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Properties and Antioxident Activity of Probiotic Yoghurt Flavored with Black Carrot, Pumpkin and Strawberry

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ABSTRACT

Customer demand on yoghurts with natural additives has been dramatically increased. The study was carried out to improve the flavor and nutritional value of yogurt. Black carrot, pumpkin and strawberry were added to yogurt at several concentrations. Chemical, rheological, Organoleptic, microbiological and antioxidant properties were determined in the flavored yoghurts. Viscosity in yoghurts was decreased by adding black carrot and strawberry, while increased by adding pumpkin. Syneresis of all flavored yoghurts was significantly lower than that of the plain yoghurt without sugar. Strains of lactic acid bacteria approximately exhibited the same behavior in flavored yoghurt samples. The viability of probiotic organism *B. lactis* Bb-12 was highest when 8% sugar was added. Organoleptic assessment indicated that the use of pumpkin as a flavoring material is highly recommended in the probiotic yoghurt manufacturing. Total phenolic content of the flavoring materials was highest in the strawberry jam followed by black carrot jam and was the lowest in the pumpkin jam. Flavonoid content in the flavoring materials showed a similar trend to that of total phenolic content in different flavored yoghurts. RSA % was higher in flavored probiotic yoghurt containing 0.5 and 1% pumpkin and carrots than plain yoghurt with or without sugar. The volume of yoghurt (L) required causing 50% (IC_{50}) or 90% (IC_{90}) free radical inhibition was inversely related to the RSA%. Chemical, rheological, microbiological, organolepical and antioxidant properties indicated that the use of pumpkin, strawberry and black carrot as flavoring materials in flavored yoghurt manufacturing is highly recommended.

Key words: Nutritional value, antioxidant properties, flavored yoghurts, probiotic yoghurts

INTRODUCTION

Yoghurt is the most popular fermented milk produced all over the world. It is characterized by its nutritional and beneficial effects on human (Chandan, 2007). The worldwide production and consumption of yoghurt has diametrically increased during the last quarter of last century, due to the introduction of fruit and vegetable flavored yoghurt (Chandan and Kilara, 2010). Fruits and vegetables which are added to yoghurt act as prebiotics (fibers) (Allgeyer *et al*., 2010), as flavoring and coloring agents (Salem *et al*., 2006; Smith and Hui, 2008) and as a source of natural antioxidants (Photochemical antioxidants) (Dimitrios, 2006). The photochemical antioxidants such as carotenoids, flavonoids and phenols etc., have potential health roles in the reduction of platelet aggregation, blood pressure, cardiovascular of disease and a role in modulation of cholesterol synthesis and absorption (Li, 2008). Moreover, fruits and vegetables contain protein bound

polysaccharides which may increase the levels of serum insulin, reduce the blood glucose levels and improve tolerance of glucose and hence could be developed as new anti diabetic agent (Li *et al.*, 2005).

Carrots are an excellent source of vitamin A. Carrot nutrition is exceptionally high in carotene. Carrots are also a good source of niacin (vitamin B3). Carrot provides a good source of potassium. Several fruits and vegetables have nutritional value. Pumpkin is an excellent source of vitamin A. Pumpkin contains a very good amount of beta and alpha carotene and provides a very good amount of beta cryptoxanthin. Strawberries are rich in phenols and particularly a class of phenols called anthocyanins. The anthocyanins in strawberries give the fruit its red color and acts as a powerful antioxidant that protects the bodies' cells from free radical damage. Strawberry nutrition is an excellent source of vitamin C. Strawberry is also a very good source of manganese. Strawberry nutrition contains good levels of niacin (vitamin B3) (Li, 2008).

The objective of the current study was to use black carrot, pumpkin and strawberry to improve the flavor and nutritional value of yogurt. Chemical, rheological, microbiological, organoliptical and antioxident properties were determent. Sensory acceptability of the flavored yoghurt was assessed.

MATERIALS AND METHODS

Materials: This study was carried out during the period from 2006-2007. Fresh whole caw's milk was obtained from Cairo University herd. Spray dried low heat skim milk powder was obtained from Valio Co. Helsinki, Finland. Sugar and flavoring vegetables (strawberry, black carrot and pumpkin) were purchased from the local market.

