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The Study of Probiotic Juice Product Conditions Supplemented by Culture of *Lactobacillus acidophilus* and *Bifidobacterium bifidum*

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Abstract: Probiotic bacteria are used widely in producing foods based on their positive qualities. Producing probiotic juices has been considered more in recent years. In this study, producing healthful probiotic drinks with apple and orange concentrates was assessed (brix 11 and 15). Milk and glucose, maltose and lactose were applied as growth supplements. After being produced, the products were incubated to let *Lactobacillus acidophilus* and *Bifdobacterium bifidum* grow. The samples` acidityand pH were measured as well as the number of microbes was counted using "direct microscopic count" method. Raising the juices' brix resulted in acidity elevation which wasn't an appropriate situation for bacteria to grow. Adding milk to these products made up a more suitable situation for bacterial growth than the one without concentrate but resulted in lower shelf life period. Sugars weren't effective on bacterial growth but glucose and lactose had positive effects on increasing shelf life period. The results of the questionnaire were analyzed, indicating no significant difference between odors, tastes and colors of the samples ($p<0.05$). The samples were analyzed for acidity and *Lactobacillus acidophilus* and *Bifidobacterium bifidum* numbers by non-parametric statistical test, "man-viteni" and "croscal valis" tests. It is conceived that more acidity in brix 15 than 11 was due to the orange concentrate influence on acidity in comparison with apple concentrate with no effects.

Key words: Probiotic · Juice · *Lactobacillus acidophilus* · *Bifidobacterium bifidum*

INTRODUCTION

Probiotic microbes are live organisms that are used by eating and condition application in repellent number and cause creation of one or many healthy effects on host body. The ability of probiotics to withstand the normal acidic conditions of the gastric juices and the other microorganisms, allow them to be established in the intestinal tract [1-4].

The reported health benefits include: boosting of the immune system, inhibition of the growth of pathogenic organisms, prevention of diarrhea from various causes, prevention of cancer, reduction of the inflammatory bowel movements, improvement of digestion of proteins and fats, synthesis of vitamins and detoxification and protection from toxins [5-8].

The concept of probiotics progressed around 1900, when Elie Mechnikoff hypothesized that the long and healthy lives of Bulgarian peasants were the outcome of their consumption of fermented milk and milk products. Members of the genera *Lactobacillus*, *Bifidobacterium* and *Streptococcus* are the most common probiotics used in commercial fermented and non-fermented dairy products today [9]. Among the popular probiotic foods in the Philippine market today are some infants' milk, fermented milk drinks and yogurts.

Recently, designing and producing probiotic products with herbal basis have been considered a lot

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because of both their natural healthful effects (proteins, fibers, vitamins and minerals) and inducing a lot of varieties. It seems that producing the probiotic form of these foods with considerable healthful quality will provide future researches topics. Producing probiotic juices is of dramatic importance nowadays. Totally, juices are of a high potential for turning into probiotic products in some reasons including: being healthful products by themselves, being consumed by a large variety of people and not being incompatible with some consumers' bodies such as lactose in dairy foods. Some studies have been done on probiotic orange juices [10]. The aim of the present study is produced non-dairy probiotic product, milk is suitable in terms of nutritional and market-friendly for transferring probiotics to human digestive, but to produce a good product and market-friendly which can create and maintain the define number of probiotics during the maintenance period and have sensory utility for the consumer, probiotic juices like a normal product was produced.

MATERIALS AND METHODS

Materials: Lyophilized *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were used as starter culture (CHR Hansen Company, Denmark). Orange and apple concentrate, apple and orange, sterilized low fat milk (fat %1.5) and sugar (lactose, glucose and maltose) were used.

Producing Products with Apple and Orange Concentrates by Adding Acid: At first, produced materials were assessed in brix to both use adequate concentrate and be beneficial economically. Next steps, the influences of adding citric acid to the samples were assessed.

