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Physico-Chemical, Antioxidant, Microbial and Sensory Properties of Probiotic Dairy Beverage and Fruit Juice with L. casei

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ABSTRACT

Recently, there has been an increase in demand for probiotic dairy and probiotic fruit juices due to new nutritional trends. This study was carried out to evaluate the functional properties of probiotic dairy and probiotic fruit juices (pumpkin and guava juice) with *L. casei* during storage periods (0,5 and 10 days) at refrigerator temp (2-6° C). Physicochemical (pH, minerals, T.S.S., viscosity, total phenolic content and DPPH), microbiological (Total count, *L. casei* and mold &yeast) and sensory evaluation were investigated. Four treatments were studied, probiotic milk (FM), pumpkin juice (FP), guava juice (FG) and a mix of milk, pumpkin and guava juices (FX). Results indicated that the highest value of pH was for probiotic guava (FG). The lowest value of iron was for probiotic milk (0.08mg/100g) and the highest value was for probiotic pumpkin (0.57 mg/100g). The highest contents of magnesium and potassium were for probiotic guava juice (24.53 and 458.23 mg/100g, respectively). The highest value of total phenols was for FP. Values of pH, DPPH and total phenols decreased during the storage period (10 days) for all treatments. The highest value of total antioxidant as DPPH was for FP. Total count and *L. casei* increased during the first 5 days and then decreased at the end of storage period (10 days). There was no detected growth for molds and yeasts during the cold storage period (10 days) for all treatments. The highest values of overall acceptability were for FP and the lowest values were for control treatment.

Keywords: Fermented, Milk, Guava, Pumpkin.

Introduction

According to FAO/WHO (2010), Probiotics are live microorganisms (mainly bacteria and a few numbers of yeast strains) that, when administered in the proper quantities, can improve the health of the host. The greatest probiotic carriers have traditionally been thought of as probiotic milk products; however, vegetarianism, allergies, dyslipidemia, and lactose intolerance may place certain regulations on the utilization of items containing milk products. As a result, a variety of raw ingredients have been thoroughly investigated to see if they are viable bases for producing innovative functional foods without milk **(Vasudha and Mishra, 2013).**

Due to current trends in nutrition, consumers with respect for healthy lifestyles, the percentage of vegetarians and vegans increases in developed countries nations, as well as the low cost of getting plant materials, there has recently been an increase in interest in nondairy-probiotic products (probiotic fruit juice). **(Nasef et al.,2020).**

Beans or substances containing carbohydrates can be used to make probiotic beverages. Fruit juices include a lot of sugar, which may encourage the growth of probiotics. Juices made from fruits and vegetables have been reported to be rich in the most effective probiotics delivery systems because they are rich in vitamins and antioxidants and suitable for lactose intolerant people. Components that have previously been identified as coming from cow's milk or buffalo milk are processed by fermentation with the addition of a starting culture of lactic acid bacteria (*Lactobacillus and Bifidobacterium*), such as yoghurt, or without a starter, like a curd. **(Vodnar et al., 2019)**. Pumpkin may be used to create probiotic beverages and contains a high concentration of antioxidants and nutritional fiber. **(Bhat A. and Bhat M. A. ,2013**).

The guava fruit is commonly grown in tropical and subtropical areas and is becoming more and more well-liked everywhere. Guava fruit often has an attractive aroma that is sweet and fruity **(Bashir and Abu-Goukh, 2003).** Some of the most researched and used species as probiotics include *Lactobacillus casei*, closely related species *L. paracasei*, and *L. rhamnosus*. Their primary commercial use is in the dairy industry, which results in meals with better flavor and texture **(Hill et al., 2018).**

Lactic acid fermentation enhanced the flavors and nutritional worth of fruits and vegetables while reducing their toxicity. Probiotic fruit and vegetable juices with added probiotics are supplemental foods that improve overall health. (**Aditya Chaudhary, 2019)** The development of fruit-based probiotics has made it possible for those who are lactose intolerant, allergic to casein, hypercholesterolemic, and strict vegans to eat these bacteria **(Pimentel T C, (2017).**

As a result, this study suggested that probiotic dairy and fruit beverages have many health benefits. Fruit juices could be a good substitute for probiotic products based on dairy especially the increasing of vegetarian populations in developing nations, in addition to the low cost of these beverages.