Thermophilus yoghurt culture (*Streptococcus salvarius thermophilus* and *Lactobacillus delbrueki* subsp. *bulgaricus*) in the form of freeze dried- direct Vat set (FD-DVS) was obtained from Rodia Food, France, while *Befidobacterium lactis* Bb-12 as probiotic strain was obtained from Chr. Hansehs' Laboratories, Copenhagen, Denmark. All microbial media used were obtained from Oxiod Division cf Oxiod LTD, London. Folin-Ciocalteu reagent, tannic acid and quercetin (Sigma-Aldrich Chemie, Steinheim, Germany), methyl alcohol, aluminium trichloride ($AlCl_3$), 2,2 diphenyl-1-picrylhydrozyl (DppH), FlukaChemie, Switzerland, L-cysteine-HCl (Sigma Chemical Co., St. Louis, USA) and sodium carbonate (Labosi, Paris, France) were used.

Methods

Preparation of flavoring materials: Peeled pumpkin, black carrot and strawberry were cleaned, washed and cut into small pieces mixed with the same weight of sugar (1:1) and heated till the vegetables were cooked then blended with blender. Prepared jams were kept at 5°C until used.

Experimental procedure: Preliminary experiments were carried out to choose the suitable concentration of sugar, flavoring materials, starter culture for each yoghurt and probiotic cultures. Results of preliminary experiments revealed that, addition of vegetable jams prepared with 2 and 8% sugar at 0.5, 1.0 and 1.5%, were the most preferable treatments.

Preparation of plane and flavored yoghurt: Caw's milk (4.7% lactose, 3.2% protein, 3.4% fat, 12.0% T.S and 0.17% acidity) was standardized to 14% T.S by skim milk powder. The amount of seven and half kg of milk was divided into five equal portions. The first portion was kept without any additions. The second portion was sweetened with 8.0% sucrose. Jams of these fruits were added separately to the left three portions of standardization cow's milk at 0.5, 1.0 and 1.5%.

The sugar content of the vegetable jam containing milk was adjusted to 8% sucrose by adding commercial sugar. All milk portions were heated to 90°C for 15 min. then cooled to 42°C and inoculated with 2% yoghurt culture and 2% *Bifidobacterium lactis* B6-12 culture under aseptic conditions. Yoghurt was sampled in 125 g plastic cups and incubated at 42±2°C for 4 h, then transferred to a refrigerator at 5°C to be kept for 3, 6 and 10 days.

The flavored probiotic yoghurt was chemically, rheologically, microbiology and organoleptically assessed in fresh samples, after 3, 6 and 10 days of cold storage. The antioxidants content and antioxidant acidity were estimated in fresh samples. Three replicates of flavored probiotic yoghurt were manufactured and analyzed and results were tabulated.

Analysis: The pH was measured using a laboratory pH meter (type WTW, Inolab 720, Germany). Viscosity was estimated using Brook field viscometer LTV with spindle RV5 at 150 rpm in 200 mL samples at 25°C. Syneresis was carried out according to the method of Keogh and O'Kennedy (1998). Phenolics and flavonoids content were determined as mentioned by Singleton *et al.* (1999) and Blasa *et al.* (2006), respectively. The antioxidant activity of ingredients and yoghurt samples was estimated as the radical scavenging activity (RSA%) and the volume of yoghurt required causing 50 or 90% free radical inhibition (Velazquez *et al.*, 2003).

Streptococcus thermophilus count was enumerated using M17 agar (Terzaghi and Sandine, 1975). Count of *Lactobacillus bulgaricus* was determined using MRS agar as reported by Dave and Shah (1997), while count of Bifidobacteria was determined according to Vinderola *et al.* (2000). Organoleptic assessment was carried out as mentioned by Williams (1982).

Statistical analysis was performed using MSTAT-C program (Ver.2.10, Michigan state University, USA).

RESULTS AND DISCUSSION

Chemical and rheological properties

pH value: Table 1 indicated that addition of black carrot and strawberry led to decrease the pH value in fresh to 4.10 and 4.19, respectively and after ten days of storage to 3.50 and 4.06, respectively. Addition of pumpkin increased the pH in fresh and cold stored yoghurts to 4.47 and 4.36, respectively. The decrease of pH in both black carrot and strawberry yoghurts is in accordance with the results of Lourens-Hattingh and Viljoen (2001) and Taha *et al.* (2007), who found that the addition of flavoring materials led to increase the population of the different strains of the starters' culture microorganisms. On the other hand, the increase of pH in pumpkin yoghurts maybe due to the presence of several protein-pound polysaccharides in the pumpkin (Caili *et al.*, 2007) which may increase its viscosity and cause coagulation at higher pH.

It was also found that as the percentage of added flavoring materials increases the pH of yoghurts decreases which confirms their stimulation effect on the starter microorganisms and *Befidobacterium lactis* Bb 12.

During cold storage the pH values of all products significantly decreased in the first 3 days. There after the decrease was insignificant. The results are in line with the results of Mehriz *et al.* (2007) who observed a gradual reduction in the pH value during shelf life of yoghurt samples.

