and vegetable beverages enriched with active components such as probiotics, prebiotics, phenolics, flavanoids and antioxidants are most popular among the various processed products. Food additives as probiotic lactic acid bacteria and prebiotics may exert positive effects on biochemical properties and sensory characteristics when it is used for fermentation process. Besides this, blending of two or more fruit juices is the one of the best methods to improve the nutritional quality of the juice. Moreover, new product development is possible through blending in the form of natural health drink (Jan and Marish, 2012). Pomegranate is quite important as it has a great variety of industrial and medicinal uses due to its attractive colour, distinctive flavour and being rich source of antioxidants, total phenols and tannins. Apart from this, pomegranate juice was investigated as a non-dairy probiotic drink after fermentation with selected probiotic lactic acid bacteria (Mousavi *et al.,* 2011). On the other hand, kokum is one of the underutilised fruits with lactic acid bacteria and yeasts as predominant fermentative microorganisms. When kokum juice is blended with other fruit juices rich in carbohydrates it may improve the quality with respect to flavour, biochemical characters and nutrient content of the beverage. In the present study, efforts were made to know the suitability of blended pomegranate juice as a raw material for production of lactic acid bacterial fermented drink, its nutritional and sensory quality through three systematically laid out experiments at the Department of Post-Harvest Technology and Department of Agricultural Microbiology, College of Horticulture, Bagalkot.

5.1 Development of fermented pomegranate beverage with and without kokum juice using probiotic lactic acid bacteria

Fermented beverages were prepared with different proportion of pomegranate and kokum juice using different lactic acid bacterial strains. The merit of any consumable product depends not only on its composition and nutritional value but also on sensory quality. Therefore, consumer acceptance level of fermented beverage was done by employing 9-point hedonic scale. The evaluation of lactic acid bacterial fermented beverage was done on the basis of colour, flavour, taste, consistency and overall acceptability which decide the consumer acceptability. The organoleptic evaluation of fermented pomegranate beverage was carried out by a panel of judges consisting of teachers and post graduate students of COH, Bagalkot.

Colour is the important parameter which decides the consumer preference. The score for the colour parameter was found to be numericaly highest in combination of 100% Pomegranate juice fermented by *Lactobacillus plantarum* followed by T_6 (85% PJ + 15% KJ + *Lactobacillus plantarum*; 7.81) (Table 1 and Fig.1). There was no much visual difference observed by the panellist for colour. However, treatments with fermented beverages scored highest may be due to its attractive bright colour.

Flavour is the combined perception of taste, aroma and mouthfeel (Benoit, 2004). Score for flavour was not significantly differed among the treatments. However, maximum score (7.14) for flavour was recorded in T_3 (100% PJ + *Lactobacillus plantarum*; 7.05). Minimum score for the flavour was observed in T_{12} (75% PJ + 25% KJ + *Lactobacillus plantarum*; 6.51).

Highest score (8.30) for taste was recorded in T_6 (85% PJ + 15% KJ + *Lactobacillus plantarum*). Least score for the taste was recorded in T_{13} (65% PJ+35% KJ + *Lactobacillus delbrueckii*; 7.12). The taste of the fermented beverage with higher concentration of kokum juice was disliked slightly compared to lower concentration of kokum juice. This might be due to the higher sourness of the juice. Similar results were obtained by Sivudu *et al.* (2014) in mixed watermelon and tomato juice fermented by lactobacilli.

Numerically highest score for consistency was observed in T_6 (85% PJ + 15% KJ + *Lactobacillus plantarum*; 7.16) and lowest in T_{13} (65% PJ + 35% KJ + *Lactobacillus delbrueckii;* 5.62).

Highest overall acceptability (7.30) was observed in T_6 (85% PJ + 15% KJ + *Lactobacillus plantarum*). This may be due to highest score for taste, consistency and colour in comparison with the other treatments. The minimum score for overall acceptability was noticed in T_{13} (65% PJ + 35% KJ + *Lactobacillus delbrueckii*; 6.12).

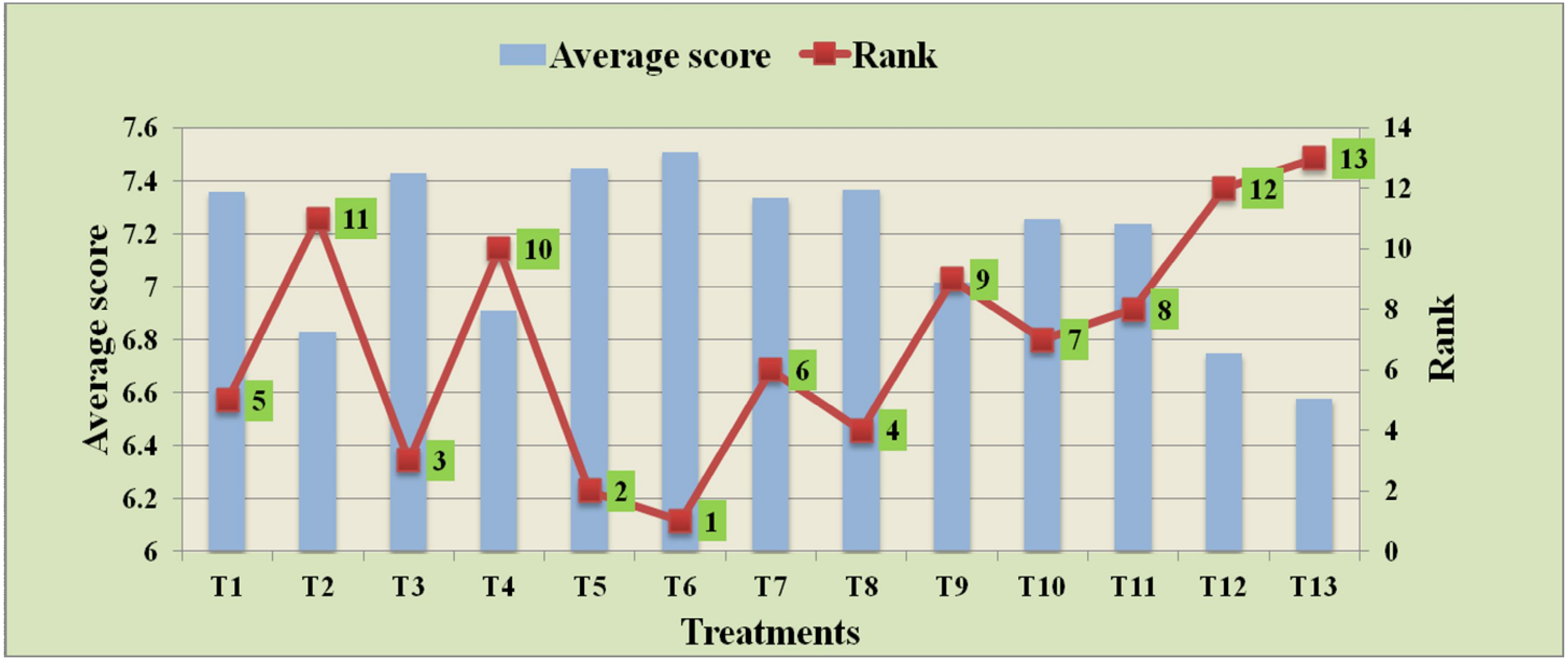


Fig. 1: Average score and ranking of different treatments of fermented pomegranate beverage with and without kokum juice

T_1 - 100% UPJ
T_2 - 100% PJ + *La*
T_3 - 100% PJ + *Lp*
T_4 - 100% PJ + *Ld*
UPJ - Uninoculated pomegranate juice
PJ - Pomegranate juice
KJ – Kokum juice

T_5 - 85% PJ + 15% KJ + *La*
T_6 - 85% PJ + 15% KJ + *Lp*
T_7 - 85% PJ + 15% KJ + *Ld*
T_8 - 75% PJ + 25% KJ + *La*
a - Lactobacillus acidophilus
Lp - Lactobacillus plantarum

T_9 - 75% PJ + 25% KJ + *Lp*
T_{10} - 75% PJ + 25% KJ + *Ld*
T_{11} - 65% PJ + 35% KJ + *La*
T_{12} - 65% PJ + 35% KJ + *Lp*
T_{13} - 65% PJ + 35% KJ + *Ld*
Ld - Lactobacillus delbrueckii

5.2 Biochemical quality and sensory evaluation of fermented pomegranate beverage with and without kokum juice during storage

Fermented beverage of pomegranate juice alone and with different proportion of kokum juice were prepared using three strains of probiotic lactic acid bacteria. Uninoculated pomegranate juice was taken as control. The beverages were subjected for biochemical, microbial and sensory analysis. The results obtained in this experiment are discussed here under.

5.2.1 Citric acid and lactic acid (%)

Analysis of acid content in the fermented beverage is necessary to ensure the quality of the beverage. The treatment mean values of citric acid and lactic acid per cent over different storage period ranged from 0.33 to 1.64 per cent and 0.06 and 2.25 per cent respectively (Table 2 and Fig. 2). Highest citric acid (1.64%) and lactic acid (2.35%) were produced in T_{11} (65% PJ + 35% KJ + *Lactobacillus acidophilus*). The higher acidity was correlated with the decrease in pH of the fermented beverage. The increase in the citric acid equivalent and a concomitant increase in lactic acid after fermentation (initial period of storage) and during further storage period might be due to the metabolic activity of the probiotic LAB as reported by Tayo and Akpeji (2016). The increase in citric acid and lactic acid content was observed in all the fermented juices up to 30 days of storage. This result was similar to the study conducted by many researchers (Sapna *et al*., 2002; Nosrati *et al*., 2014). Moraru *et al.* (2007) also reported that changes in the pH of the medium and lactic acid development are due to the production of organic acid by LAB culture. Gaanapriya *et al.* (2013) reported that the increase in lactic acid will inhibit the activity of the spoilage microorganisms. However, the acidity of uninoculated juice decreased as the storage period advanced. The decrease in the acidity of the uninoculated juice could be attributed to chemical interaction between organic constituents of the beverage induced by temperature and action of enzymes as observed by Palaniswamy and Muttukrishan (1974). Higher citric acid and lactic acid content was observed in 30 DAS (0.99% and 1.45% respectively). After 30 days of storage, marginal decrease in citric and lactic acid content was observed in fermented juices which might be due to the lower metabolic activity of LAB.

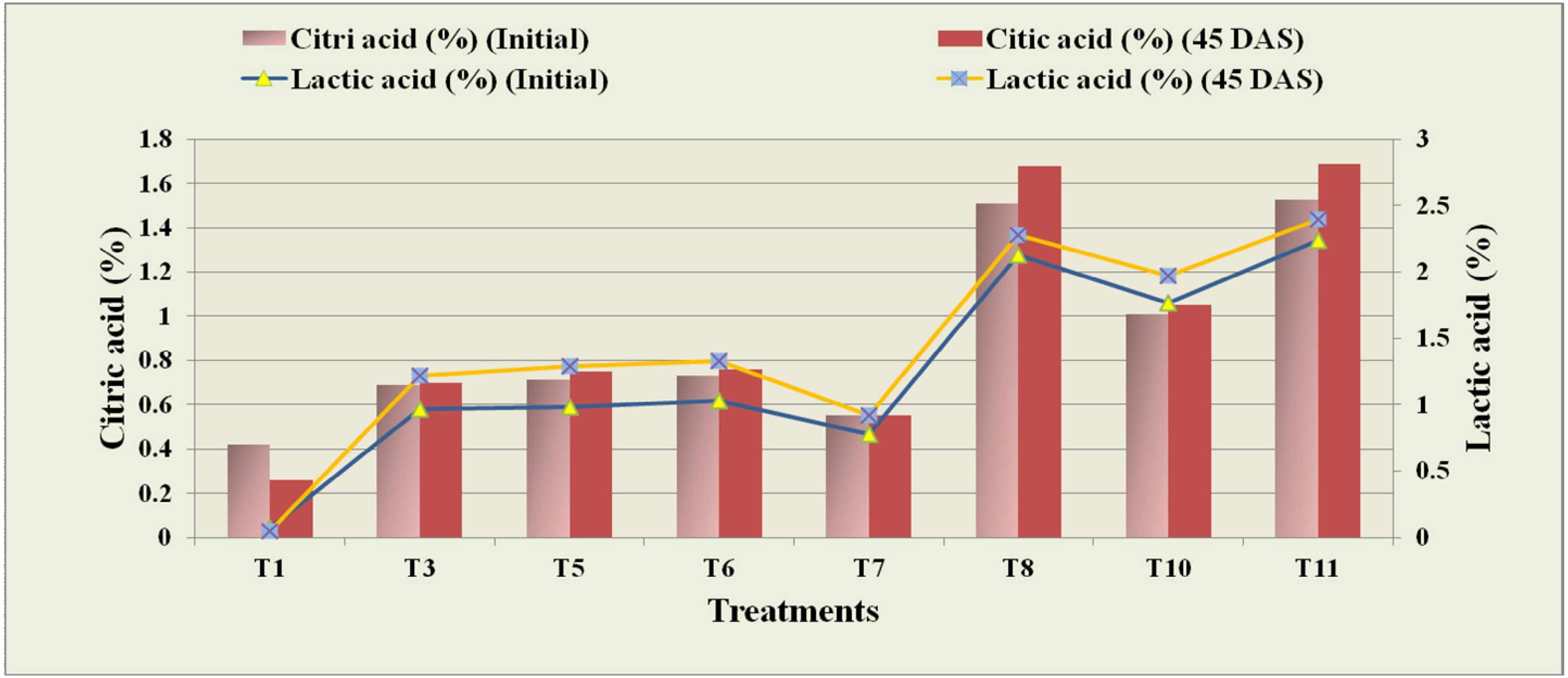


Fig. 2: Effect of treatments and storage period on citric acid and lactic acid content of fermented beverage of pomegranate with and without kokum juice

T_1 - 100% UPJ
T_3 - 100% PJ + *Lp*
T_5 - 85% PJ + 15% KJ + *La*
T_6 - 85% PJ + 15% KJ + *Lp*
T_7 - 85% PJ + 15% KJ + *Ld*
T_8 - 75% PJ + 25% KJ + *La*
T_{10} - 75% PJ + 25% KJ + *Ld*
T_{11} - 65% PJ + 35% KJ + *La*

UPJ - Uninoculated pomegranate juice
La - Lactobacillus acidophilus
PJ - Pomegranate juice
Lp - Lactobacillus plantarum
KJ - Kokum juice
Ld - Lactobacillus delbrueckii

Karovicova *et al*. (2003) reported that lactic acid can be suppressed by butyric bacteria activity slowly in an acidified medium.