The products with apple and orange concentrate were produced in 4 brixes: 11, 13, 15 and 17. Increasing the brix resulted in better tastes of the samples. In the producing process, acidity and pH were tested every 3 days.

The Acids were measured by titration acidity and were showed by gram/liter or citric acid percentage. The brix on acid ratio is an important index which is used for taste description, depending on the fruits ripening [11].

Producing Products with Apple and Orange Concentrates Without Adding Acid: It was done exactly like the last step (brix 11 and 15), but without any citric acid.

Producing Products with Apple and Orange Fruits by Adding Acid: The only difference of this step with the previous ones was using apple and orange juices instead of their concentrates.

Producing Products with Apple and Orange Concentrates with *Lactobacillus Acidophilus* (Brix 11 and 15): Four 1-liter containers were used to produce juices containing *Lactobacillus acidophilus* and an appropriate amount of sugar, water and concentrate were added to the products till the desirable brix was made. Then, 0.33 grams of *Lactobacillus acidophilus* was directly added to all containers. All of them were incubated at 38 °C and the acidity and pH tests were done nearly every 2 hours. The samples were refrigerated after six hours at 2 °C. The produced probiotic juices were directly analyzed for counting the microbes every two days [13-17].

Producing Products with Apple and Orange Concentrates with *Bifidobacterium Bifidum* (Brix 11 and 15): All the process was similar to the previous one, except using *Bifidobacterium bifidum* instead of *Lactobacillus acidophilus*.

Producing Products with Apple and Orange Concentrates with *Lactobacillus Acidophilus* and *Bifidobacterium Bifidum* by Adding Milk (0, 5, 10 and 15%): At this level, milk was used as a probiotic bacteria initiator. In 4 containers, milk was added as well as sugar, water and concentrate. The first one was regarded as the control group and to other ones 5, 10 and 15% milk were added respectively 5%, 10% and 15%. The incubation was performed at 38°C and pH and acidity tests were done every 2 hours. After 6 hours, they were refrigerated at 2°C [13-17]. The produced probiotic juices were directly analyzed for counting the microbes every 3 days.

Producing Products with Apple and Orange Concentrate with *Lactobacillus Acidophilus* and *Bifidobacterium Bifidum* Bacteria by Adding Glucose, Lactose and Maltose: The only difference of this step was adding 30% of glucose, lactose and maltose.

Measuring the Shelf Life Periods of the Products: For this purpose, 1000 grams of all produced compounds were refrigerated for 6 days and their shelf life time length was measured on the 1^{st}, 3^{rd} and 6^{th} day. They were also tested for formalin, acidity, pH and sensory properties.

RESULTS

Tables 1 and 3 introduce acidity progress speed and pH in orange and apple juice containing probiotic bacteria of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in brixs 11 and 15 at the production time.

Tables 2 and 4 introduce acidity, pH and microbe's measurement using "direct microscopic count" method at the shelf life period of product.

Tables 5 and 7 introduce acidity progress speed and pH at the production time of apple and orange juice containing *Lactobacillus acidophilus* bacteria by adding milk (0, 5, 10 and 15%) in brix 11 and tables 6 and 8 show period lasting of products in refrigerator by defining acidity, pH and microbes measurement by "direct microscopic count" method.

Tables 9 and 11 introduce acidity progress speed and pH at the production time of apple and orange juice containing *Bifidobacterium bifidum* by adding milk (0, 5, 10 and 15%) in brix 11 and tables 10 and 12 introduce shelf life period of products in refrigerator by defining acidity, pH and measurement of microbes as direct.

Table 13 introduces acidity progress speed and pH at the production time of apple and orange juice containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* by adding glucose sugar in brix 11 and table 14 shows introduction of products in refrigerator in shelf life period.

Table 15 introduces acidity progress speed and pH at the production time of apple and orange juice containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* by adding maltose sugar in brix 11 and table 16 illustrates introduction of products in refrigerator in shelf life period.