Viscosity: The results in Table 1 showed that addition of black carrot and strawberry significantly decreased the viscosity of the flavored yoghurts after ten days of cold storage to 285 and 399 cp, respectively. In contrary, the addition of pumpkin significantly increased the viscosity of the yoghurts after ten days of cold storage to 502 cp. The highest viscosity values recorded for pumpkin

Table 1: pH, viscosity and syneresis of plain, pumpkin, black carrot and strawberry flavored probiotic yoghurts during storage at 5°C

	Vegetable solid levels	pH					Viscosity (cp.)					Syneresis (%)				
Treatments	Solids level	Fresh	3	6	10	Mean	Fresh	3	6	10	Mean	Fresh	3	6	10	Mean
Plain (-)	0.00	4.50	4.30	4.20	4.10	4.27^{C}	406.0	443	410	518	444^{a}	28.0	32.0	27.0	27.0	28^{a}
Plain (+)	0.00	4.43	4.39	4.30	4.10	4.31^{a}	362.0	500	416	414	423^{a}	23.0	23.0	26.0	23.0	24^{b}
Pumpkin	0.5%	4.53	4.47	4.44	4.34	4.45^{b}	426.0	454	409	493	445^{c}	26.0	26.0	26.0	23.0	25^{b}
	1.0%	4.57	4.37	4.43	4.35	4.43^{b}	450.0	521	443	541	488^{b}	24.0	26.0	23.0	22.0	24^{bc}
	1.5%	4.47	4.36	4.47	4.36	4.42^{b}	488.0	536	458	545	506^{b}	19.0	25.0	24.0	22.0	22^{c}
Mean		4.50^{A}	4.38^{A}	4.37^{B}	4.25^{C}		426.0^{C}	490^{B}	427^{C}	502^{A}		23.0^{B}	27.0^{A}	25.0^{AB}	23.0^{B}	
Plain (-)	0.00	4.50	4.30	4.20	4.10	4.27^{a}	406.0	443	410	518	444^{a}	27.8	31.9	26.6	26.7	28^{a}
Plain (+)	0.00	4.43	4.39	4.30	4.10	4.31^{a}	362.0	500	416	414	423^{a}	23.1	23.1	25.7	23.1	24^{b}
Black Carrots	0.5%	4.12	3.60	3.70	3.50	3.73^{b}	287.7	306	351	312	314^{b}	22.9	25.4	24.1	25.1	25^{b}
	1.0%	4.11	3.90	3.60	3.30	3.73^{b}	285.6	299	322	337	311^{b}	22.5	26.8	23.4	24.9	24^{b}
	1.5%	4.10	3.80	3.70	3.50	3.78^{b}	293.2	315	335	344	322^{b}	23.6	23.5	24.8	27.5	25^{b}
Mean		4.25^{A}	4.00^{B}	3.90^{B}	3.70^{B}		326.0^{B}	372^{A}	367^{A}	385^{A}		24.0^{B}	26.0^{A}	25.0^{AB}	26.0^{AB}	
Plain (-)	0.00	4.50	4.30	4.20	4.10	4.27^{a}	406.0	443	410	518	444^{a}	27.8	31.9	26.6	26.7	28^{a}
Plain(+)	0.00	4.43	4.39	4.30	4.10	4.31^{a}	362.0	500	416	414	423^{a}	23.1	23.1	25.7	23.1	24^{b}
Strawberry	0.50%	4.32	4.15	4.20	4.13	4.20^{b}	331.0	373	349	328	345^{bc}	24,8	28.4	27.8	26.8	27^{a}
	1.0%	4.22	4.01	4.05	4.05	4.08^{c}	323.0	372	355	425	369^{b}	28.2	30.7	31.0	29.3	30^{a}
	1.5%	4.19	3.98	4.06	4.06	4.07^{c}	284.0	341	364	311	324^{c}	25.0	31.2	30.9	29.7	29^{a}
		4.33^{A}	4.17^{B}	4.16^{B}	4.01^{B}		341.0^{B}	405^{A}	378^{A}	399^{A}		26.0^{A}	29.0^{A}	28.0^{A}	27.0^{A}	

Means designated with the same letters either capital or small in the same raw or columns are not significantly different at 0.05 level of probability, -: Without sugar, +: With sugar

flavored yoghurt may be due to the presence of stabilizer agents (dietary fibers) in pumpkin which has the ability to bind water tending to increase the consistency of the products by increasing the water-binding capacity (Caili *et al*., 2007). The highest viscosity values for flavored yoghurt were recorded for samples containing 1.5% pumpkin, 1.5% black carrot and 1% strawberry. Upon storage the viscosity of plain and flavored yoghurt samples was significantly increased during the first 3 days of storage period, while they significantly decreased in plain and pumpkin yoghurt. Contrary to that, the viscosity of black carrot and strawberry increased till the end of storage period.