Material and methods

Preparation of bacterial strain

Starter strain Lactobacillus casei subsp. casei ATTC393 got from Microbiology Dairy Lab., National Research Centre. L. casei subsp. casei, was individually activated by three successive transfers in modified MRS then incubated at 37°C for 48 h. After excellent coagulate formation formed that contained 106 CFU/g, the cultures were stored in a refrigerator at 4 °C to be used.

Pumpkin juice preparation

Pumpkins (*Cucurbita moschata* Duch.) at commercial mature were brought from local market in Tanta, Egypt and stored at 4 °C. The pulp was chopped into cubes (3 cm3) after removing the peel and seeds. After that, the pre-cut pumpkins were blanched for 10 minutes in boiling water (1:3 w/w) and mixed in the blender then filtered with cheese clothes, after that pasteurized at 80°C for 15 min and subsequently cold at 4°C.

Preparation of guava juice

Guava was cleaned in running water. Guava juice was produced from freshly picked and completely ripe guava fruit, blended in a sterile stainless-steel blender filtered with cheese clothes After that pasteurized at 80°C for 15 min and subsequently cold at 4°C **(Vieira and Silva, 2014).**

Preparation of mixed milk, pumpkin and guava juices

Mix milk, pumpkin juice and guava juice at ratio of 1:1:1.

Physicochemical analysis of probiotic milk and fruit juice

pH value determination

All fruit juice samples' pH values were tested using a digital pH meter. (model3505-JENWAY - UK) after (0 times, 5 days, 10 days) at 25°C as described in **AOAC (2012).**

Total soluble solids determination

A refractometer Carl Zeiss, Jena (Germany), was used to measure the total soluble solids (T.S.S.) at 20 °C. According to **AOAC (2012),** a correction was applied for different temperatures, and the results were presented as °Brix at 20°C.

Viscosity determination

Viscosity was determined using a Brookfield DV-E viscometer. As stated by Denin-Djurdjevic et al. (2002), all samples were run on Spindle No. 3 at 30 rpm. At a temperature of 5°C, the product's viscosity was measured.

Total phenolic content determination

The Folin-Ciocalteu method determined the total phenolic content **(Zilic et al., 2012).** In general, the extract (100 L) was put into a test tube, the volume was adjusted to 3.5 mL with distilled water, and the Folin-Ciocalteau reagent was added to oxidize the extract. After 5 minutes, 1.25 mL of a 20% aqueous sodium carbonate (Na_2CO_3) solution was added to the mixture to neutralize it. The absorbance was measured at 725 nm towards a solvent blank after 40 minutes. A calibration curve made using gallic acid and represented as mg of gallic acid equivalent (mg GAE) per g of sample was used to calculate the total phenolic content.

Determining the radical DPPH scavenging activity

According to **Hwang and Do Thi (2014)**, the stable DPPH* was used to calculate the extracts' potential to scavenge free radicals. For DPPH, the final concentration was 200 M, while the reaction volume was 3.0 mL. after 60 minutes of incubation in the dark, the absorbance was measured at 517 nm against a blank of pure methanol. The following equation was used to determine the percentage of the DPPH free radical that was inhibited:

$$\text{Inhibition } (\%) = 100 \times \frac{\text{A control} - \text{A sample}}{\text{A control}}$$

Results were presented in milligrams of Trolox equivalents (TE) per gram of material. If the observed DPPH value exceeded the linear range of the standard, more dilution was required.

Milk, Pumkin juice, Guava juice and Mixed (milk, pumpkin juice and guava juice) → Heat treatment at 80°C for 15 min → Cooling into 4°C →

Heat all samples for 40°C → Adding starter (*L.casei* ATTC393) at ratio 2%

Incubation at 40°C for 24 hr → cooling into 4°C → Storing for 10 days at 4°C

Fig.1 Flowchart for producing probiotic fruit juice and milk.

Microbial analysis

The viable counts were determined using the plate coating method., *L. casei* was determined using MRS agar containing 0.06% bile salt **(Chen et al., 2011)**. Results are given as colony forming units (CFU/ml) of the sample.