Table 17 shows acidity progress speed and pH at the production time of apple and orange juice containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* by adding lactose sugar in brix 11 and table 18 shows introduction of products in refrigerator in shelf life period.

Table 1: pH and acidity in the producing products with apple and orange concentrates with Lactobacillus acidophilus (brix 11 and 15)

	Bx=11 orange Lact obacillus acidophilus		Bx=15 orange Lact obacillus acidophilus		Bx=11 apple Lact obacillus acidophilus		Bx=15 apple Lact obacillus acidophilus	
Hour	pH	acidity	pH	acidity	pH	acidity	pH	acidity
0	3.1	0.1	3	0.4	3.8	0.07	3.45	0.1
2	3	0.2	2.88	0.4	3.6	0.08	3.5	0.1
4	2.92	0.2	2.85	0.4	2.5	0.08	3.5	0.1
6	2	0.2	2.81	0.4	2.5	0.1	3.45	0.1
-	-	P>0.05	-	P>0.05	-	P>0.05	-	P>0.05

Table 2: pH, acidity and bacterium count in the producing products shelf life with apple and orange concentrates with Lactobacillus acidophilus (brix 11 and 15)

	Bx=11 orange Lactobacillus acidophilus			Bx=15 orange Lactobacillus acidophilus			Bx=11 apple Lactobacillus acidophilus			Bx=15 apple Lactobacillus acidophilus		
Day	pH	acidity	microbe	pH	acidity	microbe	pH	acidity	microbe	pH	acidity	microbe
1	2.98	0.2	4.5×1010	2.91	0.4	0.7×1010	3.66	0.07	0.7×1010	3.58	0.1	2.25×1010
3	2.98	0.2	1×1010	2.91	0.5	1×1010	3.53	0.08	1.75×1010	3.48	0.1	0.5×1010
6	2.98	0.1	1.25×1010	2.83	0.4	1.75×1010	3.52	0.1	0.7×1010	3.46	0.2	0.5×1010
-	-	-	P>0.05	-	P>0.05	-	-	P>0.05	-	-	P>0.05	-

Table 3: pH and acidity in the producing products with apple and orange concentrates with Bifidobacterium bifidum (brix 11 and 15)

	Bx=11 orange Bifidobacterium Bifidum		Bx=15 orange Bifidobacterium Bifidum		Bx=11 apple Bifidobacterium Bifidum		Bx=11 orange Bifidobacterium Bifidum	
Hour	pH	acidity	pH	acidity	pH	acidity	pH	acidity
0	2.90	0.2	2.83	0.4	3.58	0.1	3.39	0.1
2	2.96	0.2	2.91	0.5	3.72	0.1	3.63	0.1
4	2.90	0.2	2.96	0.5	3.69	0.1	3.56	0.1
6	2.89	0.2	2.89	0.4	3.60	0.08	3.5	0.1
-	-	P>0.05	-	P>0.05	-	P>0.05	-	P>0.05

Table 4: pH, acidity and bacterium count in the producing products shelf life with apple and orange concentrates with Bifidobacterium bifidum (brix 11 and 15)

	Bx=11 orange Bifidobacterium Bifidum			Bx=15 orange Bifidobacterium Bifidum			Bx=11 apple Bifidobacterium Bifidum			Bx=15 apple Bifidobacterium Bifidum		
Day	pH	acidity	microbe	pH	acidity	microbe	pH	acidity	microbe	pH	acidity	microbe
1	3.05	0.2	1×1010	2.95	04	1.25×1010	3.71	0.08	0.7×1010	3.56	0.1	0.5×1010
3	3.11	0.3	0.7×1010	3.03	05	1.5×1010	3.89	0.1	0.1×1010	3.71	0.1	0.2×1010
-	-	P>0.05	-	-	P>0.05	-	-	P>0.05	-	-	P>0.05	-