5.2.2 pH

pH reduction is of great importance for the quality of the end product. The mean pH of the treatments ranged from 2.48 to 3.54 per cent (Table 3 and Fig. 3). Lower pH was observed in all the fermented juices compared to unfermented juice. The result indicated that fermentation by LAB strains resulted in increased acidity of the juice. Irrespective of storage period, lowest mean pH of the treatments was recorded in T_{11} (65% PJ + 35% KJ + *Lactobacillus acidophilus*; 2.48) followed by T_8 (75% PJ + 25% KJ + *Lactobacillus acidophilus*; 2.55) and T_5 (85% PJ + 15% KJ + *Lactobacillus acidophilus*; 2.56). The juices fermented by *Lactobacillus acidophilus* followed by *Lactobacillus plantarum* showed lower pH than *Lactobacillus delbrueckii*. Similar results were obtained by Yoon *et al*. (2005) in red beet juice fermented by different LAB stains (*Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus delbrueckii* and *Lactobacillus casei*). The pH of all the treatments decreased constantly during the storage period with lowest being at 30 DAS (2.85) except in unfermented juice. This indicates that LAB strains are able to produce acids even at refrigerated temperature (4°C). Decrease in the pH during storage may be due to the microbial activity and lactic acid production. The results obtained are in conformity with the findings of Pereira *et al.* (2011) in LAB fermented cashew apple juice and Fonteles *et al.* (2011) in cantaloupe juice. Kalita *et al.* (2015) reported that conversion of sugar into organic acids during fermentation resulted in decreased pH in litchi juice fermented by *Lactobacillus acidophilus, Lactobacillus plantarum* and *Lactobacillus rhamnosus.*

5.2.3 Total soluble solids (° brix) and sugars (%)

Among the different treatments, total soluble solids (TSS) were lowest (10.51° brix) in T_{11} (65% PJ + 35% KJ + *Lactobacillus acidophilus*) which was on par with T_8 (75% PJ+25% KJ + *Lactobacillus acidophilus*; 10.98° brix) (Table 3 and Fig.3). *Lactobacillus acidophilus* in the presence of more than 15 per cent of kokum juice resulted in the more reduction of total soluble solids. However, treatments with 15 per cent of kokum juice fermented by *Lactobacillus plantarum* showed lower TSS (T_6; 85%

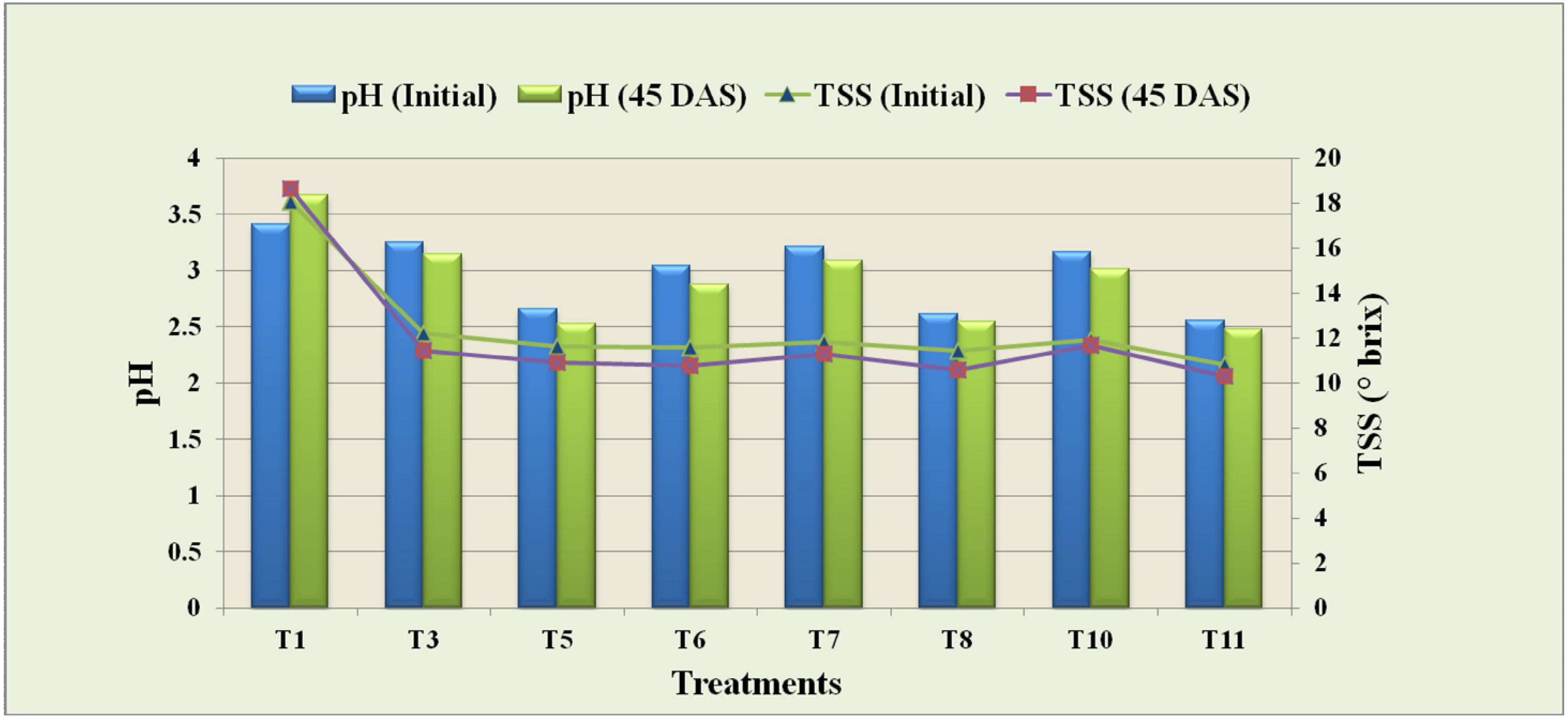


Fig. 3: Effect of treatments and storage period on pH and total soluble solids of fermented beverage of pomegranate with and without kokum juice

T_1 - 100% UPJ
T_3 - 100% PJ + *Lp*
T_5 - 85% PJ + 15% KJ + *La*
T_6 - 85% PJ + 15% KJ + *Lp*
T_7 - 85% PJ + 15% KJ + *Ld*
T_8 - 75% PJ + 25% KJ + *La*
T_{10} - 75% PJ + 25% KJ + *Ld*
T_{11} - 65% PJ + 35% KJ + *La*

UPJ - Uninoculated pomegranate juice
PJ - Pomegranate juice
KJ - Kokum juice
La - Lactobacillus acidophilus
Lp - Lactobacillus plantarum
Ld - Lactobacillus delbrueckii

J+15% KJ + *Lactobacillus plantarum*; 11.09° brix) than T_5 (85% PJ + 15% KJ + *Lactobacillus acidophilus*; 11.18° brix). Maximum TSS (18.42° brix) was observed in the unfermented juice (T_1; Uninoculated pomegranate juice). In case of storage period, minimum TSS was noticed at 45 DAS (11.95° brix) and maximum TSS (12.44° brix) at initial period. The result of the study confirmed that LAB strains were able to grow in fruit matrices which depend on the substrate used, the oxygen content, other nutrients and the final acidity of the fruit matrix. Similar findings were reported by Yoon *et al.* (2005) in the fermentation of beet juice by beneficial lactic acid bacteria.

Among the treatments, significantly minimum total sugar (10.60%) and reducing sugar content (2.89%) was observed in T_{11} (65% PJ + 35% KJ + *Lactobacillus acidophilus*) followed by T_8 (75% PJ + 25% KJ + *Lactobacillus acidophilus*; 10.79% and 3.31%) and T_6 (85% PJ + 15% KJ + *Lactobacillus plantarum;* 11.03% and 3.38%) (Table 4 and Fig. 4). The lactic acid cultures rapidly fermented the juice and reduced the level of sugar. Minimum reducing sugar content indicates efficiency of LAB strains in utilization of sugar during fermentation. *Lactobacillus acidophilus* in the presence of higher concentration of kokum juice (>15%) and *Lactobacillus plantarum* in the presence of lower concentration of kokum juice (0 and 15%) resulted in the better sugar consumption. This result suggested that sugar consumption by different LAB strains depends upon the substrates used in the fermentation. Similar results have been reported by Reddy *et al.* (2015) in fermentation of mango juice by probiotic lactic acid bacteria. The lowest total sugar and reducing sugar content was observed at 30 DAS (11.53% and 4.33% respectively). The bacteria rapidly consumed the substrate (sugars) in the fruit matrix and liberated the end products into the medium. The results of the study showed that the amount of total and reducing sugars was reduced during storage period as a result of fermentation by *Lactobacillus* spp. to produce organic acids. This indicated that the bacteria could utilise the sugars present in the fruit juices for cell synthesis. The result was in accordance with Kumar *et al.* (2015) in fermentation of grape, mango and melon to produce probiotic beverage by *Lactobacillus casei*. Metabolism of carbohydrates by *Lactobacillus* spp. varies from strain to strain and depends on the substrate and even on the fermentation time as reported by Hou *et al.* (2000) and Kumar *et al.* (2015). In this result, it was also found that glucose is a very good carbon and energy source for Lactobacilli. The same was observed by Wang *et al.* (2003) in

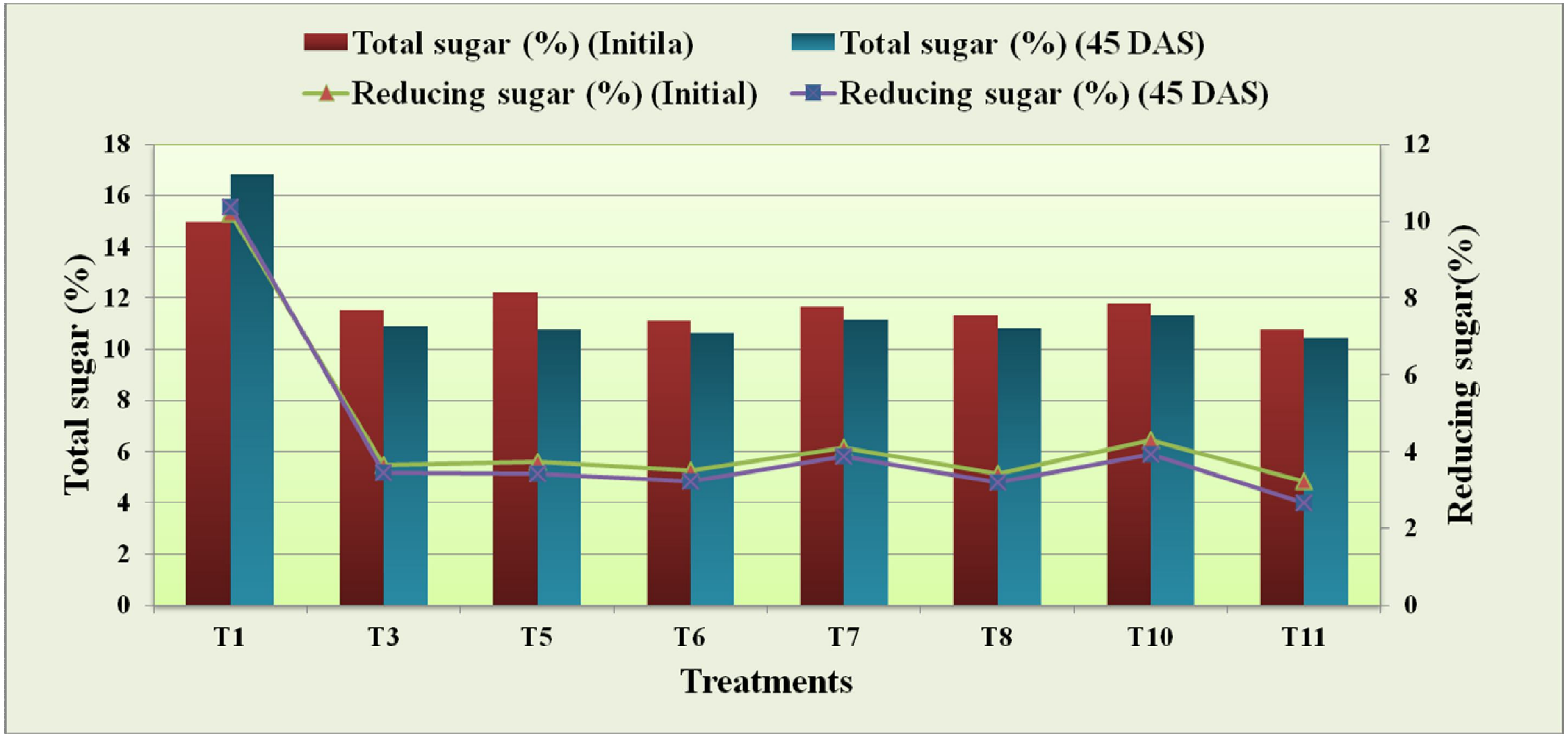


Fig. 4: Effect of treatments and storage period on total sugar and reducing sugar content of fermented beverage of pomegranate with and without kokum juice

T_1 - 100% UPJ	T_5 - 85% PJ + 15% KJ + *La*	T_7 - 85% PJ + 15% KJ + *Ld*	T_{10} - 75% PJ + 25% KJ + *Ld*
T_3 - 100% PJ + *Lp*	T_6 - 85% PJ + 15% KJ + *Lp*	T_8 - 75% PJ + 25% KJ + *La*	T_{11} - 65% PJ + 35% KJ + *La*

UPJ - Uninoculated pomegranate juice PJ - Pomegranate juice KJ - Kokum juice
La - Lactobacillus acidophilus *Lp - Lactobacillus plantarum* *Ld - Lactobacillus delbrueckii*

fermentation of soymilk by lactic acid bacteria alone or simultaneously with Bifidobacteria. However, in unfermented juice increased sugar content was noticed as the storage period advanced which could be attributed to inversion of non-reducing sugar in the presence of acids present in the beverage. Similar results have been reported by Imtiaz *et al.* (2011) in apple and apricot blended juices, Sheela and Shruthi (2014) in Mosambi, bitter gourd and lemon blended ready to serve beverages.