Syneresis: As shown in Table 1, syneresis of all flavored yoghurts was significantly lower than that of the plain yoghurt without sugar, but not of that plain yoghurt with sugar. The increase of pumpkin led to decrease the syneresis of the produced yoghurt to 22%, while insignificant changes where noticed when black carrot and strawberry were increased.

During storage syneresis increased significantly through the first three days, this may be due to the effect of storage on the lowering pH value. Schmidt and Bouma (1992) and Richmond *et al.* (1985) indicated that there is a positive correlation between acid production and degree of syneresis in cottage cheese and yoghurt.

Microbiological properties (survival of lactic acid and probiotic bacteria)

Survival of *S. thermophilus* and *L. bulgaricus*: Table 2 showed that the strains of lactic acid bacteria approximately exhibited the same behavior in plain yoghurt without adding sugar. The count increased significantly during the first three days and then decreased significantly till the end of storage period.

Table 2: *St. thermophilus*, *Lb. bulgaricus* and *B. Lactis* Bb-12 counts of flavored probiotic yoghurts during shelf life (10 days at 5°C)

	Vegetable solid levels	*St. thermophilus*×10^7					*Lb. bulgaricus*×10^7					*B. lactis* Bb- 12×10^6				
Treatments	Solids level	Fresh	3	6	10	Mean	Fresh	3	6	10	Mean	Fresh	3	6	10	Mean
Plain (-)	0.0	25.0	52.0	28.0	13.0	29.0^a	13.0	18.0	7.0	5.0	11.0^{ab}	150.0	2.0	131.0	76.0	90.0^b
Plain (+)	0.0	35.0	21.0	25.0	13.0	24.0^a	12.0	14.0	7.0	3.0	9.0^b	468.0	4.0	66.0	127.0	166.0^b
Pumpkin	0.5%	13.0	30.0	4.0	16.0	16.0^a	8.0	11.0	14.0	4.0	9.0^b	135.0	301.0	45.0	62.0	135.0^b
	1.0%	27.0	18.0	3.0	15.0	15.0^a	40.0	34.0	34.0	2.0	27.0^a	17.0	480.0	16.0	123.0	158.0^b
	1.5%	138	25.0	3.0	3.0	43.0^a	5.0	21.0	28.0	3.0	14.0^{ab}	300.0	160.0	30.0	191.0	170.0^b
Mean		48.0^A	29.0^{AB}	17.0^{AB}	7.0^B		16.0^{AB}	20^{0A}	18.0^{AB}	3.0^B		214.0^B	189.0^B	57.0^C	115.0^{BC}	
Plain (-)	0.0	25.0	52.0	28.0	13.0	29.0^b	13.0	18.0	7.0	5.0	10.0^C	150.0	2.0	131.0	76.0	90.0^b
Plain (+)	0.0	35.0	21.0	25.0	14.0	23.0^b	12.0	14.0	7.0	3.0	8.0^C	468.0	4.0	66.0	127.0	166.0^{ab}
Black Carrots	0.5%	54.0	93.0	34.0	54.0	58.0^a	21.0	61.0	44.0	8.0	33.0^b	355.0	48.0	162.0	543.0	277.0^a
	1.0%	41.0	17.0	30.0	18.0	24.0^b	36.0	54.0	80.0	14.0	45.0^a	237.0	71.0	165.0	18.0	123.0^{ab}
	1.5%	26.0	45.0	26.0	16.0	28.0^b	31.0	51.0	57.0	18.0	39.0^{ab}	372.0	75.0	60.0	90.0	149.0^{ab}
Mean		36.0^{AB}	45.0^A	26.0^{AB}	22.9^B		22.0^B	40^A	39.0^A	9.0^C		316.0^A	40.0^B	116.0^B	170.0^{AB}	
Plain (-)	0.0	25.0	52.0	28.0	13.0	30.0^a	13.0	18.0	7.0	5.0	11.0^b	150.0	2.0	131.0	76.0	90.0^a
Plain(+)	0.0	35.0	21.0	25.0	14.0	24.0^a	12.0	14.0	7.0	3.0	9.0^b	468.0	3.0	66.0	127.0	16.0^a
Strawberry	0.5%	58.0	75.0	26.0	19.0	44.0^a	33.0	41.0	18.0	15.0	27.0^a	316.0	93.0	125.0	207.0	18.0^a
	1.0%	25.0	47.0	19.0	13.0	26.0^a	18.0	36.0	4.0	4.0	16.0^{ab}	300.0	130.0	57.0	58.0	13.0^a
	1.5%	17.0	38.0	21.1	11.0	22.0^a	20.0	25.0	10.0	10.0	16.0^{ab}	417.0	99.0	33.0	131.0	17.0^a
		32.0^{AB}	32.0^{AB}	32.0^{AB}	14.0^B	19.0^{AB}	27.0^A	7.0^B	7.0^B		330.0^A	66.0^B	83.0^B	120.0^B		