Table 5: pH and acidity in the Production products with orange concentrate with Lactobacillus acidophilus by adding milk (0, 5, 10 and 15%) in brix 11

	%0 milk, orange		%5 milk, orange		%10milk, orange		%15milk, orange	
Hour	pH	acidity	pH	acidity	pH	acidity	pH	acidity
0	2.9	0.2	3.2	0.2	3.4	0.2	3.6	3.6
2	2.9	0.2	3.1	0.2	3.4	0.2	3.5	3.5
4	2.8	0.2	3.1	0.2	3.3	0.2	3.5	3.5
6	2.8	0.2	3.1	0.2	3.3	0.2	3.5	3.5
-	-	P>0.05	-	P>0.05	-	P>0.05	-	P>0.05

Table 6: pH, acidity and bacterium count in the Production products shelf life with orange concentrate by Lactobacillus acidophilus by adding milk (0, 5, 10 and 15%) in brix 11

	%0 milk, orange			%5 milk, orange			%10 milk, orange			%15 milk, orange		
Day	pH	acidity	microbe	pH	acidity	microbe	pH	acidity	microbe	pH	acidity	microbe
1	3.06	0.2	3.75×1010	3.39	0.2	3.5×1010	3.64	0.2	3×1010	3.72	0.2	1.25×1010
3	3.4	0.2	3.25×1010	3.53	0.2	2×1010	3.83	0.2	1.5×1010	3.94	0.3	1.25×1010
6	3.19	0.2	1×1010	3.57	0.2	1.5×1010	3.81	0.2	1.5×1010	3.89	0.2	1.5×1010
-	-	P>0.05	-	-	P>0.05	-	-	P>0.05	-	-	P>0.05	-

Table 7: pH and acidity in the Production products with apple concentrate with Lactobacillus acidophilus by adding milk (0, 5, 10 and 15%) in brix 11

	%0 milk, apple		%5 milk, apple		%10milk, apple		%15milk, apple	
Hour	pH	acidity	pH	acidity	pH	acidity	pH	acidity
0	3.9	0.1	4.4	0.1	4.7	0.1	4.9	0.1
2	3.9	0.1	4.3	0.07	4.5	0.1	4.7	0.1
4	3.8	0.07	4.3	0.07	4.3	0.1	4.6	0.1
6	3.8	0.07	4.3	0.06	4.3	0.1	4.4	0.1
-	-	P>0.05	-	P>0.05	-	P>0.05	-	P>0.05

Table 8: pH, acidity and bacterium count in the Production products shelf life with apple concentrate with Lactobacillus acidophilus by adding milk (0, 5, 10 and 15%) in brix 11

	%0 milk, apple			%5milk, apple			%10milk, apple			%15milk, apple		
Day	pH	acidity	microbe	pH	acidity	microbe	pH	acidity	microbe	pH	acidity	microbe
1	3.9	0.08	1×1010	4.2	0.1	1×1010	4.2	0.1	2.25×1010	4.3	0.2	2×1010
3	3.68	0.07	1.75×1010	4.2	0.08	0.7×1010	4.2	0.1	1.75×1010	4.15	0.1	1.5×1010
6	3.45	0.08	1.5×1010	3.8	0.08	2×1010	3.9	0.1	3.5×1010	3.9	0.1	1.5×1010
-	-	P>0.05	-	-	P>0.05	-	-	P>0.05	-	-	P>0.05	-

Table 9: pH and acidity in the Production products with orange concentrate by Bifidobacterium bifidum by adding milk (0, 5, 10 and 15%) in brix 11

	% 0milk, orange		%5milk, orange		%10milk, orange		%15milk, orange	
Hour	pH	acidity	pH	acidity	pH	acidity	pH	acidity
0	3.36	0.2	3.6	0.3	4	0.2	4	0.2
2	3.3	0.2	3.5	0.2	3.9	0.2	4	0.2
4	3.2	0.2	3.5	0.2	3.9	0.2	3.8	0.2
6	3.2	0.2	3.5	0.2	3.8	0.2	3.8	0.2
-	-	P>0.05	-	P>0.05	-	P>0.05	-	P>0.05