Sensory Analysis

A sensory examination was performed. On the Hedonic scale of nine points, Panelists were requested to rate their level of liking: 1=extreme dislike, 2=dislike strongly, 3=dislike moderately, 4=dislike slightly, 5=both like and dislike, 6 = like slightly, 7= like moderately, 8=like a lot, and 9 = like extremely. To score the sample's overall acceptability, including its colour, flavor, and texture according to **Min et al. (2003).**

Statistical Analysis

The statistical analysis was carried out using SPSS 20.0. The significance of any changes ($p < 0.05$) was determined using Duncan's multiple range method.

Results and Discussion

Physicochemical properties of probiotic milk and fruits juices with *L. casei*

Total soluble solids (TSS) of probiotic milk and probiotic juices with probiotic strains (*L. casei* subsp. *casei*) during the storage period (Zero time, 5 days and 10 days) at refrigerator temp. (2-6 °C) values are shown in **Fig. (2).** There were no significant differences in the values of (TSS) for treated samples during the period of storage. These obtained results were in agreement with **Nasef et al., (2020).**

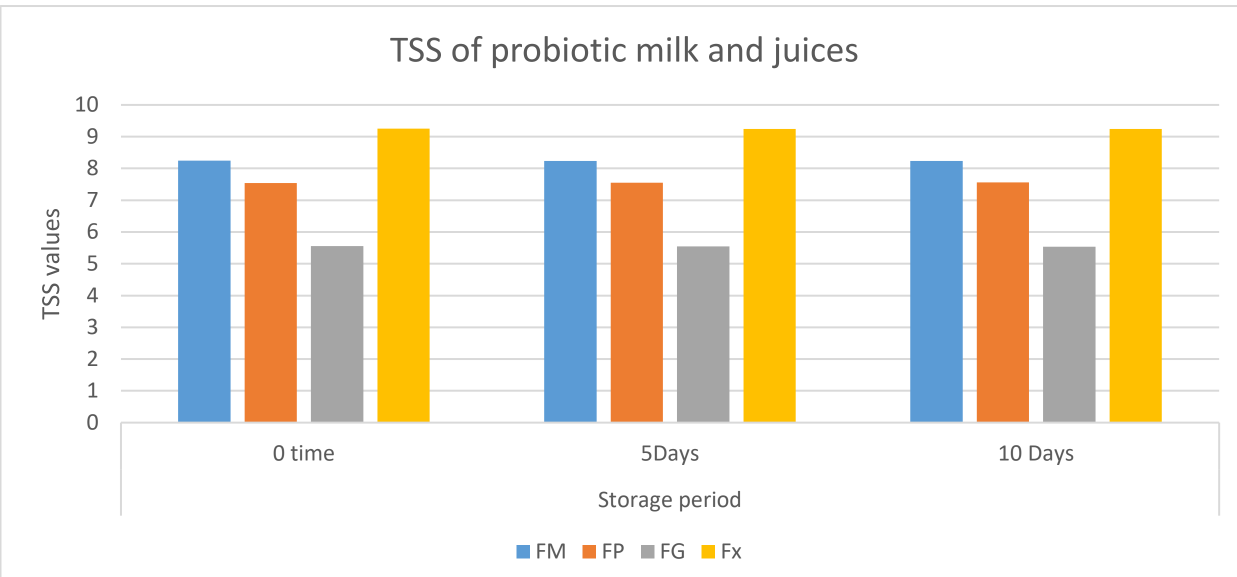

Fig.2 TSS of probiotic milk and juices during storage period (10 days)

FM= Probiotic Milk

FP= Probiotic Pumpkin juice

FG= Probiotic Guava juice

Fx= Probiotic Mix of juices with milk

Fig.3 shows pH values of probiotic milk and juices. pH value of probiotic milk was the lowest value. Lactose was transformed to lactic acid by *L.casei* during the fermentation process. The highest value of pH was for probiotic guava (FG) followed by probiotic mix (FX) and then probiotic pumpkin juice (FP). pH values of all treatments decreased during the storage period (10 days). These results were in agreement with **El-Sayed et al., (2016)** who studied the properties of carrot juice with probiotic bacteria.