Means designated with the same letter either capital or small in the same raw or columns are not significantly different at 0.05 level of probability.
-: Without sugar, +: With sugar

Similar behavior was observed in flavored yoghurt samples, but in sweetened yoghurt there was a different behavior as it was fluctuated.

The results indicated that, though the stimulation effect of all flavored ingredients used on the growth of *L. bulgaricus*. Its viability during storage for 10 days was higher as compared with those of *S. thermophilus*. The count of *S. thermophilus* was higher than that of *L. bulgaricus* in all treatments throughout the storage period. This observation is in line with observation of Birillo *et al.* (2000) who found that the count of *Streptococcus* was significantly higher than that of *Lactobacillus* in yoghurt.

It is worthy to mention that the inclusion of black carrot resulted in highest viability of *L. bulgaricuis* in the yoghurt during the first 6 days of storage followed by that yoghurts containing 1.0 and 1.5% pumpkin and ended by that of 0.5% strawberry containing yoghurt.

Survival of *Bifidobacterium* Bb-12: It is obvious from Table 2 that the addition of sucrose to yoghurt prior to processing tended to stimulate the growth of *Bifidobacterium lactis* Bb-12. The viability of probiotic organism *B. lactis* Bb-12 was the highest when 8% sugar was added.

During the storage period, the population of probiotic bacteria in all samples decreased toward the end as compared with the fresh samples except for pumpkin flavored yoghurt where its viability decreased only after three days of storage.

Sensory evaluation of plain and flavored probiotic yoghurt: Results recorded in Table 3 showed that all fresh yoghurts under investigation were highly accepted, scoring 93.3-98.0 out of 100. The most preferable fresh yoghurt was the 1.5% pumpkin containing yoghurt which scored

Table 3: Sensory evaluation score of plain, pumpkin, black carrot and Strawberry flavored probiotic yoghurts during shelf life (10 days at 5°C)

	Vegetable solid levels	Flavor (5)					Acidity (10)					Viscosity (20)				
Treatments	Solids level	Fresh	3	6	10	Mean	Fresh	3	6	10	Mean	Fresh	3	6	10	Mean
Plain (-)	0.0	47.4	46.7	44.2	45.7	46^{d}	9.9	9.8	9.0	7.3	9.0^{d}	19.2	19.2	19.8	19.3	90.0^{b}
Plain (+)	0.0	48.4	47.3	45.7	47.8	47^{b}	9.8	9.5	9.0	8.7	9.2^{b}	19.3	19.3	19.8	19.3	166.0^{b}
Pumpkin	0.5%	47.4	47.0	46.3	46.9	47^{c}	9.4	8.3	9.3	8.7	8.9^{e}	18.8	18.8	19.8	20.0	135.0^{b}
	1.0%	48.3	47.7	47.5	46.6	47^{b}	9.6	8.6	9.3	9.0	9.1^{c}	19.3	19.3	19.8	20.0	158.0^{b}
	1.5%	49.1	48.7	48.5	47.5	48^{a}	9.7	9.3	9.5	8.7	9.3^{a}	19.5	19.5	19.8	20.0	170.0^{b}
Mean		48.0^{A}	47.0^{B}	46.0^{D}	47.0^{C}		9.6^{A}	9.1^{C}	9.2^{B}	9.2^{B}		19.0^{D}	19.0^{C}	20.0^{A}	20.0^{B}	
Plain (-)	0.0	47.4	46.7	44.2	45.7	46^{bc}	9.9	9.8	9.0	7.3	9.0^{e}	19.5	19.2	198.0	19.3	20.0^{e}
Plain (+)	0.0	48.4	47.3	45.7	47.8	47^{a}	9.8	9.5	9.0	8.7	9.3^{a}	19.5	19.3	19.8	19.3	20.0^{bc}
Black carrots	0.5%	49.1	46.3	44.5	43.0	46^{bc}	9.7	9.0	8.2	7.7	8.7^{a}	19.2	20.0	19.3	19.7	2.0^{b}
	1.0%	47.0	45.3	45.3	43.6	45^{c}	10.0	8.3	8.5	8.3	8.8^{d}	20.0	19.3	19.7	20.0	20.0^{a}
	1.5%	48.0	47.3	46.3	44.1	46^{b}	10.0	9.0	9.0	8.7	9.1^{b}	20.0	19.0	20.0	20.0	20.0^{a}
Mean		48.0^{A}	47.0^{B}	45.0^{C}	45.0^{C}		9.9^{A}	9.1^{B}	8.7^{C}	8.1^{D}		19.0^{B}	19.7^{A}	19.7^{A}	19.7^{A}	
Plain (-)	0.0	47.4	46.7	44.2	45.7	46^{c}	9.9	9.8	9.0	7.3	9.0^{e}	19.5	19.2	19.8	19.3	20.0^{e}
Plain(+)	0.0	48.4	47.3	45.7	47.8	47^{a}	9.8	9.5	9.0	8.7	9.3^{a}	19.3	19.3	19.8	19.3	20.0^{e}
Strawberry	0.5%	47.7	46.7	46.8	45.3	47^{b}	8.7	9.7	9.0	8.0	8.8^{b}	20.0	20.0	19.7	20.0	20.0^{b}
	1.0%	47.0	46.3	47.6	46.2	47^{b}	9.7	8.7	9.7	8.7	9.2^{b}	20.0	20.0	20.1	20.0	20.0^{a}
	1.5%	47.0	47.0	47.6	46.1	47^{b}	9.0	9.3	9.0	7.7	8.8^{e}	20.0	20.0	19.7	20.0	20.0^{b}
		48.0^{A}	47.0^{B}	47.0^{B}	46.0^{C}		9.4^{A}	9.4^{A}	9.1^{C}	8.1^{C}		19.8^{A}	19.7^{B}	19.8^{A}	19.7^{B}	

