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CHARACTERISTICS OF SWEET ACIDOPHILUS MILK FORTIFIED WITH GRAVIOLA (*ANNONA MURICATA*) JUICE

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Abstract

Fruit yogurt has become one of the most favorable healthy food for consumers all over the world it combines the benefits of functional food and delicious taste. In this research Sweet Acidophilus milk made by adding different concentrations of Graviola juice 5%, 10% and 15% and 5% sugar to milk, using 10% *Lactobacillus acidophilus* as a starter and held at 6±2°C for 14 days. The titratable acidity and total solids of flavored Acidophilus milk have increased by increasing concentration of Graviola juice and during the storage period. Total protein and total carbohydrates in milk gradually increased for all treatments during the storage period progresses. The total plate count and *Lactobacillus acidophilus* have increased in all treatments until the end of the storage period. Sweet Acidophilus milk made with 5%, 10% Graviola juice gained the highest overall scores.
Keywords : Sweet fortified milk, *Lactobacillus acidophilus*, acidophilus milk, probiotic, fermented milk, functional foods.

Introduction

In recent years, there has been an increasing trend about incorporation of health promoting bacterial species; e.g. *Lactobacillus acidophilus* and *Bifidobacterium longum*, into fermented milk products. In fact, these bacteria having prescribed population of viable cells, when administered orally as a supplement or through food products impart a variety of beneficial health effects (Junaid *et al.*, 2013).

Consumers across the world are becoming more interested in foods with health promoting features as they gain more awareness of the links between food and health. Among the functional foods, products containing probiotics are showing promising trends worldwide (Aswal, Shukla, & Priyadarshi, 2012). Probiotics, such as *Lactobacillus spp.* and *Bifidobacterium spp.* are bacterial members of the normal human intestinal flora, which exert several beneficial effects on human health. and well-being through, production of short-chain fatty acids and improve the intestinal microbial balance, resulting in the inhibition of bacterial pathogens, reduction of colon cancer, improving the immune system and lowering serum cholesterol levels (Marhamatizadeh *et al.,* 2014)

Probiotics are recognized for their applications in dairy products, particularly yoghurts and the market for these products is still rising. To achieve the claimed health benefits, one of the most important requirements for manufacturing and marketing of probiotic yoghurt is to maintain a high number of probiotic organisms "10^6 CFU/g" at the point of consumption (Lourens-Hattingh and Viljoen, 2001). The flavors are key factors for foodstuff acceptability by consumers. Organoleptic evaluations have shown a marked preference for the fruity yoghurt. Addition of different fruit in yoghurt manufacture has been attempted increasingly. Use of fruit in yogurt makes its more delicious. This product contains both the refreshing flavor of fruit and beneficial effect of yoghurt. (Amal, Eman, & Zidan, 2016).

Yoghurt is an increasingly popular cultured dairy product in most countries. This is partly because of increased awareness of the consumers regarding possible health benefits of yoghurt. Yoghurt is easily digested, has high nutritional value, and is a rich source of carbohydrates, protein, fat, vitamins, calcium, and phosphorus. Because milk protein, fat, and lactose components undergo partial hydrolysis during fermentation, yoghurt is an easily digested product of milk (Subhra and Aruna, 2013). Lactic acid bacteria and its metabolites have shown important roles in improving microbiological quality and shelf-life of many fermented food products. Dairy products have long been consumed by consumers and provide a good example of bio-preservation (Lourens-Hattingh & Viljoen, 2001).

Graviola has a miraculous nature as "Natural Cancer Cell killer", 1,000 much stronger than to chemotherapy treatment, and reported to have a polyketide-derived fatty acid compound called annonaceous acetogens which can be inhibited the damaged cells just before they could become cancerous. Graviola (Soursop) is a juicy, acidic, and aromatic nature fruit recorded many therapeutic and nutritive properties. The fruit pulp is a rich source of fructose, and contains significant amounts of vitamins, such as C, B1, and B2 (Dias and Jayasooriya, 2017). Graviola (*Annona muricata*) is an Amazon fruit tree that grows in the tropics of North and South America. It has used for a wide range of human diseases including inflammatory conditions, rheumatism, neuralgia, diabetes, hypertension, insomnia, cystitis, parasitic infections, and cancer. Despite Graviola being used for centuries, research on its health benefits has been extremely limited (Hansra, Silva, Mehta, & Ahn, 2014).