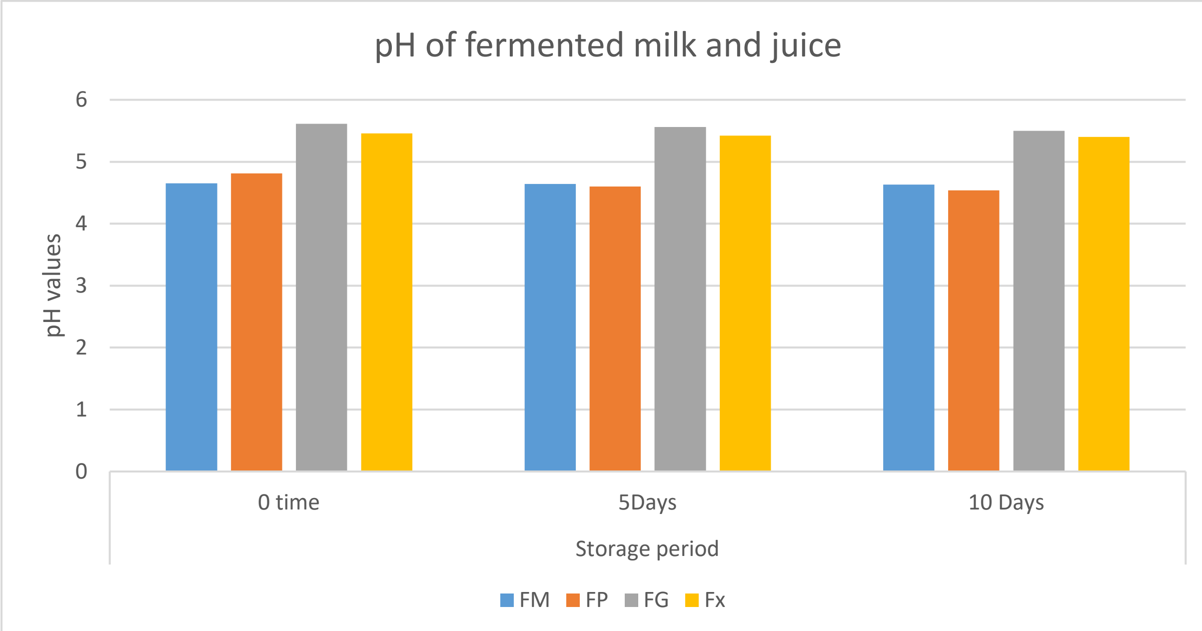

Fig.3 pH of probiotic milk and juices during storage period (10 days)

Fig.4 shows the viscosity of probiotic milk and juices during storage period (10 days). The viscosity value of FM was the highest value compared with other treatments. It may be due to the fermentation of lactose and casein which increased viscosity. Viscosity values increased during the storage period (10 days). These results were in agreement with **Costa et al., (2017)** who resulted that the viscosity of fermented fruit juices with Lactobacillus species increased of the end product storage due to rising of polysaccharides forming during storage.