Means designated with the same letters either capital or small in the same raw or column is not significantly different at 0.05 level of probability. (-) means, without sugar; (+) means, with sugar

98.0 points; followed by sweetened yoghurt (plain yoghurt with sugar) scoring 97.6 points, followed by both of 1.5% black carrot containing yoghurt and plain yoghurt without sugar which scored 97.3 points. The results also revealed that the sensory evaluation score increased as the percentage of added flavoring ingredients increased.

Upon storage at 5°C for 10 days, it was interesting to find that all pumpkin flavored yoghurts were the most preferred products during storage period where sensory evaluation score was 93-95.3 and 96.5-98 points for 0.5 and 1.5% pumpkin flavored yoghurts, respectively (Table 4).

Preparation of fruit yoghurts has been investigated by a number of researchers in different parts of world (Hossain *et al*., 2012). Sensory evaluation results of flavored yoghurts were affected by type and concentration of fruits in the yoghurts (Chandan and Kilara, 2010).

Antioxidant properties

Total phenolic contents: Results given in Table 5 indicated that the total phenolic content of the flavoring materials was the highest in the strawberry jam followed by that of black carrot and was lowest in the pumpkin. The total phenolic content was 64.67, 21.91 and 15.57 mg tannic acid equivalent (TAE)/100 g, respectively. When these jams were added to yoghurt milk, the produced yoghurts contained total phenolic in the following order: strawberry yoghurt>pumpkin yoghurt>plain yoghurt>sweetened yoghurt. Moreover, as the percentage of flavoring ingredients increases the total phenolic content increases.

Antioxidant activities and total phenolic contents of 62 fruits were evaluated. The results showed that different fruits had diverse antioxidant capacities and the variation was very large (Fu *et al*., 2011).

Table 4: Sensory evaluation score of plain, pumpkin, black carrot and strawberry flavored probiotic yoghurts during shelf life (10 days at 5°C)