Table 10: pH, acidity and bacterium count in the Production products shelf life with orange concentrate with Bifidobacterium bifidum by adding milk (0, 5, 10and 15%) in brix 11

	%0milk, orange			%5milk, orange			%10milk, orange			%15milk, orange		
Day	pH	acidity	microbe	pH	acidity	microbe	pH	acidity	microbe	pH	acidity	microbe
1	3.49	0.1	1.25×1010	3.71	0.2	0.5×1010	4.06	0.2	2.25×1010	4	0.2	2.25×1010
3	4.11	0.2	1.25×1010	4.5	0.2	1.5×1010	4.68	0.2	1.5×1010	4.82	0.2	1×1010
-	-	P>0.05	-	-	P>0.05	-	-	P>0.05	-	-	P>0.05	-

Table 11: pH and acidity in the Production products apple concentrate with Bifidobacterium bifidum by adding milk (0, 5, 10 and 15%) in brix 11

	%0milk, apple		%5milk, apple		%10milk, apple		%15milk, apple	
Hour	pH	acidity	pH	acidity	pH	acidity	pH	acidity
0	4.2	0.1	4.4	0.1	4.8	0.1	5	0.1
2	3.9	0.1	4.2	0.1	4.4	0.1	4.6	0.1
4	3.7	0.1	4.2	0.1	4.3	0.1	4.6	0.1
6	3.5	0.08	4.2	0.08	4.3	0.1	4.6	0.1
-	-	P>0.05	-	P>0.05	-	P>0.05	-	P>0.05

Table 12: pH, acidity and bacterium count in the Production products shelf life with apple concentrate with Bifidobacterium bifidum by adding milk (0, 5, 10 and 15%) in brix 11

	%0milk, apple			%5milk, apple			%10milk, apple			%15milk, apple		
Day	pH	acidity	microbe	pH	acidity	microbe	pH	acidity	microbe	pH	acidity	microbe
1	4.14	0.08	2.25×1010	4.38	0.1	1.5×1010	4.64	0.1	1.5×1010	4.69	0.1	1×1010
3	4.11	0.1	1×1010	4.5	0.1	2×1010	4.68	0.1	1.5×1010	4.82	0.1	1.25×1010
-	-	P>0.05	-	-	P>0.05	-	-	P>0.05	-	-	P>0.05	-

Table 13: pH and acidity in the Production products with orange and apple concentrate by glucose sugar with Lactobacillus acidophilus and Bifidobacterium bifidum in brix 11

	Lactobacillus acidophilus orange		Lactobacillus acidophilus apple		Bifidobacterium bifidum orange		Bifidobacterium bifidum apple	
Hour	pH	acidity	pH	acidity	pH	acidity	pH	acidity
0	2.94	0.2	3.59	0.07	2.95	0.2	3.7	0.07
2	2.88	0.2	3.6	0.08	2.89	0.2	3.5	0.08
4	2.92	0.2	3.7	0.08	2.8	0.2	3.6	0.08
6	2.9	0.2	3.7	0.08	2.8	0.2	3.6	0.07
-	-	P>0.05	-	P>0.05	-	P>0.05	-	P>0.05

Table 14: pH, acidity and bacterium count in the Production products shelf life with orange and apple concentrate by glucose sugar with Lactobacillus acidophilus and Bifidobacterium bifidum in brix 11

	Lactobacillus acidophilus orange		Lactobacillus acidophilus apple		Bifidobacterium bifidum orange		Bifidobacterium bifidum apple	
Day	pH	acidity	pH	acidity	pH	acidity	pH	acidity
1	3	0.2	3.7	0.1	3.7	0.2	3.7	0.1
3	2.8	0.1	3.5	0.08	2.8	0.2	3.57	0.08
6	2.94	0.1	3.59	0.08	2.93	0.2	3.6	0.06
-	-	P>0.05	-	P>0.05	-	P>0.05	-	P>0.05