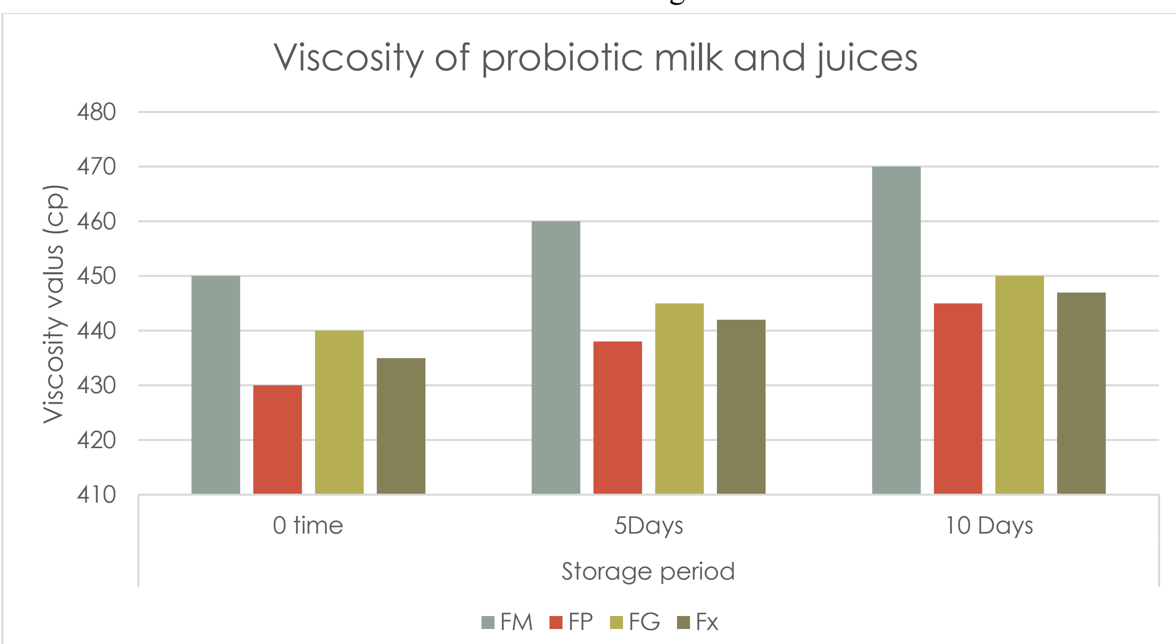

Fig.4 Viscosity of probiotic milk and juices during storage period (10 days) where: FM= Probiotic Milk, FP= Probiotic Pumpkin juice, FG= Probiotic Guava juice, Fx= Probiotic Mix of juices with milk.

Minerals of probiotic milk and juices during storage period (10 days)

Table (1) shows the minerals in probiotic milk and juices (pumpkin, guava and a mix of milk, pumpkin and guava) with L.casei. Minerals are necessary to maintain the body's optimal health and function. The lowest value of iron was for probiotic milk (0.08mg/100g) and the highest value was for probiotic pumpkin (0.57 mg/100g). The calcium and phosphorus content of probiotic milk was the highest value (145.52 mg/100g) and (85.21 mg/100g), respectively. compared with other treatments and the lowest content of calcium was for probiotic pumpkin juice (15.26 mg/100g) with significant differences.

Table (1) Minerals of probiotic milk and juices during storage period (10 days)

Samples	Minerals concentration (mg/100g)				
	Fe	Ca	P	Mg	K
FM	0.08d	145.52a	85.21a	13.14c	182.32d
FP	0.57a	15.26d	30.92d	9.17d	230.21c
FG	0.29c	18.44c	45.88c	24.53a	458.23a
Fx	0.45b	58.96b	54.06b	15.35b	290.32b

[a-d] In the same column the different superscript letter have a significant difference. (Duncan's test P<0.05) where: FM= Probiotic Milk, FP= Probiotic Pumpkin juice. FG= Probiotic Guava juice, Fx= Probiotic Mix of juices with milk.

The highest contents of magnesium and potassium were for probiotic guava juice (24.53 and 458.23 mg/100g, respectively). These obtained results are in agreement with **El-Nimr et al. (2014)** who made probiotic milk drinks from permeate with chickpea and skim milk powder.

Total phenols of probiotic milk and juices during storage period (10 days)

Fig.5 shows the total phenols of probiotic milk and juices during storage period (10 days). One of the most important components of juices, is phenolics which are directly linked to their qualities that promote health. Results indicated that the highest value of total phenols was for FP followed by FG, FX and the lowest value was for FM. Total phenols decreased during the storage period (10 days). These results were in agreement with **Shalaby and Mohamed (2022)** who studied characteristics of probiotic- enriched turnip juice during storage.