5.2.4 Antioxidant activity (%) and total phenol content (mg GAE/ 100 ml)

Highest antioxidant activity of 77.07 per cent was recorded in T_6 (85% PJ + 15% KJ + *Lactobacillus plantarum*) which was on par with T_3 (100% PJ + *Lactobacillus plantarum*; 75.96%) and lowest was noticed in uninoculated juice (Table 5 and and Fig. 5). Fermentation by *Lactobacillus plantarum* resulted in higher antioxidant activity with no significant difference between 100 per cent pomegranate and 85 per cent pomegranate juice with 15 per cent kokum juice. The antioxidant activity of fermented beverage with different proportion of fruit juice and LAB was higher than unfermented pomegranate juice. The phenolic compounds found in fresh fruit juice are generally glycosylated with sugar that on fermentation of the juice and sugar consumption by microorganism undergo deglycosylation and release of free hydroxyl groups and relevant aglycones (Mousavi *et al.,* 2013) which might be contributed to the improved antioxidant properties of the fermented juice. El-Nawawy *et al.* (2009) reported that the antioxidant activity of fermented permeate with natural fruit juices (Guava, mango and lemon juice) was higher when compared to fermented permeate without fruit juices. Similar results were also obtained by Kalita *et al.* (2015) in litchi juice fermented by probiotic lactic acid bacteria, Mousavi *et al.* (2013) in pomegranate juice using LAB strains and in *Phyllanthus emblica* fruit juice fermented using probiotic bacterium *Lactobacillus paracasei* (Peerajan *et al.,* 2016). Antioxidant activity of all the treatments decreased with time as the storage period advanced. Irrespective of treatments highest antioxidant activity was recorded at initial period (77.60%) and the least at 45 DAS (63.69%). Decrease in the antioxidant activity during storage may be linked to a lower content of phenolic compounds and ascorbic acid in stored juice as compared to fresh. These results are in conformity to the studies conducted by Filannino *et al.* (2013) in organic pomegranate juice fermented by *Lactobacillus plantarum* and Khatoon and Gupta (2015) in sweet lime and sugarcane juice fermented using

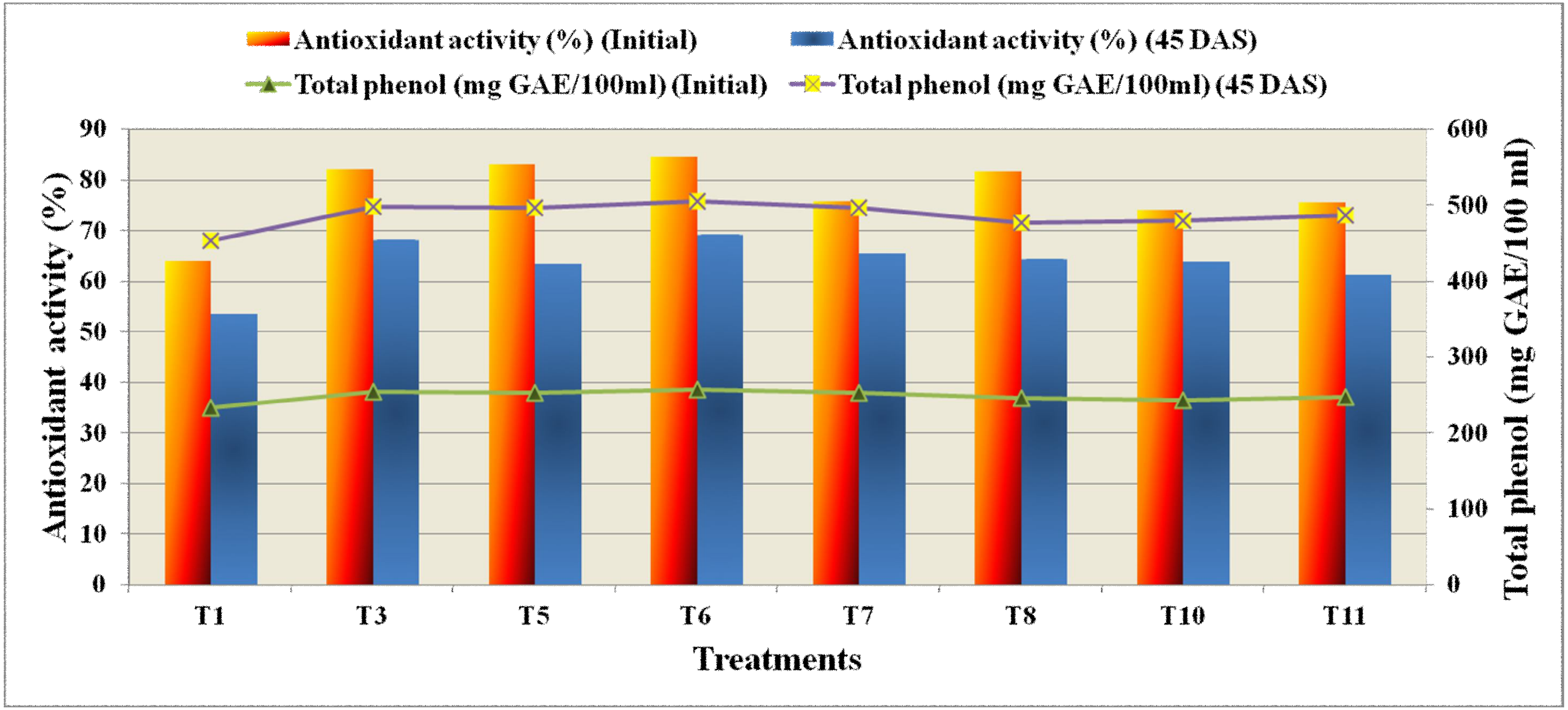


Fig. 5: Effect of treatments and storage period on antioxidant activity and total phenol content of fermented beverage of pomegranate with and without kokum juice

T_1 - 100% UPJ
T_3 - 100% PJ + *Lp*
T_5 - 85% PJ + 15% KJ + *La*
T_6 - 85% PJ + 15% KJ + *Lp*
T_7 - 85% PJ + 15% KJ + *Ld*
T_8 - 75% PJ + 25% KJ + *La*
T_{10} - 75% PJ + 25% KJ + *Ld*
T_{11} - 65% PJ + 35% KJ + *La*

UPJ - Uninoculated pomegranate juice
PJ - Pomegranate juice
KJ - Kokum juice
La - Lactobacillus acidophilus
Lp - Lactobacillus plantarum
Ld - Lactobacillus delbrueckii

Lactobacillus acidophilus. Ascorbic acid is a powerful antioxidant in fruits and can contribute to the antioxidant potential of juices as reported by Reddy *et al.* (2010). The same authors also reported that improvements in the radical scavenging effect can be related to the increase in the free form of phenolic compounds.

Among the treatments, maximum amount of total phenol content (252.00 mg GAE/100 ml) was recorded by the treatment T_6 (85% PJ + 15% KJ + *Lactobacillus plantarum*) followed by T_3 (100% PJ + *Lactobacillus plantarum*; 249.68 mg GAE/100 ml) and lowest was noticed in uninoculated juice (Table 5 and and Fig. 5). This result revealed that fermentation process by LAB is good enough to enrich the product with polyphenolic content by selected substrate and starter culture. The release of a significant amount of phenolic content is possible by blending of 85 per cent pomegranate juice with 15 per cent of kokum juice fruits by *Lactobacillus plantarum* mediated fermentation. In case of storage period, maximum score of total phenol was recorded at initial period (248.32 mg GAE/100 ml) and lowest score was recorded at 45 DAS (238.894 mg GAE/100 ml). The decrease in the total phenol content during storage period is probably due to the enzymatic oxidation of polyphenolic content by polyphenol oxidase (Altunkaya and Gokmen, 2008). These findings are in accordance with the results obtained in *Lactobacillus paracasei* HII01 mediated fermentation in *Phyllanthus emblica* fruit juice by Peerajan *et al.* (2016). Several studies reported that phenolics in the fruit significantly contributed to their antioxidant properties (Shan *et al.*, 2005; Wong *et al.*, 2006; Wu *et al.*, 2006).

5.2.5 Ascorbic acid (mg/100 ml)

Ascorbic acid is one of the important components of juices from nutritional point of view. The ascorbic acid content of the fermented juices were higher than the unfermented juice indicating fermentation by LAB increases the ascorbic acid content of juice. The mean ascorbic acid content of treatments ranged from 2.97 to 10.05 mg per 100 ml (Table 6 and Fig.6). Significantly highest ascorbic acid content (10.05 mg per 100 ml) was noticed in the treatment T_6 (85% PJ + 15% KJ + *Lactobacillus plantarum*) followed by T_3 (100% PJ + *Lactobacillus plantarum*; 9.31 mg per 100 ml) and lowest was noticed in T_1 (Uninoculated juice; 2.97 mg/100 ml) indicating that *Lactobacillus plantarum* is more efficient in producing more ascorbic acid content during fermentation. Similar results were obtained by Latha (2012) in fermentation of kokum juice by *Lactobacillus plantarum* where they reported that vitamin C content of kokum juice increased from 80 mg/100 ml to 176.5 mg/100 ml after fermentation. The ascorbic acid content of the juice decreased during the storage in all the treatments with the advancement of the storage period. This may probably due to the fact that ascorbic acid being sensitive to oxygen, light and heat was easily oxidised in presence of oxygen by both enzymatic and non-enzymatic catalyst (Mapson, 1970). This could be due to conversion of ascorbic acid to dehydroxy ascorbic acid. Both ascorbic acid and dehydro ascorbic acid are highly volatile and unstable forms of vitamin C. Similar results were also noticed by Atallah, (2015b) in fermented functional beverages of milk permeate with mango and carrot by probiotic lactic acid bacteria where ascorbic acid content decreased from 14.10 and 4.05 mg/100 g to 13.21 and 3.20 mg/100 g in mango and carrot blended beverage respectively.

5.2.6 Instrumental colour analysis (*L* a* b**)

In the instrumental colour analysis for measuring (*L* a* b**), *L* c*oordinate represents the lightness, the *a** coordinate represents greenness (-*a**) or redness (+*a**) and the b* coordinate represents blueness (-*b**) or yellowness (+*b**). Using this system, an object measured for colour would be assigned as *L*, a** and *b** value to describe the colour and specify its location in the three dimensional colour space. The distance between two colours, each having its own location in the colour space relates to a measurable colour difference (Kevin, 2002).

Irrespective of storage period, the maximum *L** value (11.79) of treatment was recorded in T_1 (Uninoculated pomegranate juice) which indicates the lighter colour of the juice. The maximum *a** value (28.14) was recorded in T_{11} (65% PJ + 35% KJ + *Lactobacillus acidophilus*). Maximum *a** value indicates the red colour of the juice (Table 7). The maximum *b** value (12.15) was also recorded in the T_{11} (65% PJ + 35% KJ + *Lactobacillus acidophilus*) (Table 8). Anthocyanins are responsible for the red colour in pomegranate and kokum juice. Anthocyanin is pH dependent (the red flavylium is stable at low pH) as the pH changes were substantial hence the colour changes of the juice (Choi *et al.*, 2002). Visually, no colour change was observed in all of the treatments during the storage period. Higher colour intensity was noticed in fermented juice when compared to unfermented juice. Increase in the red colour (a*) intensity was observed in all the treatments as the storage period advanced with maximum a* value (20.95) at 45 DAS and least at initial period (16.16). This result was well supported by Filannino *et al.* (2013) in organic pomegranate juice, in which colour intensity of organic pomegranate juice fermented by different strains of *Lactobacillus plantarum* and uninoculated pomegranate juice was found to be increased during the storage. This result was in close conformity with the study of Khatoon and Gupta (2015) in fermented sugarcane juice. Oxidation of ascorbic acid or precipitation of the pigments (Sistrunk and Cash, 1974), contributed to reduction of the luminosity, giving a darker appearance to the juice. With time, the increase in the intensity of the dark colours, such as the red decreased the yellow intensity as reported by Sandi *et al.* (2004) in passion fruit juice.

5.2.7 Lactic acid bacteria population (cfu/ml)

The lactic acid bacterial population was analyzed in beverages during initial period and after storage period of 30 days (Table 9 and Fig. 6). Among the treatments, highest LAB population (2.91×10^6 cfu/ml) were observed in T_6 (85% PJ + 15% KJ + *Lactobacillus plantarum*) which was statistically on par with T_8 (75% PJ + 15% KJ + *Lactobacillus plantarum*; 2.58×10^6 cfu/ml) and bacterial population was not detected in T_1 (Uninoculated pomegranate juice). The survival of *Lactobacillus* spp. varied due to the probiotic strain used as a result of different sensitivity to environmental stresses of these bacteria such as low pH and high titratable acidity (Mortazavian *et al.,* 2006). During storage, highest LAB population was detected at initial period (2.79×10^6 cfu/ml) and lowest at 30 DAS (0.58×10^6 cfu/ml). Juices fermented by *Lactobacillus acidophilus* showed higher population during initial period of storage but after 30 days population reduced drastically which may be due to higher acidity of the juice produced by *Lactobacillus acidophilus.* The results of this study confirm the findings of Sheehan *et al.,* (2007) indicating that the pH decreased with time and led to a faster decrease in the number of viable bacteria in fruit juices fortified with probiotic lactic acid bacteria. Yanez *et al.* (2008) reported that an increase in acidity as a result of the fermentation process can reduce the survivability the probiotic lactic acid bacteria. Therefore, variations in bacterial stability observed in this study may be due to pH, fruit juice composition or oxygen present. Similar results were obtained by Ozcan *et al.* (2015) in

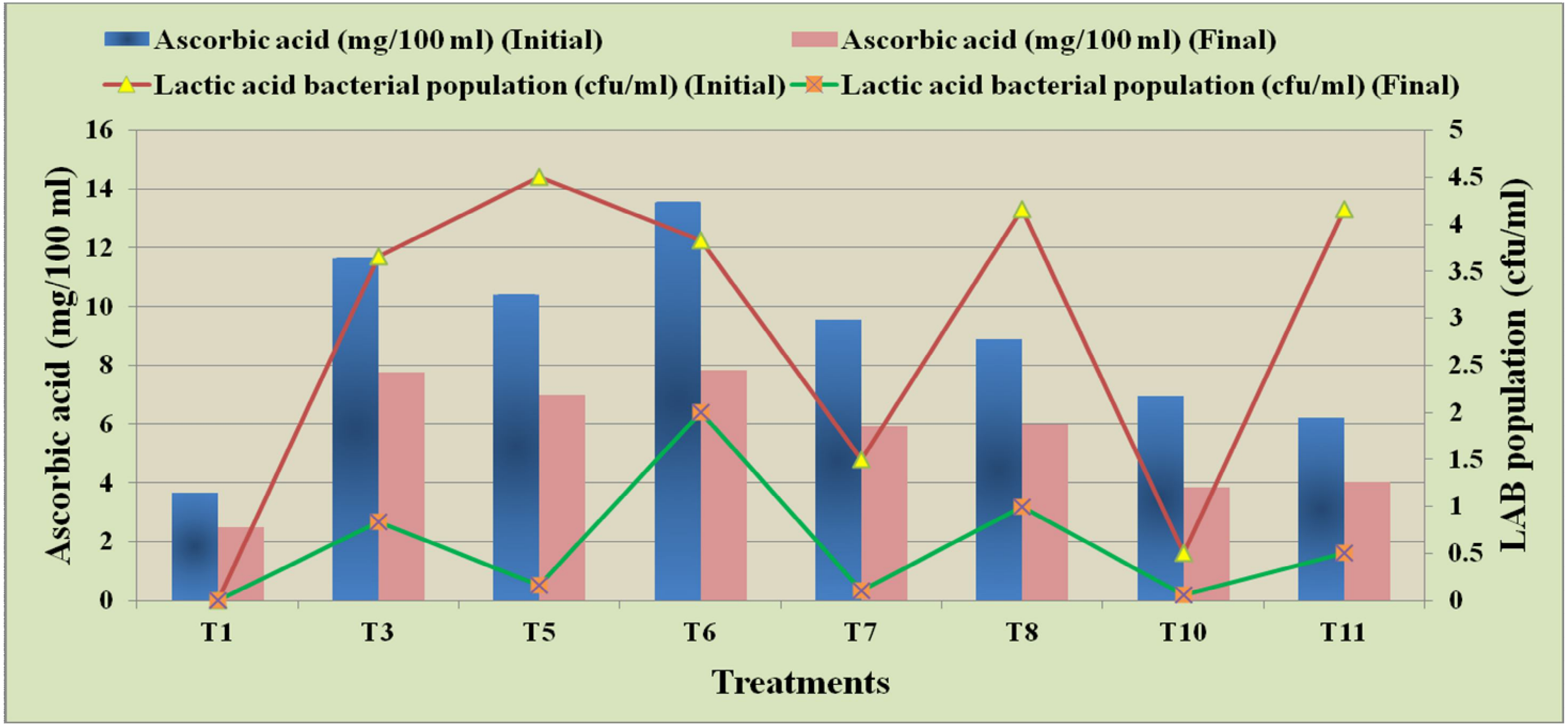


Fig. 6: Effect of treatments and storage period on ascorbic acid and LAB population (X 10^6 cfu/ml) of fermented beverage of pomegranate with and without kokum juice