Fortification ensures safest method by which manufacturers can deliver health-promoting, nutritionally dense food products. Fermented dairy products are the most consumed healthy and nutritious food around the world. Therefore, it offers an appropriate potential to convey nutritious ingredients to the human diet. Fortification of fermented dairy products is considered as an emerging technology as it considers the issues of the role of fermented dairy products in quality of life and on the reduction of the risk of chronic diseases. The risks associated with fortification are minimal except if good manufacturing practices are not followed. Improved understanding of interactions between food ingredients and health and ingenuity of food technologists in food formulation and fabrication will contribute to the advances in fortification of fermented dairy products (Jalal *et al.,* 2016). Several studies have shown that Graviola are easy to produce, commercialize, and have excellent sensory acceptance, a beyond-adequate nutritional profile and claim to functional and therapeutic benefits.

Thus, the goals of this work were:

- To develop desirable healthy Acidophilus milk using Graviola as a sweetener and natural prebiotic and by incorporating Probiotic cultures.
- Studying the impact of adding Graviola as a type of Prebiotics on the characteristics of Acidophilus milk.

This study suggested that the consumption of Graviola can be useful for increasing the concentrations of biologically active substances in the body.

Materials and Methods

Materials

Buffalo's milk was obtained from Masr EL-kheir in Arab EL Awamer, Assuit, Egypt. *Lactobacillus acidophilus* was obtained from El-Azhar University. Graviola fruit was obtained from local market in Assuit city, Egypt.

Methods

Preparation of Graviola:

Fresh fruit of Graviola washed thoroughly in cold running tap water and cut off and the seed removed, each half roughly cut into thick slices using a thin food knife. The Graviola juice obtained by blending in blender with sieves then the fruits mixed. The Graviola juice transferred into glass jars, pasteurized in a water bath (85–90°C, 15 min), cooled in an ice-water bath for 20 minutes and stored at 4°C prior to use. (Ismail, 2015)

Manufacture of Sweet Graviola Acidophilus Milk:

Buffalo's milk was heat treated at 90±1ºC for 5 min and cooling to 40-41°C then divided into four parts. The first part was set as control (T1) and Graviola then added to the other parts as follow: T_2: Adding 5% Graviola, T_3: Adding 10% Graviola and T_4: Adding 15% Graviola. after that the sugar was added (5%) then the milk inoculated with 10% active starter culture of *Lactobacillus acidophilus* the mix distributed into plastic cups then incubated at 42°C till 4 hours and storages at 7±1°C for 14 days (Karki, 2012)

Chemical Analysis

The samples of acidophilus milk were analyzed for the titratable acidity according to (A.O.A.C, 2000). Protein was estimated from the crude nitrogen content of the samples determined by the Kjeldahl method and the moisture by oven-drying (IDF, 1993). fat contents were determined in the control treatment by using Gerber method, while in the other treatments by using Soxelt method according to (A.O.A.C, 2000) and (IDF, 1986), respectively. Crude fiber was determined according to (Zinash *et al.,* 2013). Total carbohydrates were calculated by Anthrone method according to (A.O.A.C, 2000) and according to (FAO, 2003) using the following equation:-

% Total Carbohydrates = 100 – (%Moisture +%Ash +%Fat +%Protein).

Antioxidant compounds (Vitamins A, C, and D) for the acidophilus milk were determined by High Performance Liquid Chromatography (HPLC), Agilent Packared (1200 series) equipped with auto sampling injector solvent degasser ultraviolet (UV) detector and quarter HP pump (1200 series), according to (Bakr, Mohamed, Tammam, & El-gazzar, 2015). For fruit samples, vitamin A and D were determined according to (Danish Official, 1996), At RCFF (Regional Center for Food and Feed) in Agricultural Research Center, Giza, Egypt. Vitamin C was determined according to (Danish Official, 1999), At RCFF (Regional Center for Food and Feed) in Agricultural Research Center, Giza, Egypt .