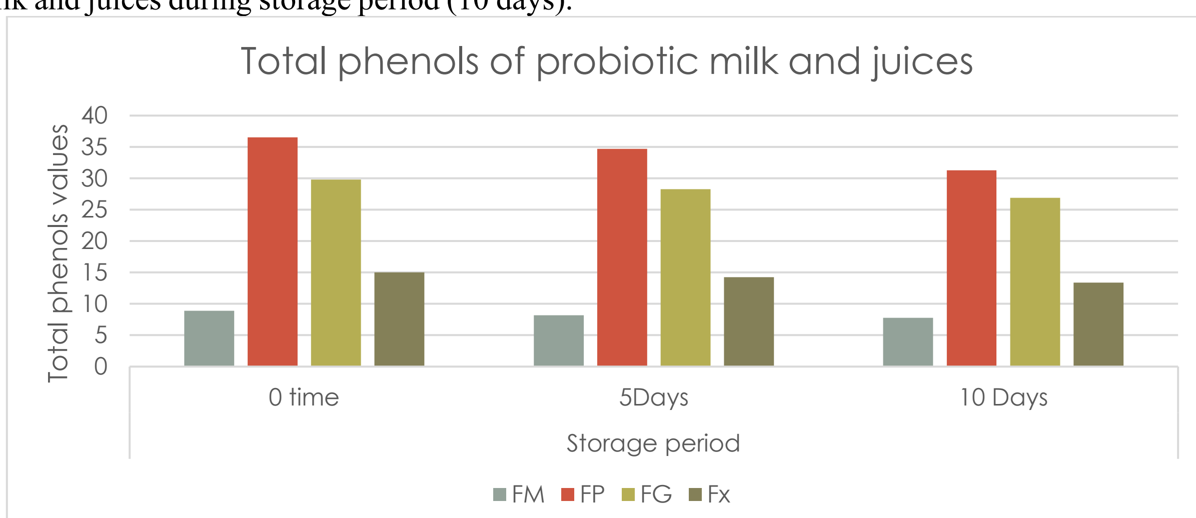

Fig.5 Total phenols of probiotic milk and juices during the storage period (10 days) where: FM= Probiotic Milk, FP= Probiotic Pumpkin juice. FG= Probiotic Guava juice, Fx= Probiotic Mix of juices with milk

DPPH of probiotic milk and juices during the storage period (10 days)

Table 2. displays the DPPH values of probiotic milk and juices during the storage period (10 days). The results showed that the highest value of total antioxidant as DPPH was for FP followed by FG, FX and the lowest value was for FM. It may be due to the amount of total phenols. The phenolic compounds play a big part in the antioxidant action because they supply the radical the hydrogen atom from the hydroxyl group and create a stable phenoxyl radical in the process **(Aksoy et al. 2013)** DPPH values decreased during the storage period (10 days). These results were in agreement with **El-Sayed and Ramadan (2020)** who studied the properties of beverages made with probiotic rice milk and cactus pear during the storage period (12 days).

Table (2) DPPH of probiotic milk and juices during storage period (10 days)

Samples	Storage period		
	0 time	5 Days	10 Days
	DPPH (mg TE/100 ml)		
FM	2.97^{Da}	2.67^{Db}	2.56^{Dc}
FP	40.34^{Aa}	38.32^{Ab}	36.02^{Ac}
FG	3.51^{Ca}	3.29^{Cb}	3.11^{Cc}
Fx	8.09^{Ba}	7.69^{Bb}	7.30^{Bc}

$^{(A-D)}$ In the same column different uppercase superscripts represent significant differences. ($P < 0.05$). $^{(a-e)}$ In the same row different lowercase superscripts represent significant differences ($P < 0.05$) where: FM= Probiotic Milk, FP= Probiotic Pumpkin juice. FG= Probiotic Guava juice, Fx= Probiotic Mix of juices with milk.

Microbial content of probiotic milk and juices during the storage period (10 days)

Table (3) shows the total count, *L.casei* count and mold& yeast as (log CFU/ml) in probiotic milk and juices. Results showed that the total count and L.casei increased in first 5 days and then decreased at the end time of storage period (10 days). It may be due to the increasing of acidity during storage time. Probiotic count kept rising above the acceptable level (6 log cfu/ml). The highest mean of total count and *L.casei* was for FX treatment. It may be due to the high nutritional and minerals in this treatment which improve the activity of lactic and probiotic bacteria. These results were in accordance with **Ismail et al., (2017)** who stated that Guava pulp increases the lactic acid bacteria and Bifidobacteria's viability in Rayeb milk. Mould and yeast growth was not detected over the 10 days cold storage period for all treatments It may be due to the sanitary practices during the preparation and storage of probiotic milk and juices with *L.casei* **(Aneja et al., 2014).**

Sensory evaluation of probiotic milk and juices during storage period (10 days).

Figures 6,7,8 and 9 show sensory evaluation of probiotic milk and juices during storage period (10 days) (color, flavor, texture and overall acceptability). Results indicated that the highest values of the color, flavor, texture and overall acceptability were for FP and the lowest values were for control treatment. The presence of beta-carotene and other carotenoid pigments in pumpkins is indicated by the fruit's bright yellow color. Organic pigments called carotenoids can be present in plants' chromoplast spontaneously. Plants contain pigments called carotenoids, which are typically yellow to red in color. The sour flavor and characteristic pumpkin smell can be detected in the probiotic beverage made from pumpkin juice. The typical smell of pumpkin comes from the raw material itself, that is pumpkin. Sensory evaluation values decreased for all treatments during the storage period (10 days). These results were in agreement with **Nazir *et al.* (2020).**