Table 15: pH and acidity in the Production products with orange and apple concentrate by maltose sugar with Lactobacillus acidophilus and Bifidobacterium bifidum in brix 11

	Lactobacillus acidophilus orange		Lactobacillus acidophilus apple		Bifidobacterium bifidum orange		Bifidobacterium bifidum apple	
Hour	pH	acidity	pH	acidity	pH	acidity	pH	acidity
0	3.04	0.2	3.8	0.06	2.97	0.2	3.7	0.07
2	2.98	0.2	3.7	0.07	2.8	0.2	3.7	0.07
4	2.9	0.2	3.6	0.07	2.8	0.2	3.7	0.07
6	2.8	0.2	3.4	0.1	2.8	0.2	3.5	0.07
-	-	P>0.05	-	P>0.05	-	P>0.05	-	P>0.05

Table 16: pH, acidity and bacterium count in the Production products shelf life with orange and apple concentrate by maltose sugar with Lactobacillus acidophilus and Bifidobacterium bifidum in brix 11

	Lactobacillus acidophilus orange		Lactobacillus acidophilus apple		Bifidobacterium bifidum orange		Bifidobacterium bifidum apple	
Day	pH	acidity	pH	acidity	pH	acidity	pH	acidity
1	2.8	0.2	3.7	0.07	3.7	0.2	3.7	0.1
3	2.9	0.2	3.3	0.08	2.9	0.2	3.5	0.08
6	2.9	0.2	-	-	2.9	0.2	-	-
-	-	P>0.05	-	P>0.05	-	P>0.05	-	P>0.05

Table 17: pH and acidity in the Production products with orange and apple concentrate by lactose sugar with Lactobacillus acidophilus and Bifidobacterium bifidum in brix 11

	Lactobacillus acidophilus orange		Lactobacillus acidophilus apple		Bifidobacterium bifidum orange		Bifidobacterium bifidum apple	
Hour	pH	acidity	pH	acidity	pH	acidity	pH	acidity
0	3	0.2	3.7	0.07	3.2	0.2	3.7	0.05
2	3	0.2	3.7	0.08	2.9	0.2	3.7	0.07
4	2.9	0.2	3.6	0.08	2.9	0.2	3.6	0.07
6	2.9	0.1	3.6	0.08	2.1	0.2	3.6	0.07
-	-	P>0.05	-	P>0.05	-	P>0.05	-	P>0.05

Table 18: pH, acidity and bacterium count in the Production products shelf life with orange and apple concentrate by lactose sugar with Lactobacillus acidophilus and Bifidobacterium bifidum in brix 11

	Lactobacillus acidophilus orange		Lactobacillus acidophilus apple		Bifidobacterium bifidum orange		Bifidobacterium bifidum apple	
Day	pH	acidity	pH	acidity	pH	acidity	pH	acidity
1	2.8	0.2	3.5	0.1	2.8	0.2	3.6	0.1
3	2.8	0.2	3.5	0.06	2.9	0.2	3.6	0.07
6	2.8	0.2	3.28	0.1	2.9	0.2	3.36	0.08
-	-	P>0.05	-	P>0.05	-	P>0.05	-	P>0.05

DISCUSSION

In this study, the possibility of producing probiotic products based on juices was assessed. At first, producing with apple and orange concentrates in different brixes: (brix11; brix 13; 15 and 17) was done to choose the best one which both is justifiable economically and makes an appropriate situation for bacterial growth.

It was seen that adding citric acid inconvenienced the situation for bacterial growth. So, to provide more convenient acidity and better pH for bacterial growth, adding the acid wasn't performed anymore. Apple and orange fruits were then used to produce juices which resulted in shorter shelf life period which is not justifiable economically. So, it was concluded that products without acid, in brixes 11 and 15, both were suitable financially and had better taste and odor.