T_1 - 100% UPJ
T_3 - 100% PJ + *Lp*
T_5 - 85% PJ + 15% KJ + *La*
T_6 - 85% PJ + 15% KJ + *Lp*
T_7 - 85% PJ + 15% KJ + *Ld*
T_8 - 75% PJ + 25% KJ + *La*
T_{10} - 75% PJ + 25% KJ + *Ld*
T_{11} - 65% PJ + 35% KJ + *La*

UPJ - Uninoculated pomegranate juice
PJ - Pomegranate juice
KJ - Kokum juice
La - Lactobacillus acidophilus
Lp - Lactobacillus plantarum
Ld - Lactobacillus delbrueckii

fruit based (apple and bluberry) fermented dairy beverages made with *Lactobacillus acidophilus* and *Lactobacillus rhamnosus.* The result of this study was in accordance with the findings of Yoon *et al.* (2004) in fermented tomato juice. Dogahe *et al.* (2015) reported that in pineapple, apple and mango juice mixture LAB population was reduced after two weeks during storage at 4°C temperature. Pakbin *et al.* (2014) reported that *Lactobacillus plantarum* and *Lactobacillus delbrueckii* were capable of surviving in the conditions of low pH and high acidity in fermented peach juice during cold storage (four weeks) at 4°C. In the present study, *Lactobacillus plantarum* showed highest population after 30 days of storage in T_6 (85 % PJ + 15% KJ + *Lactobacillus plantarum*; 2.00×10^6 cfu/ml).

5.2.8 Sensory evaluation

As there were some changes in physico-chemical constituents of fermented beverages during storage period of 45 days, these beverages need to be evaluated for their palatability by consumers. Therefore, the organoleptic evaluation was conducted at 15 days interval by a panel of semi trained judges. Sensory evaluation was done on 9 point hedonic scale. The evaluation of juice was done on colour, taste, flavour, consistency and overall acceptability (Table 10, 11, 12) (Fig. 7 and 8).

The mean colour scores of all the treatments decreased from 7.90 to 7.66; flavour scores 7.84 to 7.63; taste scores 7.65 to 7.00; consistency scores from 8.05 to 7.30, overall acceptability scores decreased from 7.85 to 7.40 during 45 days of storage at 4°C. Colour is the key attribute to influence the sensory acceptance. The physico-chemical analysis showed intensification of red colour with the addition of the probiotic LAB, which was not detected in the sensory evaluation. All fermented beverages were acceptable for the colour at the same level. Fruit fibres and flavour compounds might contribute to the desired flavour of the final product. A tendency of higher scores for beverages fermented by *Lactobacillus plantarum* was observed. However, the flavour of the juice fermented by *Lactobacillus delbrueckii* was less appreciated compared to the juice fermented by the *Lactobacillus plantarum* and *Lactobacillus acidophilus*. A fermented dairy taste and flavour were received by panellists as the result of fermentation by LAB. Similar results were obtained by Luckow and Delahunty (2004) in fermented blackcurrant juice in which the authors reported that the sensory

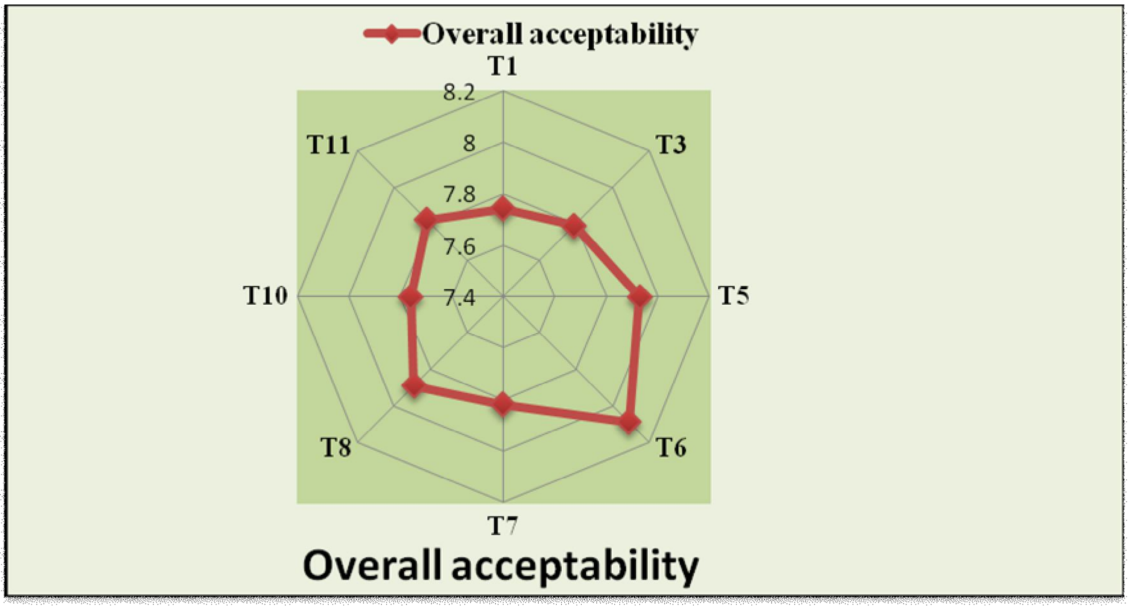


Fig.7 : Overall acceptability of fermented pomegranate beverage with and without kokum juice during initial period of storage

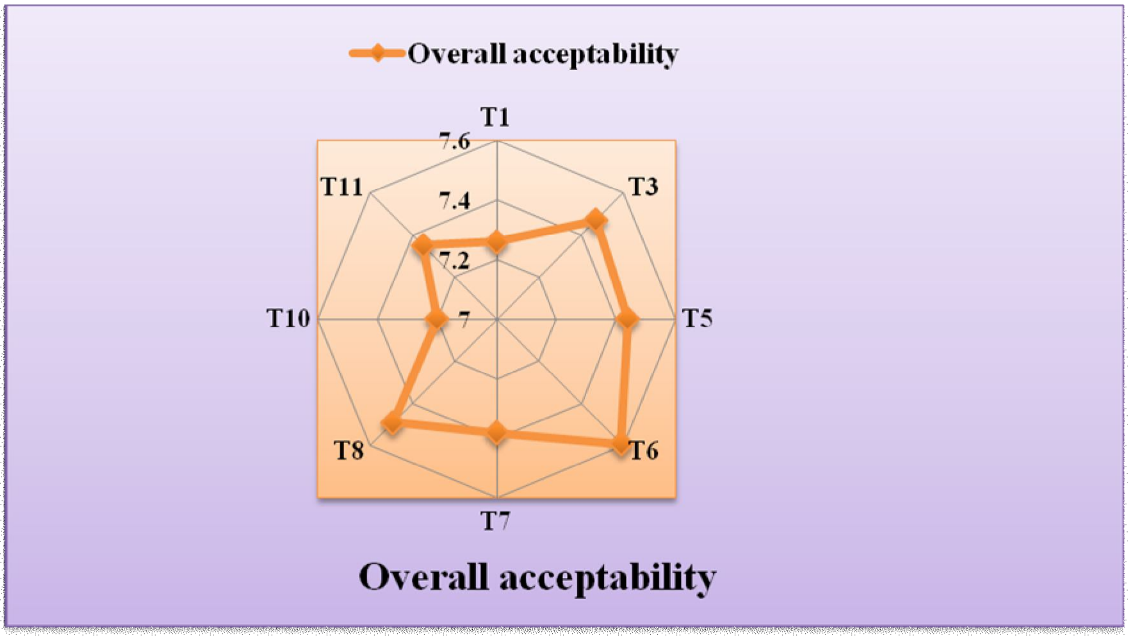


Fig. 8: Overall acceptability of fermented pomegranate beverage with and without kokum juice after 45 DAS

T_1 - 100% UPJ
T_3 - 100% PJ + *Lp*
T_5 - 85% PJ + 15% KJ + *La*
T_6 - 85% PJ + 15% KJ + *Lp*
T_7 - 85% PJ + 15% KJ + *Ld*
T_8 - 75% PJ + 25% KJ + *La*
T_{10} - 75% PJ + 25% KJ + *Ld*
T_{11} - 65% PJ + 35% KJ + *La*

characteristic of juice was perfumery, dairy in odour, sour and savoury in flavour. Furthermore production of lactic acid by Lactobacillus may have reinforced the sweet in mouth feeling. In the present study, the overall acceptability was strongly correlated with the taste and flavour but not with the visual appearance. Pimentel *et al.* (2015) reported that the sensory characteristic of fermented clarified apple juice was dairy in odour and sour in flavour.

5.3 Effect of different prebiotics on fermentation of pomegranate juice with kokum blend using probiotic lactic acid bacteria during storage

5.3.1 Citric acid and lactic acid (%)

The treatment mean values of citric acid and lactic acid per cent ranged from 0.74 to 0.84 per cent and 0.99 and 1.32 per cent respectively (Table 13 and Fig. 9). Highest citric acid (0.84%) and lactic acid (1.32%) were produced in T_7 (85% PJ + 15% KJ + *Lactobacillus plantarum*+ 5% lactose). Blending with honey and lactose produced higher acidity in the juice by bacterial fermentation when compared to fermentation of juice without any prebiotics blend. Higher sugar consumption and higher acidification was observed in the treatments with prebiotics. This indicated the availability of fermentable sugar favouring lactic acid bacteria. The increase in citric acid and lactic acid content was observed in all the treatments up to 45 days of storage. Rotar *et al.* (2015) reported that the content of lactose decreased and lactic acid increased during storage (lactose is decomposed into lactic acid) in goji berry and honey blended yoghurt. Tayo and Akpeji (2016) also reported that after one week of storage, the lactic acid production ranged from 53.14–91.88 mg/ml in probioticated pineapple juice. The result was also in close conformity with the study of Costa *et al.* (2013) in fermentation of sonicated pineapple beverage, Latha (2012) in fermented kokum juice with blend of 5 per cent honey and 20 per cent sweet potato. Moraru *et al.* (2007) reported that changes in the pH of the medium and lactic acid development are due to the production of organic acid by LAB culture. Similar results were obtained by Sapna *et al.* (2002) in spice beverage, Nosrati *et al.* (2014) in fermentation of vegetable juice and Sivudu *et al.* (2014) in fermentation of watermelon and tomato juice by LAB.

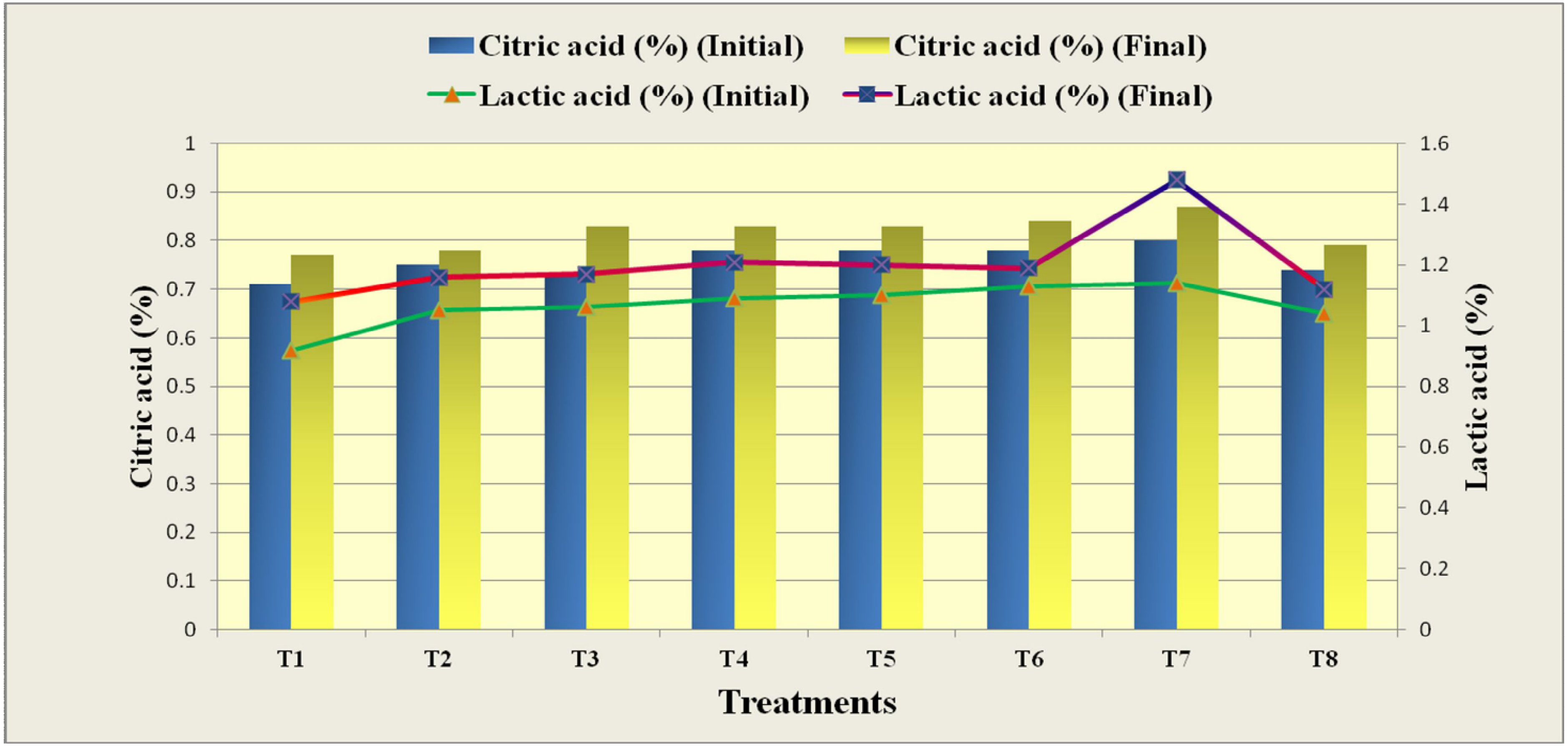


Fig. 9: Effect of prebiotics on citric acid (%) and lactic acid (%) content of fermented pomegranate beverage blended with kokum juice as influenced by treatments and storage period

T_1 - 85% PJ + 15% KJ + *Lp*
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H
PJ - Pomegranate juice
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H
KJ - Kokum juice
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L
Lp - *Lactobacillus plantarum* (2373)
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H + 2%L
H – Honey **L** - Lactose

5.3.2 pH

The mean pH of the treatments ranged from 2.79 to 3.18 (Table 14 and Fig. 10). The pH depends upon the acids and sugar content. Irrespective of storage period, lowest mean pH of the treatments was recorded in T_7 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 5% lactose; 2.79) followed by T_6 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 3% lactose; 2.88), T_4 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 5% honey; 2.93). Fermentation by lactic acid bacteria reduced the pH of all the treatments to lowest when blended with 5 per cent lactose followed by 3 per cent lactose, 5 per cent honey and higher pH observed in the treatment without prebiotics. Similar results of reduction in pH were observed in the study conducted by Latha (2012) in the kokum juice blended with honey and sweet potato. The result indicated that addition of prebiotics resulted in decreased pH of the juice when compared to the fermented juice without any prebiotics (T_1; 85% PJ + 15% KJ + *Lactobacillus plantarum*). The pH of all the treatments decreased during the storage period. This indicates that LAB strains are able to produce various organic acids in the juices vigorously during refrigerated storage (4°C). Decrease in the pH during storage may be due to activity of LAB. The results obtained are in conformity with the findings of Hashemiravan *et al.* (2015) in fermented mixture of malt extract and red fruit juice and Fonteles *et al.* (2011) in watermelon juice. According to the study of Ding and Shah (2008) the dead probiotic cells could release enzymes for hydrolyzing sugars in the fruit juice, thus lowering the pH. This might be the reason for decreased pH content of fermented beverages in the present study.