Table (3) Microbial content of probiotic milk and juices during storage period (10 days)

	Storage period		
Treatments	**0 time**	**5 Days**	**10 Days**
Total count log (CFU/ml)			
FM	6.52^{Dc}	6.72^{Da}	6.62^{Db}
FP	7.55^{Cb}	7.85^{Ca}	7.12^{Cc}
FG	7.70^{Bc}	7.91^{Ba}	7.75^{Bb}
Fx	8.52^{Ac}	9.05^{Aa}	8.98^{Ab}
***L. casei* log (CFU/ml)**			
FM	6.20^{Db}	6.40^{Da}	6.10^{Dc}
FP	7.11^{Cb}	7.32^{Ca}	7.04^{Cc}
FG	7.45^{Bc}	7.55^{Bb}	7.70^{Ba}
Fx	8.12^{Ac}	8.96^{Aa}	8.66^{Ab}
Mold and yeasts			
FM	ND	ND	ND
FP	ND	ND	ND
FG	ND	ND	ND
Fx	ND	ND	ND

$^{(A-D)}$ In the same column different uppercase superscripts represent significant differences. ($P < 0.05$). $^{(a-e)}$ In the same row Different lowercase superscripts represent significant differences ($P < 0.05$). where: FM= Probiotic Milk, FP= Probiotic Pumpkin juice. FG= Probiotic Guava juice, Fx = Probiotic Mix of juices with milk

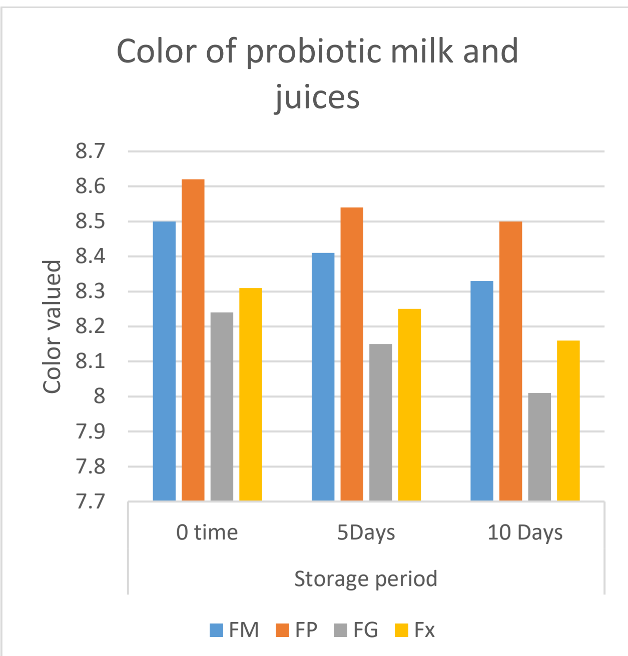

Fig.6 Color of probiotic milk and juices during storage period (10 days) where: FM= Probiotic Milk, FP= Probiotic Pumpkin juice, FG= Probiotic Guava juice, Fx= Probiotic Mix of juices with mil

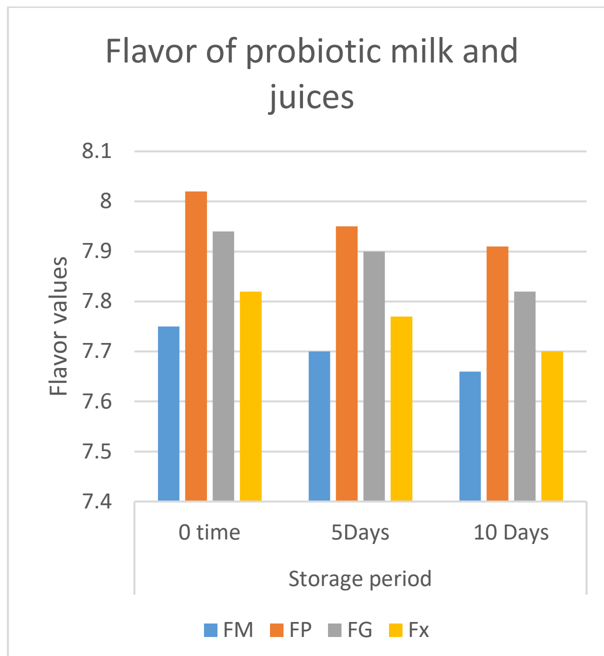

Fig.7 Flavor of probiotic milk and juices during the storage period (10 days) where: FM= Probiotic Milk, FP= Probiotic Pumpkin juice. FG= Probiotic Guava juice, Fx= Probiotic Mix of juices with milk