	Vegetable solid levels	Flavor (5)					Acidity (10)					Viscosity (20)				
Treatments	Solids level	Fresh	3	6	10	Mean	Fresh	3	6	10	Mean	Fresh	3	6	10	Mean
Plain (-)	0.00	10.0	10.0	10.0	10.0	10.0^{a}	3.33	3.33	3.33	3.33	3.3^{c}	96.4	95.2	92.2	94.10	93.8^{c}
Plain (+)	0.00	10.0	10.0	10.0	10.0	10.0^{a}	6.47	6.47	6.67	6.47	6.5^{b}	97.6	69.0	94.5	5.50	89.1^{d}
Pumpkin	0.50%	8.6	9.3	9.7	10.0	9.4^{c}	9.6	9.5	9.30	9.60	9.5^{ab}	93.3	93.0	94.5	95.30	94.0^{c}
	1.0%	9.2	9.8	10.0	10.0	9.7^{b}	9.5	9.7	10.00	9.50	9.7^{a}	95.9	95.2	96.7	95.50	95.8^{b}
	1.5%	9.8	10.0	10.0	10.0	9.9^{a}	9.5	9.7	10.00	9.50	9.7^{a}	97.3	97.2	97.8	96.50	97.2^{a}
Mean		9.5^{C}	9.8^{B}	9.9^{A}	10.0^{A}		7.7^{A}	7.7^{A}	7.90^{A}	7.70^{A}		96.0^{A}	89.0^{D}	95.0^{B}	95.00^{C}	
Plain (-)	0.0	10.0	10.0	10.0	10.0	10.0^{a}	3.3	3.3	3.30	3.30	3.3^{c}	96.4	95.3	92.2	91.40	94.0^{b}
Plain (+)	0.00	10.0	10.0	10.0	10.0	10.0^{a}	6.5	6.5	6.70	6.50	6.5^{b}	97.6	69.0	94.5	95.50	89.0^{e}
Black Carrots	0.5%	9.8	9.3	9.0	9.3	9.4^{c}	9.5	9.7	9.00	9.70	9.5^{ab}	97.3	94.3	88.5	78.70	90.0^{d}
	1.0%	9.7	10.0	9.7	9.7	9.8^{b}	10.0	10.0	9.70	10.00	9.9^{b}	96.7	93.0	91.7	90.30	93.0^{c}
	1.5%	10.0	10.0	10.0	10.0	10.0^{a}	10.0	10.0	10.00	10.00	10.0^{a}	98	95.3	94.7	91.70	95.0^{a}
Mean		9.9^{A}	9.9^{AB}	9.7^{C}	9.8^{BC}		7.9^{A}	7.9^{A}	7.70^{A}	7.90^{A}		97.0^{A}	89.0^{C}	92.0^{B}	90.00^{C}	
Plain (-)	0.00	10.0	10.0	10.0	10.0	10.0^{a}	3.3	3.3	3.30	3.30	3.3^{c}	96.4	95.3	92.2	91.40	93.0^{c}
Plain(+)	0.00	10.0	10.0	10.0	10.0	10.0^{a}	6.4	6.5	6.70	6.50	6.5b	97.6	69.0	94.5	95.50	89.0^{d}
Strawberry	0.5%	10.0	10.0	10.0	10.0	10.0^{a}	9.3	10.0	10.00	9.30	9.7^{a}	95.7	96.3	95.7	92.30	95.0^{b}
	1.0%	10.0	10.0	10.0	10.0	10.0^{a}	10.0	9.7	10.00	10.10	9.9^{a}	96.7	94.7	97.3	94.70	95.0^{a}
	1.5%	10.0	10.0	10.0	10.0	10.0^{a}	9.3	9.3	10.00	9.30	9.5^{ab}	95.3	95.7	96.0	92.30	94.0^{b}
		10.0^{A}	10.0^{A}	10.0^{A}	10.0^{A}		7.7^{A}	7.8^{A}	8.00^{A}	7.70^{A}		96.0^{A}	90.0^{D}	95.0^{B}	93.00^{C}	

Means designated with the same letters either capital or small in the same raw or column is not significantly different at 0.05 level of probability.
-: Without sugar; +: With sugar

Table 5: Phenolic content, flavonoid content and flavonoids/phenolics ratio of fresh plain, pumpkin, black carrot and strawberry flavoure probiotic yoghurts

Treatments	Vegetable solids level	Phenolics mg TAE/100 g	+ or - % in Phenolics*	Flavonoids mg QE/100 g	+ or - % in Flavonoids**	F/P Ratio***
Pumpkin jam	Pure extract	15.57	-27.24	7.32	-22.04	0.41
Carrot jam	Pure extract	21.91	2.38	9.64	9.64	0.44
Strawberry jam	Pure extract	64.67	202.19	20.69	119.81	0.32
Plain (-)	0.0	21.40	0.00	9.39	0.00	0.43
Plain (+)	0.0	19.75	-7.71	10.49	11.71	0.53
Pumpkin	0.5%	20.11	-6.03	9.53	1.47	0.47
Yoghurt	1.0%	21.53	0.61	10.24	9.05	0.48
	1.5%	22.92	7.10	10.76	14.58	0.47
Carrot	0.50%	13.28	8.79	10.11	7.66	0.43
Yoghurt	1.0%	25.13	17.43	10.94	16.50	0.44
	1.5%	27.44	28.22	12.61	34.29	0.48
Strawberry	0.5%	24.47	14.34	10.76	14.58	0.44
Yoghurt	1.0%	24.72	15.51	11.68	24.38	0.47
	1.5%	28.48	33.08	15.78	68.05	0.55