Also, the concentrations of phenolic and flavonoids compounds were estimated as the method of (Goupy *et al.,* 1999). Ash contents were determined in muffle furnace at 550˚C overnight according to (A.O.A.C, 2000). Trace elements content had been estimated according to the method described by the (A.O.A.C, 2000), using atomic absorption (Perkin – Elmer, Model 3300, USA).

Microbiological Analysis

The standard plate count technique was used to enumerate the total bacterial count of acidophilus milk samples. Appropriate dilutions of the samples were plated in duplicate on an agar medium (IDF, 1997). *Lactobacillus acidophilus* was enumerated by the method described by (Dave and Shah 1996). Yeasts and molds as (IDF, 1985a), Aerobic and Anaerobic spore forming bacteria and coliform bacteria were enumerated according to the methods of (IDF, 1985b). Psychrotrophic bacteria were estimated using standard plate count agar according to (Marshall, 2004). Plates containing 20-200 colonies were counted and the results expressed as colony-forming units per gram (CFU g-1) of sample (IDF, 1991).

Sensory Analysis

Acidophilus milk paneled tested as fresh sample and after 14 days of storage by ten panelists of staff member of Food Technology Research Section using the scores sheet (Flavor, Body and Texture, Appearance and Acidity) according to (Bakr *et al.,* 2015).

Statistical analysis

The data obtained from three replicates were analyzed by ANOVA using the SPSS statistical package program, and differences among the means were compared using the Duncan's Multiple Range test **(SPSS, 2011).** A significance level of 0.05 has chosen.

Results and Discussion

Table 1 : Chemical composition of buffalo raw milk.

%Acidity	%Moisture	%Total solids	%Total Carbohydrates	%Total protein	%Fat	%Ash
0.26	84.66	15.34	3.53	4.50	6.70	0.61

Table (1) showed the chemical composition of fresh buffalo's milk in this study, and its acidity was 0.26% and contained 4.5% protein; 6.7% fat; 0.61% ash; 84.66 % water; 15.34 % total solids and3.53% carbohydrates, These results were in good agreement with (Hashmi & Saleem, 2015; Rafiq *et al.*, 2016; Soliman, 2005; Sun *et al.*, 2014)

Table 2 : Gross chemical composition, minerals and vitamins of Graviola fruit:

Gross chemical composition %								
Acidity	**Moisture**	**Total solids**	**Total Carbohydrates**		**Total protein**	**Fat**	**Ash**	**Crude Fiber**
1.50	78.01	21.99	17.43		2.80	0.50	1.26	6.45
Elements concentrations (mg / 100g)								
Mg	**Na**	**K**	**Mn**	**Fe**	**Ca**	**Zn**	**Cu**	**P**
0.352	27.002	14.295	0.176	3.706	12.707	0.00	1.235	0.529
Some vitamins content of Graviola (mg / 100g)								
Vitamin C		**Vitamin D**				**Vitamin A**		
0.13		**0.583**				**10.58**		

Table (2) showed the chemical composition of Graviola in this study, the moisture content obtained was lower than that of conventional fruits such as Citrus lanatus fruit (89%). The higher ash value of soursop indicated that Graviola is good sources of minerals and therefore can be used in diet supplementation which will improve the mineral quality of diets. Traditionally, fiber defined as the portions of plant foods that are resistant to digestion by enzymes. The fiber classified as soluble and fermentable in the colon and insoluble fiber, which has a thickening action but may ferment to a limited extent in the colon. It is worth noting that most prebiotics are non-digestible carbohydrates that fermented in the colon. Carbohydrates act selectively to stimulate the growth of bifidobacteria, which considered beneficial for human health. These results were in partly agreement with (Siqueira *et al.*, 2015)

Table (2) showed some elements content of Graviola in this study, and it contained 0.352, 27.002, 14.295, 0.176, 3.706, 12.707, 0.00, 1.235, 0.529 and 2.294 for Mg, Na, K, Mn, Fe, Ca, Zn, Cu, P and Se, respectively.