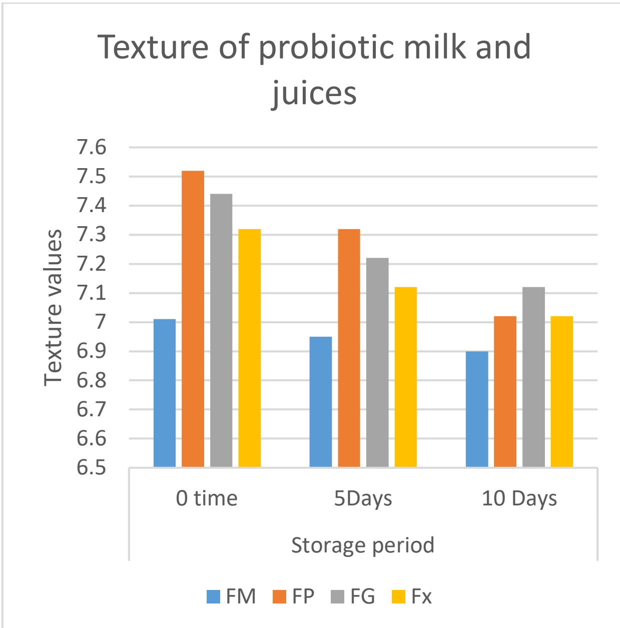

Fig.8 Texture of probiotic milk and juices during the storage period (10 days) where: FM= Probiotic Milk, FP= Probiotic Pumpkin juice. FG= Probiotic Guava juice, Fx= Probiotic Mix of juices with milk.

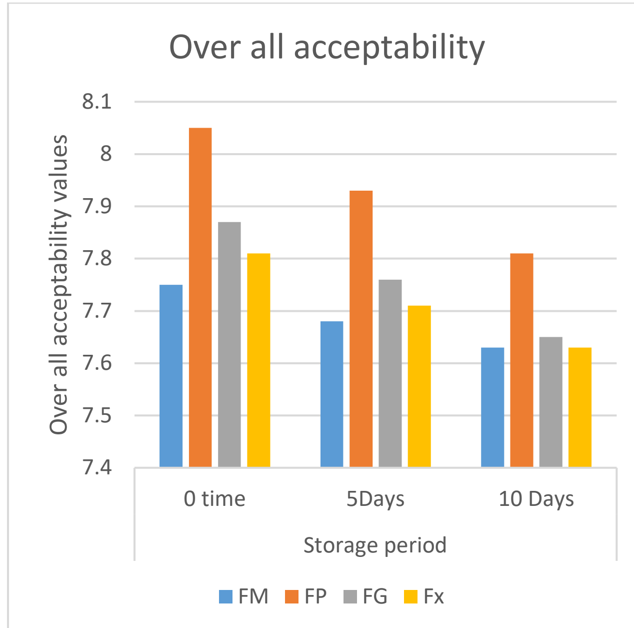

Fig.9 Over all acceptability of probiotic milk and juices during storage period (10 days) where: FM= Probiotic Milk, FP= Probiotic Pumpkin juice. FG= Probiotic Guava juice, Fx= Probiotic Mix of juices with milk

Conclusion

Lactobacillus casei is a probiotic bacteria used for probiotic dairy and probiotic fruit juices. Probiotic dairy and fruit juices are healthy functional beverages which improve absorption of nutrients. Probiotic pumpkin and guava juices were probiotic fruit juices, enabling those who dislike dairy products or have an intolerance or allergy to milk components to do so. Fruit juices serve as an effective probiotic delivery vehicle. Fruits naturally contain high antioxidant, total phenols, important macro- and microelements. Probiotic pumpkin juice enhanced flavor and color of beverages. Probiotic milk, guava juice, pumpkin juice and mixed milk of juices with *L.casei* are suitable for all people either vegetarians or not. Addition of a milk with fruit juice has improving the growth of L. casei.

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