Apple and orange fruits used to provide juices from the fruit samples were lasting little than concentrate and products were spoiled about short time. The production wasn't economical.

The changes applied on the juices (containing *Bifidobacterium bifidum* and *Lactobacillus acidophilus*) were analyzed based on acidity indexes, formalin, pH and living ability in different periods.

The incubation temperature had significant effect on the fermentation time as well as probiotic fermentation ability in fermented products. The incubation time depends directly upon the optimum temperature of bacterial growth. The evidences indicate that the optimum temperatures for *Bifidobacterium bifidum* and *Lactobacillus acidophilus* and *Streptococous thermophilus* and *Lactobacilus bolgaricus* are, 37, 40-42 and 42-45°C, respectively [10].

The results also showed that probiotic *Lactobacilus* are thermothroph and *Bifidobacterium* are mesophilic. It should be indicated that the optimum temperature for mesophilic initiator bacteria is 20-22°C20-22 [10].

In this research, the samples, first, were incubated at 38°C and then refrigerated at 2°C, considering a 6-day shelf life period (Table 1-18).

In products containing apple and orange concentrates, the acidity was boosted by increasing the brix and using more concentrate. Brix 11 was the most appropriate situation, with respect to the acidity and pH, for bacteria growth.

To compare the apple and orange products' acidity in brixes 11 and 15, non-parametric and "man-viteni" tests were used. According to table 1, there was a significant difference between the brixes 11 and 15 in acidity of orange samples containing *Lactobacillus acidophilus* bacteria ($p=0.011$), indicating more acidity in brix 15. In apple samples, the results were also the same ($p=0.046$). In table 2 – studying the samples' shelf life period - there was a significant difference between the acidity of orange samples ($p=0.043$), with no difference in apple ones ($p=0.105$). Apple concentrate increased the acidity in production procedure but had no influence on acidity in shelf life period. The acidity increase seems to be due to the microbes' number was rising. But in shelf life period, there was no change in acidity because of stable microbes' population. On the other hand, increasing the acidity of orange samples in production and shelf life period returned to the concentrate.

The Table 3 shows use of *Bifidobacterium bifidum* bacteria but during production there was meaningful difference between orange sample acidity ($p=0.011$) and acidity in brixs 15 was higher than brixs 11. But there wasn't meaningful difference between apple and orange samples with *Bifidobacterium bifidum* bacteria at shelf life period (Table 3). It seems that the high amount of acidity in brixs 15 than brixs 11 of orange samples is because of orange concentrate effect on acidity, this wasnt seen in apple concentrate, though.

Evaluating products shelf life period at the every three days the orange and apple containing concentrate along with *Lactobacillus acidophilus* bacteria in juice by brixs 11 and 15 and juice sample was lasting to 6 days by adding milk, apple and orange juice, lactose and glucose sugar and only samples containing maltose sugar was lasting to third day (Tables 2, 6, 8, 14, 16,18).

Orange and apple concentrate with *Bifidobacterium bifidum* bacteria was lasting to third day and after that there was no turbidity. Also the samples containing milk were lasting to third day by *Bifidobacterium bifidum* bacteria by adding glucose and lactose sugars for both bacterium lasting to sixth day and only maltose sugar to *Bifidobaterium bifidum* bacteria wasn't lasting to third day (Tables 4, 10,12,14,16,18).

Comparing above sample lasting, the products containing *Lactobacillus acidophilus* was lasting longer than products containing *Bifidobactrium bifidum* and only in sugar samples there was equal lasting in length in both bacteria.

Statistics showed that *Bifidobactrium bifidum* multiplication and growth is the lowest in comparison with *Lactobacillus acidophilus* and other lactic bacteria. It may result from more sensitivity to oxygen level, high acidity, low pH and more needs to growth supplements [10].