Minerals are important in human nutrition. It is well known that enzymatic activities as well as the electrolytic balance of the blood fluid are related to the adequacy of Na, K, Mg and Zn. Potassium is very important in maintaining the blood fluid volume and osmotic equilibrium. These results were in partial agreement with (Akomolafe and Ajayi, 2015; Gyamfi *et al.*, 2011). Table (2) showed some of antioxidants vitamins in Graviola used in this study and it contained 10.58, 0.583, 0.13 for vitamin A, vitamin D and vitamin C, respectively. Because of the presence of vitamin C in the Graviola, this suggests that the consumption of Graviola could provide a lot of health benefits (Akomolafe and Ajayi , 2015).

Table 3 : Phenols content of Graviola.

ppm	**Phenolic compounds**	**ppm**	**Phenolic compounds**
0.018	Coumarin	0.049	P. coumaric
470.52	Ferulic	191	Benzoic
0.008	Protocatechuic	0.905	Gallic
0.544	Catechin	3.137	Caffeine
0.167	3,4,5 methoxy cinnamic	1.223	Ellagic
0.045	Vannillic	45.371	Alpha Coumaric
68.448	Caffeic	0.002	Cinnamic
0.064	Catechol	0.099	P OH benzoic
0.224	Salicylic	2.843	Pyrogallol
4.3	Chlorogenic	0.131	4.Amino benzoic acid
		0.05	Iso Ferulic

Table (3) showed the amount of phenolic compounds in Graviola which contained P. coumaric, Benzoic, Gallic, Caffeine, Ellagic, Alpha Coumaric, Cinnamic, P OH benzoic, Pyrogallol, 4. Amino benzoic acid, Iso Ferulic, Coumarin Ferulic, Protocatchuic, Catechin, 3,4,5 methoxy cinnamic, Vannillic, Caffic, Catechol, Salicylic and Chlorogenic. Graviola is also considered an important source of functional compounds such as phenolic compounds. Phenolic substances are pharmacologically active components of plants which are capable of neutralizing free radicals, chelating metal catalysts and inhibiting the activity of oxidizing enzymes in biological systems. These results were in partly agreement with those of (Akomolafe and Ajayi, 2015; Siqueira *et al.*, 2015).

Table (4) showed the amount of Flavonoids compounds in Graviola which contained Qurectrin, Quercetin, Kaempferol, Hespertin, Naringenin, Apigenin, Naringin, Rutin, Hesperidin. These results were in good agreement with those of (Akomolafe and Ajayi, 2015).

Table 4 : Flavonoids content (ppm) of Graviola.

ppm	Flavonoids compounds	ppm	Flavonoids compounds
0.003	Apigenin	0.02	Qurectrin
0.141	Naringin	0.006	Qurectin
0.049	Rutin	0.011	Kaempferol
0.166	Hesperidin	0.018	Hespertin
		11.17	Naringenin

Table 5 : Influence of storage period on the Chemical composition of Acidophilus milk fortified with Graviola.