5.3.3 Total soluble solids (° brix) and sugars (%)

The treatment mean values of total soluble solids ranged from 10.79 to 11.88° brix (Table 14 and Fig. 10). Among the different treatments, total soluble solids (TSS) were lowest (10.79° brix) in T_7 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 5% lactose) followed by T_6 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 3% lactose; 10.96° brix) and T_5 (85% PJ + 15% KJ + *Lactobacillus plantarum*+ 2% lactose; 10.99° brix). However, different concentration of honey also resulted in reduction of TSS after fermentation and along the storage period. This might be due to the fact that bacterial strains have more access to low molecular weight carbon sources such as glucose,

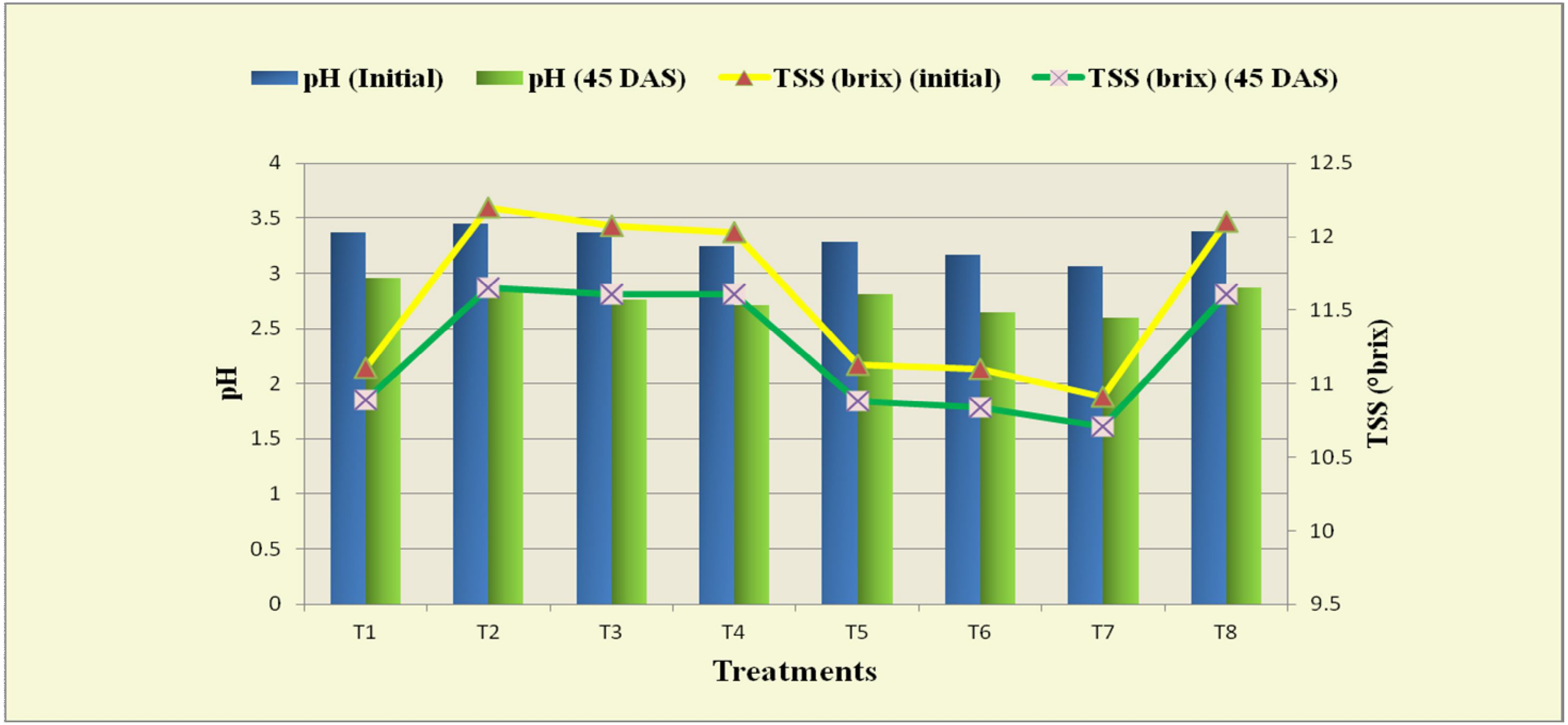


Fig. 10: Effect of prebiotics on pH and TSS (° brix) content of fermented pomegranate beverage blended with kokum juice as influenced by treatments and storage period

T_1 - 85% PJ + 15% KJ + *Lp*
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H + 2%L
PJ - Pomegranate juice
KJ - Kokum juice
Lp - *Lactobacillus plantarum* (2373)
H – Honey
L - Lactose

fructose and use them to produce acids during fermentation, so that the reducing sugars are fermented and produced lactic acid. In case of control, only marginal reduction in the TSS was observed which indicates that LAB in the presence of added sugar resulted in effective fermentation than without added sugar. Among the different storage period, lowest TSS was observed at 45 DAS (11.22° brix) and highest at initial period (11.58° brix).

Total sugar and reducing sugar content of the fermented beverage ranged from 10.74 to 11.87 per cent and 3.51 to 3.86 per cent respectively (Table 15 and Fig. 11). Among the different treatments, minimum total sugar (10.74%) and reducing sugar content (3.51%) was noticed in T_7 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 5% lactose). Minimum reducing sugar indicates efficiency of *Lactobacillus plantarum* in utilization of sugar in prebiotics added juices during fermentation. The results were similar with findings of Latha (2012), who reported that kokum juice in combination with 5 per cent honey and 20 per cent sweet potato resulted in decreased reducing sugar content from 11.45 to 6.60 per cent. Storage time significantly influenced the content of total sugars and reducing sugars. Minimum total sugar (11.17%) and reducing sugar content (3.57%) was observed at 45 DAS. Maximum total sugar (11.49%) and reducing sugar content (3.82%) was recorded at initial period. Sugar content decreased as the storage period advanced by producing organic acids which indicates that continuous utilisation of sugar by LAB during storage. This result was in accordance with findings of Mashayekh *et al.* (2015) in probiotic fermented beverage based on mixture of pineapple, apple and mango juices.

5.3.4 Antioxidant activity (%) and Total phenol (mg GAE/100 ml)

The mean antioxidant activity of treatments ranged from 76.51 to 80.40 per cent. The highest antioxidant activity (80.40%) was observed in T_4 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 5% honey) followed by T_3 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 3% honey; 78.95%) and lowest was noticed in T_1 (85% PJ + 15% KJ + *Lactobacillus plantarum*; 76.51%) (Table 16 and Fig. 12). Nutritional content of the honey and fermentation reaction by LAB might have contributed to the higher antioxidant property of beverages. Bansal *et al.* (2005) reported that honey contains good quantities of antioxidants and ascorbic acid. According to Meena *et al.* (2012),

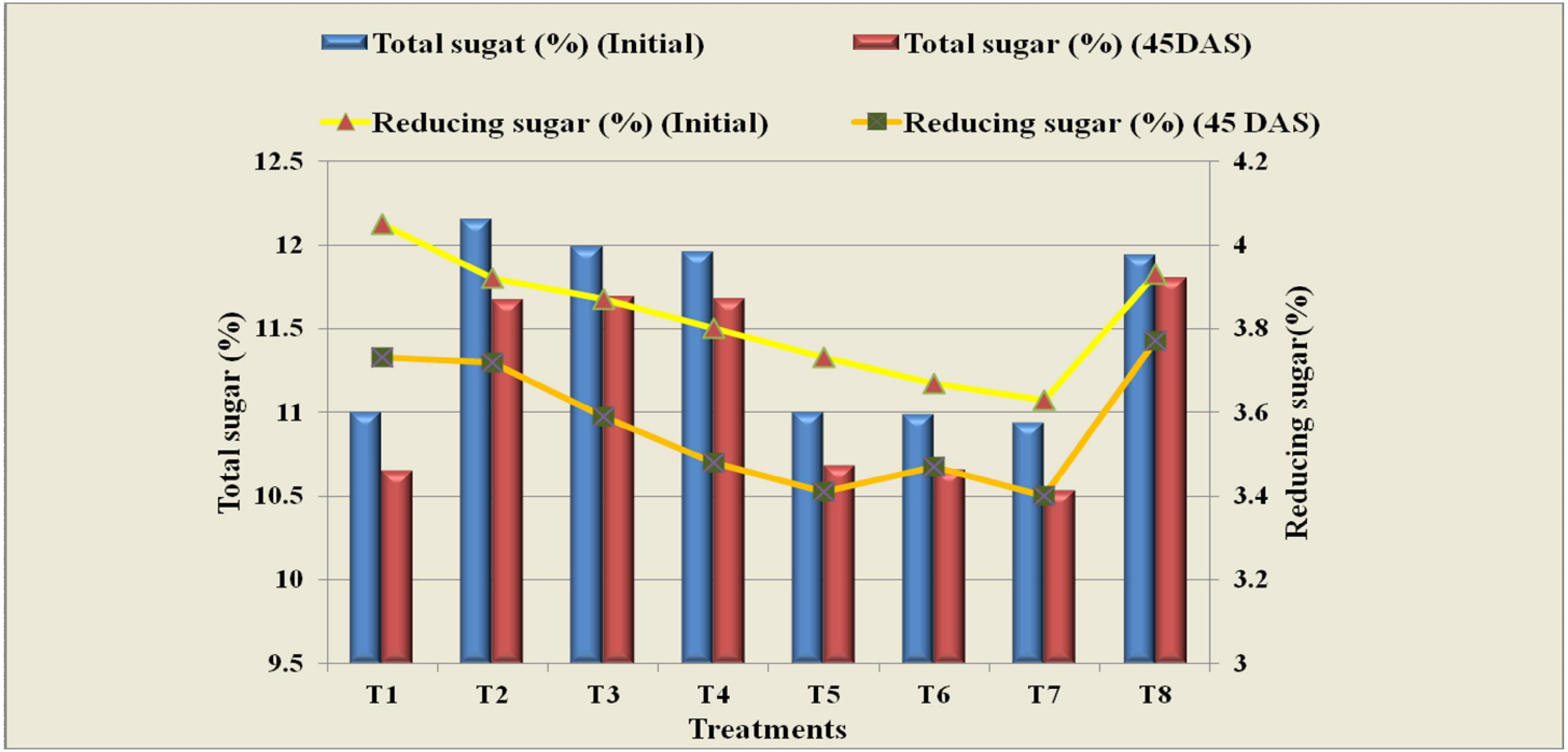


Fig. 11: Effect of prebiotics on total sugar (%) and reducing sugar (%) content of fermented pomegranate beverage blended with kokum juice as influenced by treatments and storage period

T_1 - 85% PJ + 15% KJ + *Lp*
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H + 2%L
PJ - Pomegranate juice
KJ - Kokum juice
Lp - *Lactobacillus plantarum* (2373)
H – Honey
L - Lactose

redox property of phenolic components positively influences the antioxidant properties. Antioxidant activity of all the treatments increased up to 15 days of storage, thereafter decreased up to 45 days of storage. This result was in conformity with the study of Peerejan *et al.* (2016) in fermented *Phyllanthus emblica* juice using *Lactobacillus paracasei*. Decrease in the antioxidant activity during storage may be linked to a lower content of phenolic compounds and ascorbic acid in stored juice as compared to fresh. These results are in conformity of the studies conducted by Filannino *et al.* (2013) in organic pomegranate juice fermented by *Lactobacillus plantarum* and Khatoon and Gupta (2015) in sweet lime and sugarcane juice fermented using *Lactobacillus acidophilus.*

Among the treatments, maximum amount of total phenol content (257.73 mg GAE/100 ml) was recorded by the treatment T_4 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 5% honey) followed by T_3 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 3% honey; 254.38 mg GAE/100 ml) and lowest was noticed in treatment without any prebiotics (T_1; 85% PJ + 15% KJ + *Lactobacillus plantarum*; 242.23 mg GAE/100 ml) (Table 16 an Fig. 12). Total phenol content of all the treatments with different concentration of prebiotics was higher than control (T_1). This result revealed that fermentation process by LAB along with prebiotics is good enough to enrich the product with polyphenolic content by selected substrate and starter culture. In case of storage period, total polyphenol level was gradually increased until day 15 and then the concentration was slowly reduced during further storage period. Maximum score of total phenol was recorded at 15 days after storage (251.68 mg GAE/100 ml) and lowest score was recorded at 45 DAS (243.41 mg GAE/100 ml). The decrease in the total phenol content during storage period was probably due to the enzymatic oxidation of polyphenolic content by polyphenol oxidase (Altunkaya and Gokmen, 2008). These findings are in accordance with the results obtained in *Lactobacillus paracasei* HII01 mediated fermentation in *Phyllanthus emblica* fruit juice by Peerajan *et al.* (2016).