High acidity and low pH of probiotic products are the most important factors in alleviating the probiotics' living ability. That is why the mentioned ability in sweet milk is 10 times more than fermented products. And *Bifidobacterium bifidum* lives better in ice cream and cheese than yogurt [10].

Research on probiotic milk, yogurt and honey indicated a 15-day shelf life period and a reasonable number of microbes [13-14].

Another study on malt and soya concentrate in probiotic milk and yogurt (containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum*) showed a 20-day standing period [15-17]. It is concluded that bacterial culture in milk and yogurt makes longer shelf life period than juices.

In juices, dimness and some sediment are seen for different reasons. This is called: "secondary dimness" which is made by some agents: microorganisms, proteins, tannin, araban, starch, pectin, saponin and metal ions (potassium, calcium, magnesium, iron, copper, sulphur, phosphate, sulphate). The reactions between these materials make the sediments [11].

Growth agents are compounds consumed by bacteria as direct feeding and energy sources, so compound has growth stimulant of all micro-living beings. Fore example if the compound has better growth by environment reduction potential so it is growth stimulant not growth agent [10].

One of the methods of including probiotic microorganism in human diet is adding them into milk. In this study, adding milk to the samples for supporting the proiotic bacteria made acidity reduction leading to a better growth situation than the samples without milk, but more milk-added samples didn't taste good.

According to nonparametric test and "man-viteny" test for comparing probiotic apple and orange juices, adding milk (0, 5, 10 and 15%) made no significant difference between samples with respect to shelf life period and production time, samples with 5% milk had better taste and with 10% had more reasonable bacteria number, though (Tables 5-12).

It has been showed that adding monosaccharides and disaccharides into probiotic fermented products result in strengthening some bacteria's growth, for example, *Lactobacillus acidophilus* living ability was raised even by adding a little glucose and maltose [10].

In the next step, glucose, maltose and lactose were used in probiotic juices instead of milk and no difference was seen between these and sacarose added milk (Tables 13, 14, 15, 16, 17, 18). Meanwhile, no acidity difference was seen between glucose, maltose and lactose added samples (by non-parametric "man-viteny" test). Also, the "croscal valis" test confirmed these results.

In comparison with the experiment done on malt effects on *Lactobacillus acidophilus* and *Bifidobacterium bifidum* growth in producing probiotic milk and yogurt, decreasing the time of getting to the desirable acidity and better influence on probiotic microbes than glucose, maltose and lactose sugars were seen [17].

Despite considerable advances in probiotic techniques, there is no specific and global criterion-live for these products, except in some countries such as Japan [10].

The number of probiotic live cells in each gr or m/l of product at consumption time is probiotic products basis value, so it identifies the products efficiency. The index is Biovalve and its minimum is claimed drug effects of product so called minimum of biovalue. Important suggestion and criterion-line is 10^7 by IDF in relation to probiotic [18].

To be more useful in the body, the living prbiotic bacteria number should be at least 10^7 in per gram. In this research, by "direct microscopic count" method, the number of them was estimated 10^{10}. So the products were nutritious and useful enough. And there was no significant difference between the populations of microbes between all samples.

To decrease the production expenses, increase the bacterial number and bacteria's more adaptability to the products' environments, Swenson [9] indicated that probiotic bacteria should be able to grow in fermentation period.

At the study different group's juice probiotics were studied of thickness, color and taste. Nonparametric ways were used to analyze which hasn't meaningful difference between all samples ($p<0/05$) and all products evaluated good.

Probiotics are defined as "bacteria-based products with positive effects on human health and welfare". According to the mentioned definition, non-living forms of probiotics can also have an influence on human body and their applications are not limited to edible foods. In addition, it is implied that some parts of their structures are positively helpful for body as well [13, 14].

CONCLUSIONS

As a final conclusion we can say, the samples were more lasting using *Lactobacillus acidophilus* bacteria than *Bifidobacterium bifidum* and also with the addition of glucose and lactose than maltose.

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