Titratable Acidity (%)					Storage period(days)
Mean	**T3**	**T2**	**T1**	**Control**	
2.58^{a}	2.93	2.63	2.53	2.23	**Fresh**
3.12^{b}	3.7	3.2	3.03	2.53	**3**
3.53^{b}	3.96	3.56	3.36	3.23	**7**
3.85^{ab}	4.46	3.6	3.56	3.56	**14**
	3.76^{ab}	3.25^{b}	3.12^{b}	2.88^{a}	**Mean**
Fat (%)					
Mean	**T3**	**T2**	**T1**	**Control**	
4.94^{a}	4.96	4.95	4.93	4.90	**Fresh**
4.94^{a}	5	4.94	4.93	4.90	**3**
4.9^{a}	5	4.89	4.87	4.86	**7**
4.83^{a}	4.76	4.88	4.85	4.83	**14**
	4.93^{a}	4.91^{a}	4.90^{a}	4.87^{a}	**Mean**
Total Protein (%)					
Mean	**T3**	**T2**	**T1**	**Control**	
5.62^{a}	7.55	5.29	5.16	4.51	**Fresh**
5.34^{b}	7.57	5.05	4.44	4.29	**3**
5.20^{b}	7.41	5.02	4.78	4.48	**7**
5.42^{ab}	7.49	4.28	4.26	4.78	**14**
	7.50^{ab}	4.91^{b}	4.66^{a}	4.51^{a}	**Mean**
Total Solids (%)					
Mean	**T3**	**T2**	**T1**	**Control**	
16.06^{a}	18.5	17.48	17.38	11	**Fresh**
18.13^{b}	20.70	19.26	19.14	13.41	**3**
19.78^{c}	22.82	22.40	20.39	13.49	**7**
21.27^{d}	26.10	23.45	21.94	13.59	**14**
	22.65^{c}	20^{b}	19.71^{b}	12.88^{a}	**Mean**
Ash (%)					
Mean	**T3**	**T2**	**T1**	**Control**	
0.73^{a}	0.77	0.74	0.72	0.71	**Fresh**
0.73^{a}	0.78	0.75	0.716	0.70	**3**
0.70^{a}	0.72	0.71	0.70	0.68	**7**
0.65^{a}	0.61	0.69	0.67	0.63	**14**
	0.72^{a}	0.71^{a}	0.70^{a}	0.68^{a}	**Mean**
Total Carbohydrates (%)					
Mean	**T3**	**T2**	**T1**	**Control**	
10.09^{a}	15.26	13.30	11.25	0.57	**Fresh**
10.33^{b}	15.41	14.16	11.36	0.38	**3**
10.92^{c}	15.47	14.9	12.6	0.70	**7**
11.72^{d}	17.1	15.1	13.7	0.99	**14**
	15.81^{d}	14.37^{c}	12.22^{b}	0.662^{a}	**Mean**

T_1: adding 5% Graviola, T_2: adding 10% Graviola, T_3: Adding 15% Graviola. Different letters show significant difference at $P \leq 0.05$.

Results in Table (5) showed that the mean values of titratable acidity of Sweet Graviola Acidophilus milk increased significantly by the incorporation of Graviola concentrations. This may be due to the fermentation of lactose, which produces lactic and acetic acid during fermentation and storage period. These outcomes are partly agree with those of (Hamad *et al.*, 2017).

Mean values of Fat and Protein values were increased by fortification with Graviola. This increase could be attributed to Fat and protein content of Graviola (Mbaeyi-Nwaoha and Ekere, 2014). Total solids mean values gradually increased within storage period of all treatments. This increase could be attributed to Total solids content of Graviola (Mbaeyi-Nwaoha and Ekere, 2014). During storage period carbohydrates content of different samples gradually increased due to fermentation process. In the same trend, there were significant increases in Ash content by the incorporation of Graviola, and this may be due to the high content of ash in Graviola. These results in agreement with (Aliyev, 2006; Mohamed Nour-Eldin Farid Hamad, 2017)

Table 6 : Influence of storage period on the bacteriological analysis of Sweet Acidophilus Milk fortified with Graviola.

Storage period (days)				**Samples**	**Microbiological Properties (c.f.u $\times 10^5$ /g)**
14	**7**	**3**	**Fresh**		
174	170	160	160	Control	Total bacterial count
189	188	185	172	T1	
199	193	190	184	T2	
198	195	194	192	T3	
54	50	41	40	Control	*Lb. acidophilus*
57	55	41	40	T1	
58	56	41	40	T2	
60	58	45	43	T3	
ND	ND	ND	ND	control	Yeasts & Moulds
ND	ND	ND	ND	T1	
ND	ND	ND	ND	T2	
ND	ND	ND	ND	T3	
ND	ND	ND	ND	control	Psychrotrophic bacteria
ND	ND	ND	ND	T1	
ND	ND	ND	ND	T2	
ND	ND	ND	ND	T3	

T_1: adding 5% Graviola, T_2: adding 10% Graviola, T_3: Adding 15% Graviola, ND: not detected.

Results in Table (6) showed that TBC and *Lb. acidophilus* counts of control and Sweet Graviola Acidophilus milk have increased by the increasing of concentration of Graviola until the end of the storage period (14 days). *Lb. acidophilus* as a probiotic remained above (10^6) CFU.g^{-1} until the end of storage. Adding 5, 10, 15% Graviola improved microbiological properties of Acidophilus milk.