*, ** + or - % in Phenolics or flavonoids = increase or decrease in Phenolics or flavonoids as compared with control (plain), ***F/P Ratio: flavonoids/Phenolics ratio, -: Without sugar, +: With sugar, TAE: Tannic acid Equivalent phenolics, QE: Quercetin equivalent flavonoids

Total flavonoid content: As it seen in Table 5, the flavonoid content in both the flavoring materials and the flavored yoghurts showed a similar trend to that of total phenolic content. The

Table 6: Antioxidant activity (RSA %), IC_{50} and IC_{90} of fresh plain, pumpkin, black carrots and strawberry Jam or flavored probiotic yoghurts

Treatments	Vegetable solids level	RSA (%)*	+ or - % in RSA**	Vol. (L) IC_{50}***	Vol. (L) IC_{90}****
Pumpkin jam	Pure extract	42.73	49.98	1.17	2.11
Carrot jam	Pure extract	45.35	59.18	11.0	1.98
Strawberry jam	Pure extract	46.80	64.27	1.07	1.92
Plain (-)	0.0	28.49	0.00	1.76	3.16
Plain (+)	0.0	30.52	7.13	1.64	2.95
Pumpkin	0.50%	22.67	-20.43	2.21	3.97
Yoghurt	1.0%	26.45	-7.16	18.9	3.40
	1.50%	36.34	27.53	1.38	2.48
Carrot	0.50%	26.47	-6.14	1.87	3.37
Yoghurt	1.0%	27.62	-3.05	1.58	2.84
	1.50%	31.69	11.23	1.42	3.26
Strawberry	0.50%	35.17	23.44	1.35	2.56
Yoghurt	1.0%	36.92	29.58	1.25	2.44
	1.50%	40.12	40.82	1.10	2.24

*RSA % = Radical Scavenging activity %, **+ or - % in RSA = increase or decrease in RSA as compared with control (plain). ***Vol. (L) IC_{50}, ****VOL. (L) IC_{90} = Volume of yoghurt (Liter) achieving 50% or 90% free radical inhibition. -: Without sugar, +: With sugar

total flavonoid content was significantly the highest in strawberry containing yoghurt followed by that containing black carrot and pumpkin. This may be due to the higher content of total flavonoid in strawberry jam than that in black carrot and pumpkin jams.

Also, the results showed that the total flavonoid content of the flavored yoghurts were 15.78, 12.61 and 10.76 mg QE/100 g with strawberry, black carrot and pumpkin, respectively. Which were higher than that of plain yoghurt with or without sugar (10.49 and 9.39 mg QE/100 g, respectively).

Antioxidant activity

Antioxidant activity expressed as RSA%: Table 6 showed the antioxidant activity of the experimental yoghurts and flavouring ingredients expressed as radical scavenging acidity (RSA%). The results indicated that RSA% of flavoring ingredients was highest in the strawberry jam (46.80%) followed by that of black carrot (45.35%) and pumpkin jam (42.73%). The results are in agreement with those given by Salem *et al.* (2006) and Taha *et al.* (2007) who observed that strawberries have shown the highest antioxidant activity among the other fruits and vegetables used as flavoring material in milk origin beverage or flavored yoghurt.

The results revealed that yoghurts containing 1.5% of strawberry, pumpkin and black carrot showed the highest percentage of RSA, 40.12, 36.34 and 31.69%, respectively. Upon increasing the concentration of added flavoring ingredients, the RSA% increased. This is in agreement with Prior *et al.* (1998) who illustrated a linear relationship between total antioxidant capacity and flavonoid and phenolic contents.

Volume of yoghurt required to cause 50% (IC_{50}) or 90% (IC_{90}) free radical inhibitions: The volume of yoghurt (L) required causing 50% (IC_{50}) or 90% (IC_{90}) free radical inhibition (Table 6) was inversely related to the RSA%. It means that as the RSA% of the pure flavoring materials or that of the experimental products increased the volume in liters required to

cause either 50 or 90% free radical inhibition decreased. IC50 in strawberry, pumpkin and black carrot flavored yoghurts were 1.10, 1.38 and 1.42 L, respectively. While IC90 was in strawberry, pumpkin and black carrot flavored yoghurts were 2.24, 2.48 and 3.26 L, respectively.

CONCLUSION

Black carrot, pumpkin and strawberry improved the flavored and nutritional value of probiotic yoghurt. Chemical, rheological, microbiological, organolepical and antioxidant properties indicated that the use of pumpkin, strawberry and black carrot as a flavoring material in flavored yoghurt manufacturing is highly recommended.

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