5.3.5 Ascorbic acid (mg/100 ml)

Ascorbic acid (Vitamin C) is a water-soluble antioxidant found in fruits and vegetables. The mean ascorbic acid content of treatments ranged from 6.72 to 10.52 mg per 100 ml (Table 17 and Fig. 13). Significantly highest ascorbic acid content (10.52 mg

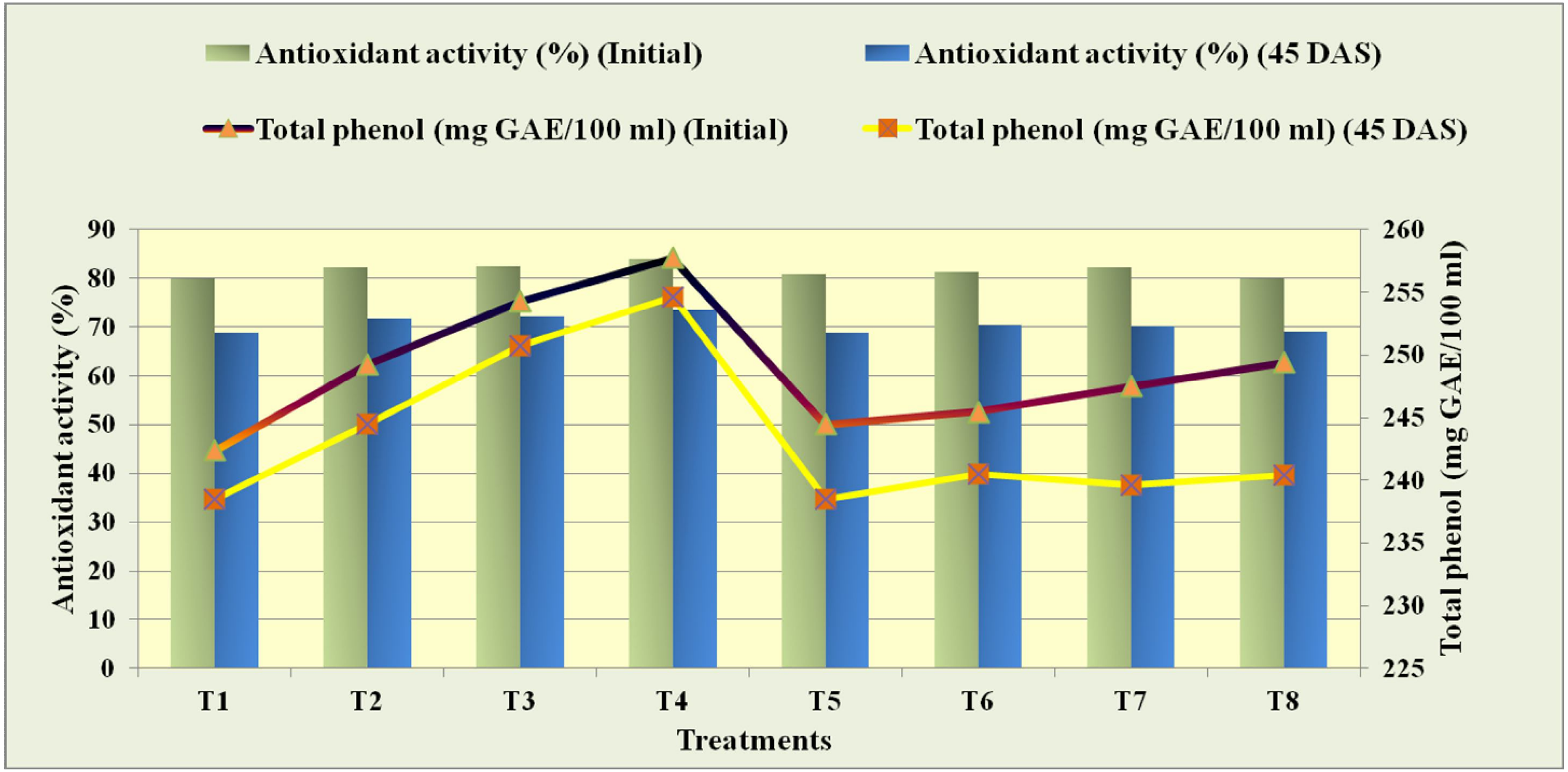


Fig. 12: Effect of prebiotics on antioxidant activity (%) and total phenol content (mg GAE/ 100 ml) content of fermented pomegranate beverage blended with kokum juice as influenced by treatments and storage period

T_1 - 85% PJ + 15% KJ + *Lp*
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H + 2%L

PJ - Pomegranate juice KJ - Kokum juice ***Lp*** - *Lactobacillus plantarum* (2373) **H** – Honey **L** - Lactose per 100 ml) was noticed in the treatment T_4 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 5% honey) followed by T_3 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 3% honey; 10.12 mg per 100 ml) and lowest was noticed in T1 (85% PJ + 15% KJ + *Lactobacillus plantarum*; 6.72 mg per 100 ml). Lactic acid bacterial fermentation of pomegranate with kokum blended juice and 5 per cent honey influenced much on ascorbic acid enhancement. Similar results were obtained by Latha (2012) in fermentation of kokum juice with blend of honey by *Lactobacillus plantarum.* The ascorbic acid content of the juice decreased during the storage in all the treatments with the advancement of the storage period. This may probably due to the fact that ascorbic acid being sensitive to oxygen, light and heat was easily oxidised in presence of oxygen by both enzymatic and non-enzymatic catalyst (Mapson, 1970). This could be due to conversion of ascorbic acid to dehydroxy ascorbic acid. Both ascorbic acid and dehydro ascorbic acid are highly volatile and unstable forms of vitamin C. Similar results were also noticed by Atallah (2015b) in fermented functional beverages of milk permeate with mango and carrot by probiotic lactic acid bacteria.

5.3.6 Protein content (%)

The mean protein content of treatments ranged from 0.17 to 1.93 per cent. The highest protein content (1.93%) was observed in T_4 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 5% honey) followed by T_3 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 3% honey; 1.77%) and lowest was noticed in T_1 (85% PJ + 15% KJ + *Lactobacillus plantarum*; 0.17%) (Table 17 and Fig. 13). Addition of honey and fermentation by LAB might be the reason for increased protein content of beverages. According to Bansal *et al.* (2005), honey contains proteins with proline as maximum and Kour *et al.* (2000) reported that honey composed of approximately 0.5 g/100 g of protein content. Protein content of beverages decreased as the storage period advanced. More reduction in protein content was observed at the end of the storage period. The decreased protein content might be due to the proteolytic activity of the starter cultures. This finding is in accordance with the study of El-Nawawy *et al.* (2009) in probiotic beverages of natural fruit juices.

5.3.7 Instrumental colour analysis (*L* a* b**)

Irrespective of storage period, the maximum *L** value (14.43) of treatment was recorded in T_7 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 5% lactose) which indicates the lighter colour of the juice (Table 18). The maximum *a** value (17.85) was recorded in T_2 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 2% honey). Maximum *a** value indicates the red colour of the juice. The maximum yellowness (*b** value; 12.83) was recorded in the T_4 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 5% honey) (Table 19). This might be due to the increased concentration of honey which resulted in increased yellowness of the beverage. This result was well supported by Peerajan *et al.* (2016) in fermented *Phyllanthus emblica* juice by *Lactobacillus paracasei.* However, no visual colour change was observed in all of the treatments during the storage period. Along the storage period decease in *L** value was noticed and it might be due to the oxidation of ascorbic acid or precipitation of the pigments (Sistrunk and Cash, 1974) which contributed to reduction of the luminosity, giving a darker appearance to the juice. Increase in the red colour (*a**) intensity was observed in all the treatments as the storage period advances with maximum *a** value (18.27) at 45 DAS and least at initial period (13.18). A possible reason for the darkened colour is due to the resultant fermentation. This result was well supported by Filannino *et al.* (2013) in organic pomegranate juice fermented by different strains of Lactobacillus plantarum and Khatoon and Gupta (2015) in fermented sugarcane juice. According to Askin and Atik (2016), the temperature during processing and storage of juices can cause changes in the colour of the product.

5.3.8 Lactic acid bacteria population (cfu/ml)

The lactic acid bacterial population was analyzed in all the fermented beverages during storage period of 45 days (Table 20 and Fig. 14). Among the treatments, highest LAB population (5.79×10^6 cfu/ml) was observed in T_7 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 5% lactose) followed by T_6 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 3% lactose; 5.29×10^6 cfu/ml) and lowest bacterial population was observed in T_1 (85% PJ + 15% KJ + *Lactobacillus plantarum*; 1.79×10^6 cfu/ml). The result of the study clearly indicated more survival of LAB when lactose was used as prebiotics. The results of this study are in accordance with Barbosa *et al.* (2016) who reported the higher survival rate for LAB cells grown in the presence of lactose, followed by glucose and fructose in dried orange juice (powder). Addition of honey also showed higher bacterial population than control which might be due to the presence of variety of oligosaccharides (Chow, 2002 and Bansal *et al*., 2005). Among different concentration of honey, highest population of 4.40×10^{6} cfu/ml was observed in T_4 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 5% honey). Similar results were observed by Rotar *et al.* (2015) in goji berries and honey blended yoghurt fortified with LAB (10^{6}-10^{7} log cfu/ml) during 21 days of storage. The maximum cell survivality was observed when the amount of prebiotic substance was at a higher concentration. This result shows stimulatory and protective effect of lactose and honey on survivality of LAB in the beverage. Similar results were obtained in fermentation of honey-enriched milk using Bifidobacterium spp. (Varga *et al*., 2012) and in fermentation of kokum juice blended with 5 per cent honey and 20 per cent sweet potato (Latha, 2012). However, addition of honey resulted in lesser lactic acid bacterial survivality when compared to addition of lactose which might be due to the bactericidal effect exerted by honey in the juice medium (Rotar *et al*.,2015).

Survival of LAB cells above 10^{6} cfu/ml were observed up to 30 days of storage in all the treatments. Maximum cell viability was observed at initial period (7.86×10^{6} cfu/ml). After 30 days of storage 3.40×10^{6} cfu/ml was observed. The glass packages due to its low oxygen permeability favours the survival of LAB during storage period. A general reduction in the viability of LAB during storage might be attributed to the reduction in the sugar level in the juice samples, metabolism of the probiotic LAB, accumulation of organic acid as some metabolites and storage temperature (Yoon *et al.*, 2004) in fermented tomato juice. Yanez *et al.* (2008) reported that an increase in acidity as a result of the fermentation process can reduce the survivability and viability of the probiotic bacteria. Shah (2001) equally reported that the viability of probiotic organisms is dependent on factors such as oxygen level in the product, oxygen permeation of the package, fermentation time, and storage temperature. Furthermore, it may also affected by lactic acid produced during cold storage (4°C). Similar results were observed by Tayo and Akpeji (2016) in fermentation of pineapple juice by LAB. The result of present study is in close conformity with the study of Goderska *et al.* (2007) where the author reported that the best survivability of the *Lactobacillus acidophilus* DSM 20079

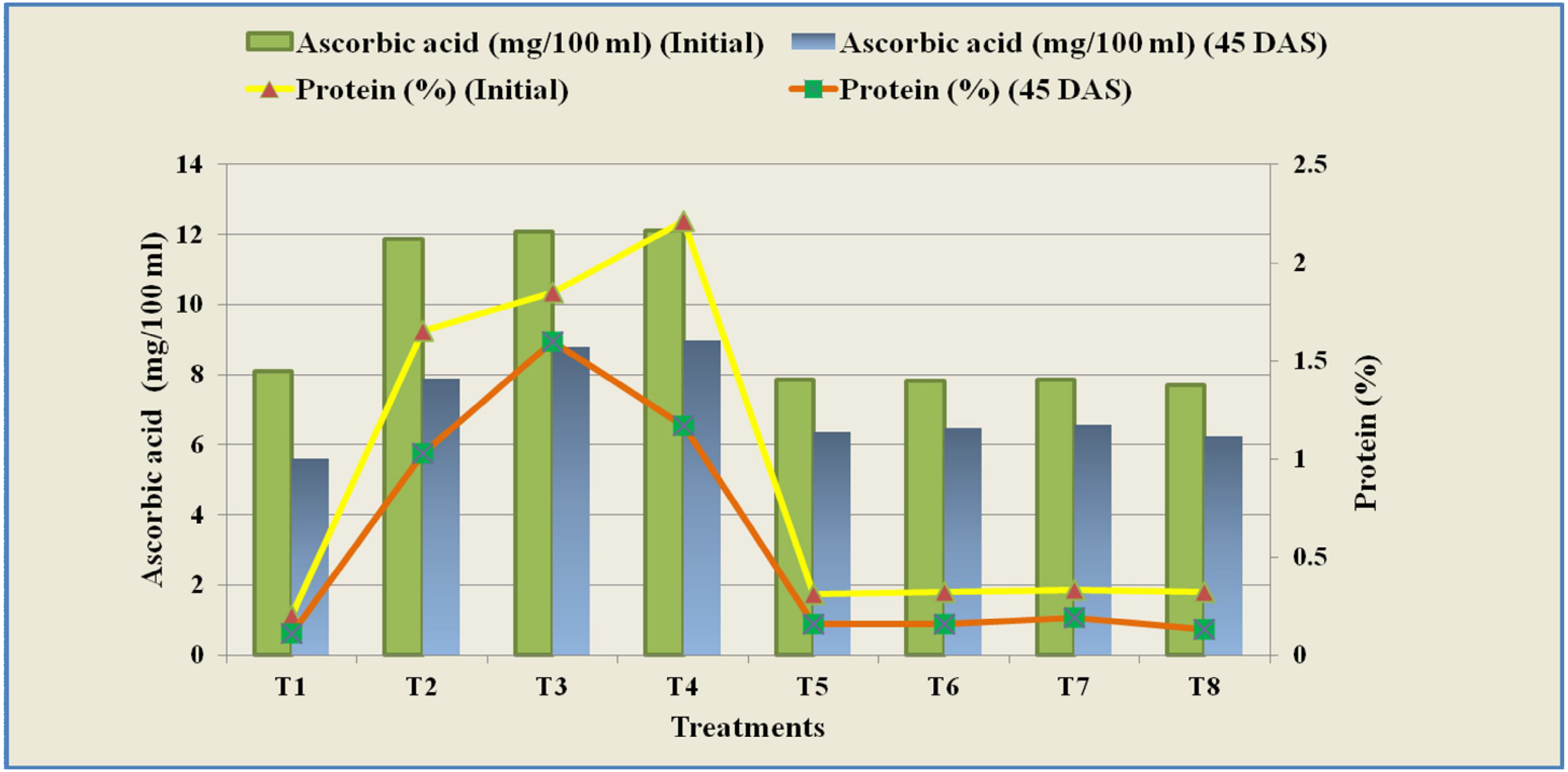


Fig. 13: Effect of prebiotics on ascorbic acid (mg/100 ml) and protein (%) content of fermented pomegranate beverage blended with kokum juice as influenced by treatments and storage period

T_1 - 85% PJ + 15% KJ + *Lp*
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H + 2%L

PJ - Pomegranate juice KJ - Kokum juice ***Lp*** - *Lactobacillus plantarum* (2373) **H** – Honey **L** - Lactose