According to international standards, the total viable count in a probiotic product must be at least 10^6 /g (Junaid *et al.*, 2013) at the time of consumption.

The counts of yeasts and molds and psychrotrophic bacteria were not detected till the end of storage period; this might be due to the severity of heat treatments in addition to the antimicrobial effects of Graviola. On the other hand, the Coliform bacteria counts, Aerobic and Anaerobic spore forming bacteria has not detected in both fresh and at all treatments until the end of storage period. This might be due to the severity of heat treatments of milk and the role of Probiotics bacteria in preservation of products, which associated with their ability to produce a range of antimicrobial compounds. These results are in agreement with those of (Subhra and Aruna, 2013) who observed that the increase in the probiotic cell counts and the survival of culture in the fruit drink makes it suitable as a probiotic fruit drink. (Hamad *et al.*, 2017) showed that the probiotic Guava fruit drink contained lactic acid bacteria could serve as a healthy drink for consumers with a dairy allergy, beneficial to gut health, prevention of diarrhea. It has been reported that the addition of fruit juices or pulps might be deleterious to the survivability of some species in food products, particularly due to acidity and the presence of antimicrobial compounds, The variances in survival were interpreted by the metabolic activity of probiotic bacteria in different fermented products, which might be affected by the composition and availability of nitrogen and carbon sources in growth media (Abou-Dobara, Ismail, & Refat, 2018).

Table 7 : Influence of storage period at 7±1°C on the organoleptic properties of Acidophilus Milk fortified with Graviola.

Sensory Properties	Samples	Storage period (days)		
		Fresh	14	Mean
Flavor (45)	Control	43	37.5	40.25[a]
	T1	43.2	37.9	40.55 [b]
	T2	43.5	34.3	38.90[c]
	T3	41.8	34.3	38.05 [c]
	Mean	42.88[a]	36 [b]	
Body and Texture (30)	Control	27.2	24.6	25.90[a]
	T1	27.4	25.9	26.65[b]
	T2	28.15	24.2	26.18[c]
	T3	26.90	23.10	25[d]
	Mean	27.41[a]	24.45[b]	
Appearance (15)	Control	13.4	11.9	12.65[a]
	T1	13.81	12.4	13.11[b]
	T2	13.65	11.3	12.48[c]
	T3	13.05	10.6	11.83[d]
	Mean	13.48[a]	11.55[b]	
Acidity (10)	Control	8.65	5.7	7.18[a]
	T1	8.45	5.5	6.98[b]
	T2	8.5	5.5	7[b]
	T3	8.3	5.3	6.8[d]
	Mean	8.48[a]	5.88[b]	
Overall Scores (100)	Control	92.25	79.7	86[a]
	T1	92.86	83.2	88.03[b]
	T2	93.8	75.3	84.55[c]
	T3	90.05	73.3	81.68[d]
	Mean	92.24[a]	77.88[b]	

Table 7. The mean scores of acidity, appearance, Body and Texture in plain acidophilus milk and flavored acidophilus milk had significant difference ($p<0.05$)

Flavor serves as a preliminary parameter for the acceptance of food and indicates the fitness of milk products for consumption. Table 9 shows that the Acidophilus milk with different treatments was rated by the panelists. These results were good during storage. At the beginning and the end of storage period, the difference of flavor among different milk treatments was very slight. Flavor means an overall integrated perception of taste and aroma associated with the product (Junaid *et al.*, 2013). The overall acceptability during storage is considered reasonable for a fermented dairy product. These quality findings may be useful for dairy industries to produce a new variety of dairy products.

Conclusion

In summary, the addition of Graviola to Acidophilus milk had a positive impact on the chemical, microbiological and sensory properties of the forfeited fermented product. From the results obtained in this study, it can conclude that Acidophilus milk combined with Graviola at 5%, 10% was the most preferred among the flavored Acidophilus milk treatments.

Based on the findings, the researchers recommended that Graviola could replace the use of Flavoring materials in the production of flavored Acidophilus milk to improve the nutrition value of the product. This product may offer additional health benefits over Acidophilus milk alone due to the added nutrients.

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