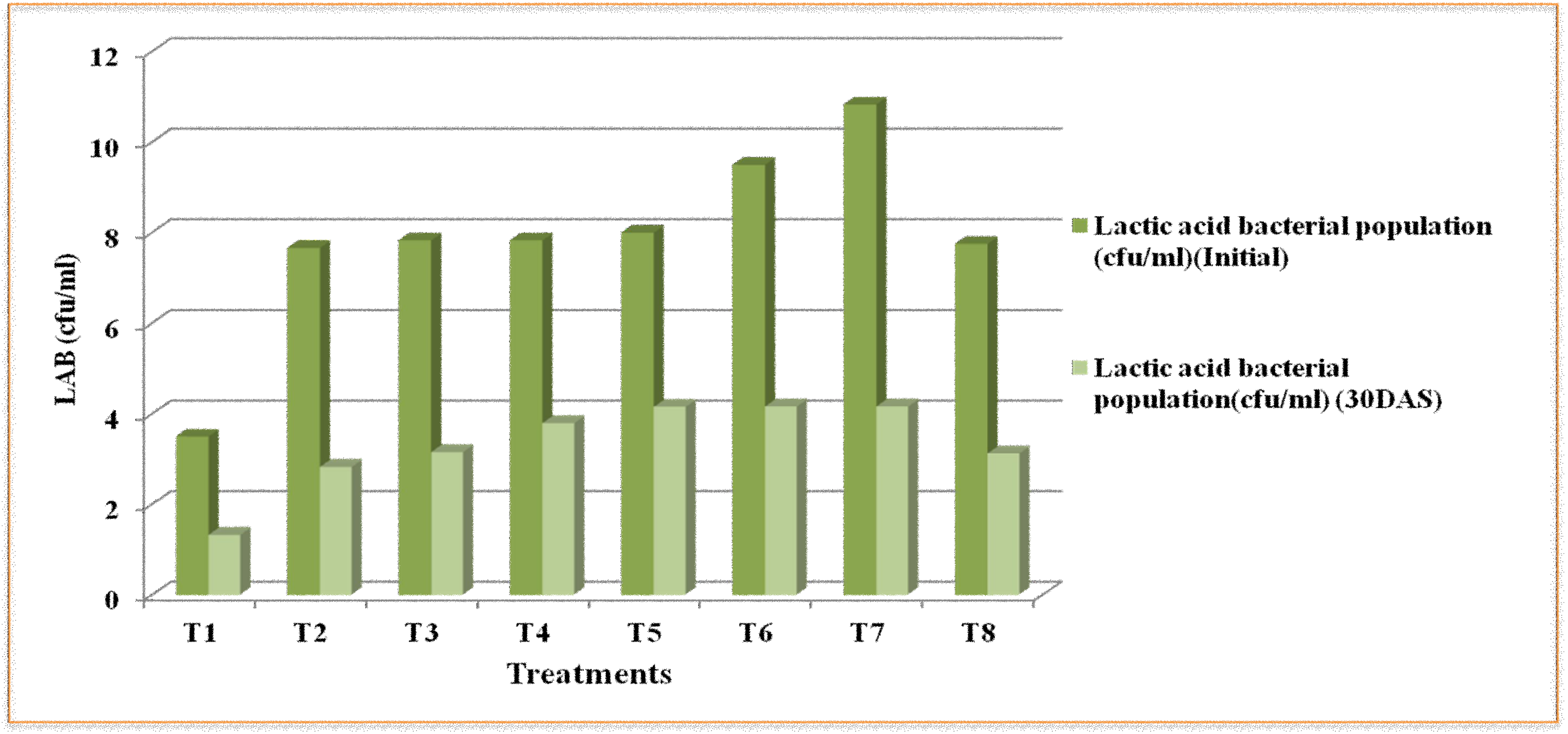


Fig. 14: Effect of prebiotics on lactic acid bacterial population (× 10^6 cfu/ml) of fermented pomegranate beverage blended with kokum juice as influenced by treatments and storage period

T_1 - 85% PJ + 15% KJ + *Lp*
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H + 2%L
PJ - Pomegranate juice
KJ - Kokum juice
Lp - *Lactobacillus plantarum* (2373)
H – Honey
L - Lactose

bacteria in fermented carrot juice up to the 28th day of refrigerated storage was obtained when oat gruel was added as prebiotic substance. Post (1996) reported that some compounds produced during lactic acid fermentation such as lactic acid, diacetyl and acetaldehyde could be associated with the loss of viability of the added probiotic bacteria.

5.3.9 Sensory evaluation

Organoleptic evaluation is generally the final guide of the quality from the consumer's point of view. Thus the evaluation was applied on the lactic acid bacteria fermented beverages. Colour, taste, flavour, consistency and overall acceptability were evaluated.

The mean colour scores of all the treatments decreased from 7.80 to 7.66; flavour scores 7.41 to 6.92; taste scores 7.73 to 6.71; consistency scores from 7.83 to 7.71, overall acceptability scores decreased from 7.70 to 7.27 during 45 days of storage at 4°C (Table 21, 22, 23) (Fig. 15 and 16). A tendency of higher scores for fermented beverages blended with honey was observed T_4 (85% PJ + 15% KJ + *Lactobacillus plantarum* + 5% honey). Fermented juices with honey had more acceptable taste and flavour than the juice with lactose and sugar free juice. This might be due to organic acid and characteristic aroma compounds formation through consumption of fructose and glucose by lactic acid bacteria. The aroma is formed by non-volatile acids, volatile acids, carbonyl compounds and miscellaneous compounds (Tamime and Robinson, 2000). These results are in conformity with the observations of Sivudu *et al.* (2014) in the probioticated mixed watermelon and tomato juice. The flavour of the fermented juice with lactose was less appreciated than the fermented juice without prebiotics (Control). Beverages blended with honey resulted in more sweet taste due to presence of oligofructose had released free sugars into the medium (fructose) which increased the intensity of the sweet taste detected by the panellist. Similar results were observed in the study conducted by the Pimentel *et al.* (2015) in clarified apple juice with oligofructose and sucralose as sugar substitute fermented by *Lactobacillus paracasei*. Profir *et al.* (2015) reported that the best average values were obtained by vegetable juice enriched with 2-3 g/100 ml freeze dried vegetables pulp and 5-7.5 g/100 ml of honey. The overall acceptability was strongly correlated with the taste and flavour but not with the visual

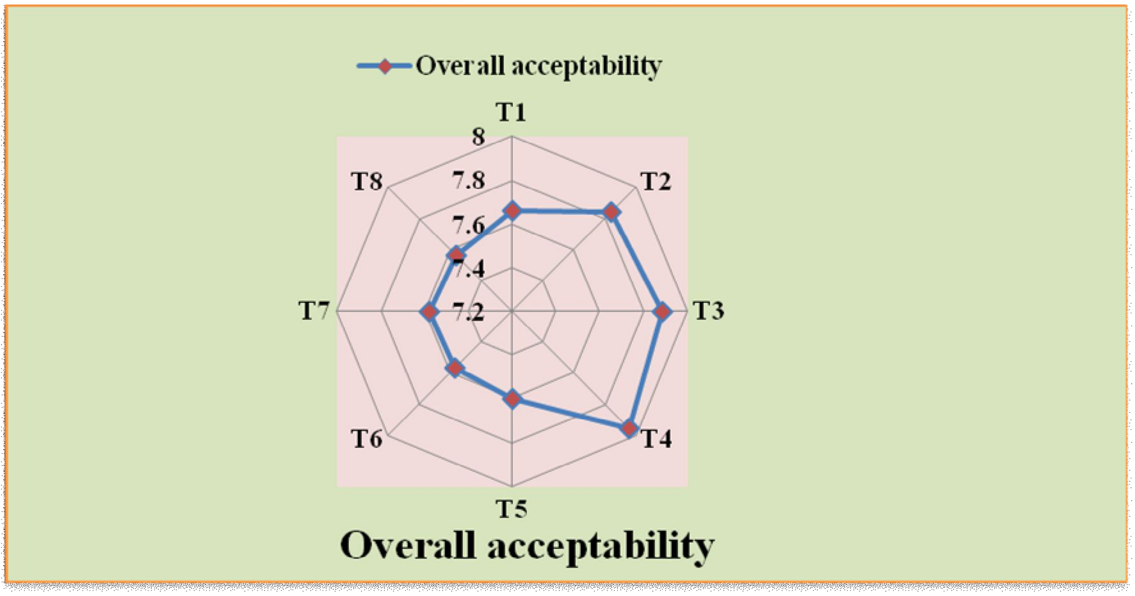


Fig. 15: Effect of prebiotics on overall acceptability of fermented pomegranate beverage blended with kokum juice at initial period of storage

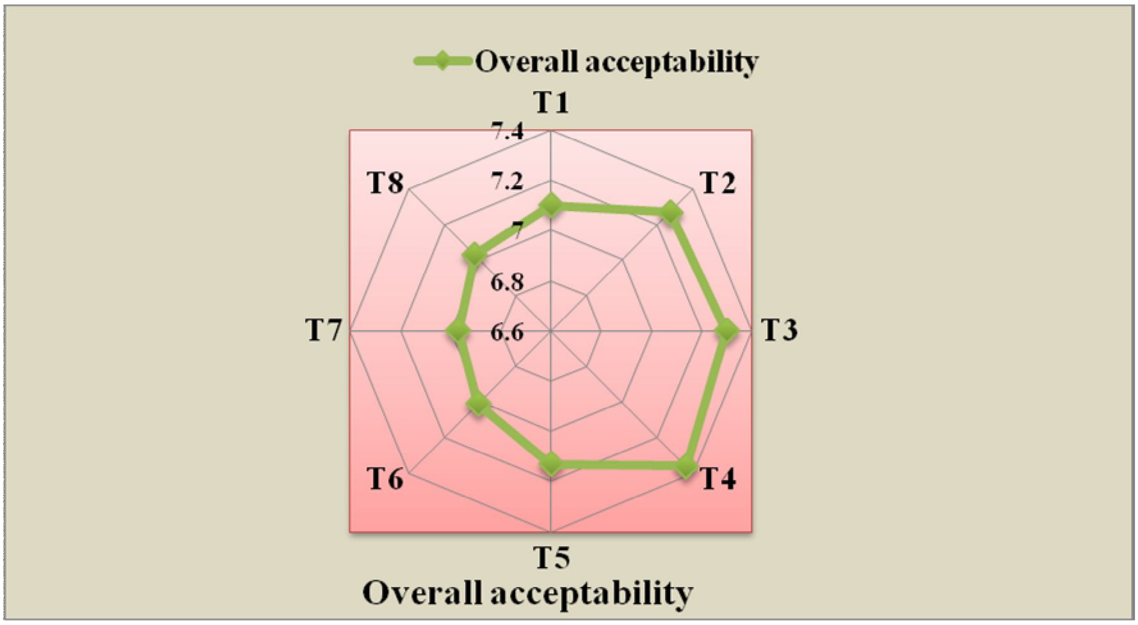


Fig. 16: Effect of prebiotics on overall acceptability of fermented pomegranate beverage blended with kokum juice after 45 days of storage

T_1 - 85% PJ + 15% KJ + *Lp*
T_2 - 85% PJ + 15% KJ + *Lp* + 2% H
T_3 - 85% PJ + 15% KJ + *Lp* + 3% H
T_4 - 85% PJ + 15% KJ + *Lp* + 5% H
T_5 - 85% PJ + 15% KJ + *Lp*+ 2% L
T_6 - 85% PJ + 15% KJ + *Lp*+ 3% L
T_7 - 85% PJ + 15% KJ + *Lp*+ 5% L
T_8 - 85% PJ + 15% KJ + *Lp*+ 2% H + 2%L

appearance. The scores of all sensory characters of lactic acid bacteria fermented beverage decreased during storage period of 45 days which might be due to the biochemical changes and production of organic acids through the activity of microorganisms resulting in increased acidity. Rodbotten *et al.* (2009) reported that acidity is an attribute that decreases the consumer acceptance of juices and the higher intensity in sweet taste suppresses the perception of acidity.

Future line of work

1. Studies on bioavailability of vitamins and nutrients through lactic acid bacteria fermentation of fruit juices can be carried out

2. Lactic acid bacteria mediated fermentation by blending kokum pulp extract with other fruit juices can be carried out

3. Effect of different fermentation condition and different fruit substrates to improve shelf life and survivality of lactic acid bacteria during different storage conditions and packaging material can be studied

6. SUMMARY AND CONCLUSIONS

An investigation entitled “Studies on fermentation of pomegranate juice by lactic acid bacteria” was carried out in the laboratory of Department of Post-Harvest Technology, College of Horticulture, Bagalkot during the year 2016-2017. The present study was carried out to standardise the protocol for preparation of lactic acid bacterial fermented pomegranate beverage blended with kokum and effect of prebiotics on fermentation of pomegranate juice blended with kokum to study the biochemical, microbial shelf life and storage stability of these products as influenced by the treatments and storage period. Salient features of the present investigation are summarised here under.

6.1 Development of fermented pomegranate beverage with and without kokum juice using probiotic lactic acid bacteria

In the present experiment, fermented beverage was prepared with different proportion of pomegranate juice and kokum juice using three lactic acid bacterial strains.

Among the different treatments, 100 per cent pomegranate juice fermented by *Lactobacillus plantarum* (T_3) obtained maximum score for colour (7.83) and flavour (7.14) followed by the treatment with 85 per cent pomegranate juice blended with 15 per cent kokum juice fermented by *Lactobacillus plantarum* (T_6). Maximum score for consistency (7.16), taste (8.30) and overall acceptability (7.30) was observed in the treatment with 85 per cent pomegranate juice blended with 15 per cent kokum juice fermented by *Lactobacillus plantarum* (T_6).

Overall highest average score (7.51) was found in treatment with 85 per cent pomegranate juice blended with 15 per cent kokum juice fermented by *Lactobacillus plantarum* (T_6). Out of thirteen treatments evaluated, eight treatments were most acceptable by panelists *viz.*, T_6 (85% PJ + 15% KJ + *Lactobacillus plantarum*), T_5 (85% PJ + 15% KJ + *Lactobacillus acidophilus*), T_3 (100% PJ + *Lactobacillus plantarum*), T_8 (75% PJ + 25% KJ + *Lactobacillus acidophilus*), T_1 (Uninoculated pomegranate juice), T_7 (85% PJ + 15% KJ + *Lactobacillus delbrueckii*), T_{10} (75% PJ + 25% KJ +

Lactobacillus delbrueckii) and T_{11} (65% PJ + 35% KJ + *Lactobacillus acidophilus*) with average scores of 7.51, 7.45, 7.43, 7.37, 7.36, 7.34, 7.26 and 7.24 respectively. These best eight treatments were taken for further storage studies for biochemical composition, microbial and sensory qualities.

6.2 Biochemical quality and sensory evaluation of fermented pomegranate beverage with and without kokum juice during storage

Out of thirteen treatments, best eight treatments from sensory evaluation were taken for storage studies and were analysed for biochemical, sensorial and microbial parameters.

Analysing the fermented pomegranate beverage with and without kokum juice during storage period for biochemical and microbial parameters signified significant differences between the treatments. Among the different treatments over the storage period of 45 days, treatment with 65 per cent pomegranate juice blended with 35 per cent kokum juice fermented by *Lactobacillus acidophilus* (T_{11}) showed maximum mean citric acid (1.64%) and lactic acid (2.25%) content, minimum pH (2.48), TSS (10.51° brix), total sugar (10.60%) and reducing sugar (2.89%) content. During storage, mean scores for citric acid and lactic acid percent increased from initial period up to 30 days of storage viz., 0.89 to 0.99 per cent and 1.24 to 1.45 per cent, respectively. pH, TSS, total sugar and reducing sugar content decreased significantly from 2.99 to 2.85, 12.44 to 11.95° brix, 11.91 to 11.61 per cent and 4.52 to 4.37 per cent respectively.

Among the different treatments, highest mean value for ascorbic acid (10.05 mg/100 ml), antioxidant activity (77.07%) and total phenol content (252.00 mg GAE/100 ml) was found in treatment with 85 per cent pomegranate juice blended with 15 per cent kokum juice fermented by *Lactobacillus plantarum* (T_6). Significant decrease in the mean scores of ascorbic acid, antioxidant activity and total phenol content was observed which varied from 8.87 to 5.60 mg/100 ml, 77.60 to 63.69 per cent and 248.32 to 238.894 mg GAE/100 ml respectively during the storage period of 45 days.

Among the different treatments, lowest mean for lightness (*L** value) (10.47) and highest mean for *a** and *b** values (28.14 and 12.15 respectively) were noted in treatment with 65 per cent pomegranate juice blended with 35 per cent kokum juice fermented by *Lactobacillus acidophilus* (T_{11}). However no visual difference was observed between the treatments and all treatments were acceptable with respect to colour. During storage, decreased trend was observed in mean scores for *L** and b* values from initial period to 45 DAS *viz.,* 14.17 to 8.37 and 13.49 to 8.48, respectively. Increased trend was observed in mean scores for redness (*a** value) from initial period to 45 DAS *viz.*, 16.16 to 20.95.

With respect to lactic acid bacterial population, highest (2.91×106 cfu/ml) mean was observed in treatment with 85 per cent pomegranate juice blended with 15 per cent kokum juice fermented by *Lactobacillus plantarum* (T_6). During storage, mean highest population was observed at initial period (2.79×10^6 cfu/ml) and the least at 30 DAS (0.58 × 106 cfu/ml).

Organoleptic score for colour (7.83), flavour (7.77), taste (7.82), consistency (7.97) and overall acceptability (7.85) was highest in treatment with 85 per cent pomegranate juice blended with 15 per cent kokum juice fermented by *Lactobacillus plantarum* (T_6). During storage period the mean scores decreased from initial value of 7.99 to 7.66, 7.84 to 7.63, 7.65 to 7.00, 8.05 to 7.30 and 7.85 to 7.40 for colour, flavour, taste, consistency and overall acceptability respectively. It indicates an eventual decrease in consumer's acceptance due to changes in biochemical constituents of beverage during storage. Considering the performance with respect to colour, flavour, taste, flavour and overall acceptability of fermented beverage it was understood that all the beverages were more acceptable during initial period of storage.

6.3 Effect of different prebiotics on fermentation of pomegranate juice with kokum blend using probiotic lactic acid bacteria during storage

With respect to combination of juices and LAB strain, the best treatment from experiment II was selected for experiment III based on biochemical, sensory and microbial properties. Different concentration of prebiotics (honey and lactose) was used in the fermentation process and analysed their effect on biochemical, sensorial and microbial parameters.

Among the different treatments over the storage period of 45 days, treatment with 85 per cent pomegranate juice blended with 15 per cent kokum juice and 5% lactose fermented by *Lactobacillus plantarum* (T_7) showed highest mean citric acid (0.84%) and lactic acid (1.32%), minimum pH (2.79), TSS (10.79° brix), total sugar (10.74%) and reducing sugar (3.51%) content. During storage period mean citric and lactic acid content increased from initial period to 45 DAS *viz.,* 0.76 to 0.82 per cent and 1.07 to 1.20 per cent, respectively. The mean scores of pH, TSS, total sugar and reducing sugar content decreased significantly during storage period from initial period to 45 days *viz.,* 3.29 to 2.78, 11.58 to 11.22° brix, 11.49 to 11.17 per cent and 3.82 to 3.57 per cent respectively.

Irrespective of storage period, the highest mean antioxidant activity (80.40%), total phenol content (257.73 mg GAE/100 ml), ascorbic acid (10.52 mg/100 ml and protein content (1.93%) was observed in treatment with 85 per cent pomegranate juice blended with 15 per cent kokum juice and 5 per cent honey fermented by *Lactobacillus plantarum* (T_4). During storage period, antioxidant activity and total phenol content was found to be highest at 15 DAS (83.43% and 251.68 mg GAE/100 ml respectively). Among interactions, the maximum antioxidant activity (85.81%) and total phenol content (261.00 mg GAE/100ml) was recorded in T_4S_2. During storage period of 45 days mean scores of ascorbic acid and protein content decreased significantly from an initial value of 9.42 to 7.12 mg/100 ml and 0.90 to 0.63 per cent.

The highest mean lightness (L^*) value was observed in treatment with 85 per cent pomegranate juice blended with 15 per cent kokum juice and 5 per cent lactose fermented by *Lactobacillus plantarum* (T_7; 14.43). Highest mean score for redness (a^*) and yellowness (b^*) values were found in treatment 2 per cent honey (T_2; 17.85) and 5 per cent honey (T_4; 12.83) respectively. It was found that addition of 5 per cent honey resulted in increased yellowness of fermented beverage. .During storage period highest mean score for L^* and b^* values were observed at initial period (17.45 and 14.11 respectively) where as redness (a^*) value of beverage increased during storage with highest score at 45 DAS (18.27).

The highest mean probiotic lactic acid bacterial population (5.79×10^6 cfu/ml) was observed in treatment with 85 per cent pomegranate juice blended with 15 per cent kokum juice and 5 per cent lactose fermented by *Lactobacillus plantarum* (T_7). Among different concentration of honey, treatment mean with 5 per cent honey (T_4) showed maximum LAB counts (4.40×10^6 cfu/ml). During storage period, highest lactic acid bacterial population was observed at initial period (8.70 × 106 cfu/ml) and lactic acid bacterial population was not detected after 45 days. However, mean population after 30 DAS was found to be 3.40 × 106 cfu/ml.

Significantly highest mean score for overall acceptability (7.70) was found in treatment with 5 per cent honey (T_4). Significantly highest mean score for flavour (7.41) and taste (7.73) was found in treatment with 5 per cent honey (T_4). No visual colour difference was observed but numerically highest mean colour score was found in treatment with 3 per cent honey (T_3). Numerically highest score for consistency (7.83) was observed in treatment with 5 per cent honey. During storage period of 45 days, mean organoleptic scores decreased from initial value of 7.92 to 7.62 for colour, 7.60 to 6.67 for flavour, 7.43 to 6.72 for taste, 7.91 to 7.67 for consistency and 7.71 to 7.14 for overall acceptability. Fermented beverage with lactose was least appreciated due to higher acidity.

Conclusion

1. Among the three species of lactic acid bacteria used for fermentation of juices *Lactobacillus plantarum* found to be the best for fermentation of pomegranate juice with 15 per cent kokum juice with respect to enhancement of nutrients, superior sensory and microbial properties.

2. Fermentation of pomegranate-kokum blended juice with honey as prebiotic showed superior biochemical characteristics, sensory properties and also supports the survival of LAB particularly at 5 per cent concentration.

3. Fermentation of pomegranate-kokum blended juice with lactose resulted in comparatively higher lactic acid bacterial population with higher survivality in 5 per cent lactose, but less appreciated with respect to biochemical characteristics and sensory properties.

4. Microbial processing of kokum juice into non alcoholic fermented beverages can minimize the extent of post harvest losses in kokum during glut season

5. Fermentation of juice by lactic acid bacteria serves as an alternative method for preserving fruit juices without the use of chemical preservative.

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Appendix I. Score card for evaluation of fermented beverages

Name of the product:

Name of the panel member:

Date:

Score system: 9 point hedonic scale

Rating: 1-dislike extremely
2-dislike very much
3-dislike moderately
4-dislike slightly
5-neither like nor dislike
6-like slightly
7-like moderately
8-like very much
9-like extremely

Treatments	**Colour***	**Flavour***	**Taste***	**Consistency***	**Overall acceptability***
Comments :					

Signature of the evaluator

Appendix II: Biochemical properties of different combinations of pomegranate and kokum juice before LAB inoculation

Juice combinations	pH	Citric acid (%)	Lactic acid (%)	TSS (° brix)	Total sugar (%)	Reducing sugar (%)	Antioxidant activity (%)	Total phenol (mg GAE/ 100 ml)	Vitamin C (mg/ 100 ml)
100% PJ	3.40	0.40	0.08	18	17.85	10.25	64.04	235.34	3.71
85% PJ + 15% KJ	3.35	0.43	0.10	18	17.74	10.22	64.14	235.65	3.70
75% PJ + 25% KJ	3.30	0.49	0.12	18	17.62	10.20	65.21	236.00	3.67
65% PJ + 35% KJ	3.28	0.51	0.14	18	17.58	10.17	65.34	236.21	3.65

UPJ - Uninoculated pomegranate juice**;** **PJ -** Pomegranate juice; **KJ -** Kokum juice; **LAB** - Lactic acid bacteria

Appendix III: Yield of juice and waste index of pomegranate fruit

	Pomegranate juice
Yield of juice (%)	**48.43**
Waste index (%)	**51.56**

Appendix IV. Details of cultures / strains used in the study:

Sl. No.	Cultures	NCIM catalogue number	Source
1.	*Lactobacillus acidophilus*	2903	NCIM, Pune
2.	*Lactobacillus plantarum*	2373	NCIM, Pune
3.	*Lactobacillus delbrueckii*	2025	NCIM, Pune

NCIM - National collection of industrially important microorganism

Appendix V: Detailed information of honey and lactose used in the experiment

Honey

Product Name	: Dabur Honey
Make	: DABUR INDIA LIMITED
Lot No.	: BDO737
Packed Date	: 01/17

Lactose

Product Name	: Lactose monohydrate
Make	: HIMEDIA
CAS No.	: 10039-26-6
Synonym	: Milk sugar

Appendix VI: Cost of ingredients for production of one litre of lactic acid bacteria fermented pomegranate beverage blended with kokum juice and honey

Sl No.	Items	Quantity required(for one litre beverage)	Rate (Rs.)	Amount (Rs.)
1	**Pomegranate fruits**	2.12 kg	80/kg	169.60
2	**Dried kokum rind**	10 g	70/kg	0.70
3	**Honey**	50 g	740/kg	37.00
4	**Glass bottle with cap**	5 bottles	5	25.00
5	**MRS media**	3 g	6440/kg	19.92
	Total cost/litre			252.22

Note: The above ingredients were calculated as per best treatments (85% pomegranate juice + 15% kokum juice + 5% honey + *Lactobacillus plantarum*) in the present study

STUDIES ON FERMENTATION OF POMEGRANATE JUICE BY LACTIC ACID BACTERIA

SHUBHADA, N. **2017** **Dr. RUDRESH, D. L.**
Major Advisor

ABSTRACT

The experiment entitled "Studies on fermentation of pomegranate juice by lactic acid bacteria" was conducted in the Department of Post-Harvest Technology, College of Horticulture, Bagalkot during the academic year 2016-17.

Fermented beverages were prepared with different proportions of pomegranate and kokum juice using different lactic acid bacterial (LAB) strains. Among the thirteen treatments, maximum score for consistency (7.16), taste (8.30) and overall acceptability (7.30) was observed in the treatment with 85% pomegranate juice (PJ) blended with 15% kokum juice (KJ) fermented by *Lactobacillus plantarum* (T_6).

The fermented pomegranate beverage with and without KJ were analysed for biochemical and microbial parameters during storage period. Among the different treatments, highest mean value for ascorbic acid (10.05 mg/100 ml), antioxidant activity (77.07%), phenol content (252.00 mg GAE/100 ml), LAB population (2.91×10^6 cfu/ml) and overall acceptability (7.85) were found in treatment with 85% PJ blended with 15% KJ fermented by *Lactobacillus plantarum* (T_6).

In the experiment III, best treatment (T_6: 85% PJ + 15% KJ fermented by *Lactobacillus plantarum*) from experiment I was blended with different concentration of prebiotics (honey and lactose). Among different concentration of prebiotics, blended pomegranate beverage with 5 per cent honey as prebiotic and fermented by *Lactobacillus plantarum* (T_4) showed the highest mean antioxidant activity (80.40%), phenol content (257.73 mg GAE/100 ml), ascorbic acid (10.52 mg/100 ml) and protein content (1.93%) during 45 days of storage period. The mean LAB population of 4.40×10^6 cfu/ml was observed in T_4 during storage period of 45 days.

In conclusion, the *Lactobacillus plantarum* fermented beverage, prepared by blending of 85% pomegranate juice + 15% kokum juice with 5% honey (prebiotic) showed superior biochemical and sensory properties up to 45 days at 4^oC. The blended beverage also supported the survival of lactic acid bacteria up to 30 days under refrigerated condition.

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««zsÀ ¯ÁåQÃÜ DªÀÄè¨ÁåQÖÃjAiÀÄ ¥ÀæªÉÃzsÀUÀ¼À §¼ÀPÉAiÀÄ zÁ½A¨É ªÀÄvÀÄÛ PÉÃPÀ gÀ¸ÀUÀ¼À ««zsÀ ¥ÀæªÀiÁtzÀ°è«Ä±Àæt ªÀÄr ºÀÄzÀÄV¹ ¥Á¤AiÀÄUÀ¼À£ÀÄß vÀAiÀiÁj¸À¯ÁUÀÄvÀÛzÉ. MlÄÖ ºÀÄ½ªÀÄ¥ÀgÀ G¥ÀÖ¼ÁgÀUÀ¼À°è UÀjµÀÖ ¹ÛgÀvÉ (7.16), gÀÄa (8.30) ªÀÄvÀÄÛ MmÁÖgÉ ¹éÃPÁgÁºÀðvÉAiÀÄ£ÀÄß (7.30) ¯ÁåQÃÜ DªÀÄè¨ÁåQÖÃjAiÀÄ ¥ÀæªÉÃzsÀ ¯ÁåPÉÆÖÃ¨Á¹¯ï¸ï ¥ÁèAmÁgÀªÀiï¤AzÀ ºÀÄzÀÄV¹zÀ ¥Á¤AiÀÄzÀ°è(±ÉÃ 85 zÁ½A¨ÉgÉ¸À ªÀÄvÀÄÛ ±ÉÃ 15 PÉÃPÀ gÀ¸À: T6) zÁR°¸À¯ÁVzÉ.

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