World Applied Sciences Journal 30 (5): 567-574, 2014
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2014.30.05.14103

Effect of Concentrated Pomegranate on Probiotic Yoghurt

A.M. Hassanein, Eman T.A. Yousef and Hoida A.M. El-Shazly

Department of Dairy Research, Food Technology Research Institute,
Agricultural Research Center (ARC), Giza, Egypt

Abstract: Effect of adding different levels of concentrated pomegranate on chemical, rheological and sensory properties of yoghurt was investigated concentrated pomegranate at the rate of 0.25, 0.5 ,0.75 ,1, 1.25 and 1.5% were added after treated thermally. The experimental yoghurt was compared with control yoghurt produced from whole milk adjusted to 3% fat. The chemical composition, pH, titratable acidity, iron, calcium, diacetyl & acetyl methyl carbonyl, syneresis, penetration, total count, coliform, yeast & mould and organoleptic properties were evaluated yoghurts at zero time after 3, 6, 9 and 12 days of storage at refrigerator. Addition of concentrated pomegranate caused a significant decrease in pH and moisture while titratable acidity, syneresis and penetration were increased. Titratable acidity, moisture and syneresiss were decreased during storage of the yoghurt while penetration, diacetyl and acetyl methyl carbonyl were increased. The yoghurt containing 1% of concentrated pomegranate showed slight differences in sensory characteristics than that of control yoghurt.

Key words: Concentrated pomegranate · Yoghurt · Chemical composition · Sensory evaluation

INTRODUCTION

Yoghurt is the most popular fermented milk produced in Egypt and worldwide. Its consumption in Egypt has been increased tremendously. The value of yoghurt in human nutrition is based, not only on the nutritive value of the milk from which it is made but also on the beneficial effect of intestinal microflora, improved lactose tolerance, protection against gastrointestinal infections, effective treatment for specific types of diarrhea, improved immunity, cholesterol reduction and protection against cancer [1]. The natural plain yoghurt is produced by adding lactic acid bacteria the induce the lactic fermentation [2] and according to Codex Standard (243-2003) yoghurt is classified as fermented milks and could contain a maximum of 50% (m/m) of non dairy ingredients (such as fruits and vegetables as well as juices purees, pulps. Pomegranate arils provide 12% of the Daily Value (DV) for vitamin C and 16% DV for vitamin K per 100g serving and contain polyphenols, such as ellagitannins and flavonoids. Pomegranate arils are excellent sources of dietary fiber which is entirely contained in the edible seeds. People who choose to discard the seeds forfeit nutritional benefits conveyed by the seed fiber and micronutrients [3]. Polyphenols in most fruits such as pomegranate are recognized as the major class of phytochemicals with antioxidant activity [4]. The bioavailability of polyphenols in milk is somewhat controversial [5]. Some early studies claimed that maximum antioxidant capacity and hence better health benefit could be gained by ingesting milk proteins-phenols complex [6] however Serafini *et al.* [7] reported reduced bioavailability of phenolics after ingestion with milk. Despite limited research data, manufacturers and marketers of pomegranate juice have liberally used evolving research results for product promotion, especially for putative antioxidant health benefits. In February 2010, the FDA issued a Warning Letter to one such manufacturer, POM Wonderful, for using published literature to make illegal claims of unproven antioxidant and anti-disease benefits [8].

The present investigation was planned to study the effect of adding different levels of concentrated pomegranate on chemical, rheological and sensory properties of yoghurt.

MATERIALS AND METHODS

Materials: Fresh cow's milk used in this study was obtained from the Food Technology Research Institute, Ministry of Agriculture. Concentrated pomegranate was obtained from Yammama, Lebnan. Mixed starter culture

Corresponding Author: A.M. Hassanein, Department of Dairy Research, Food Technology Research Institute, ARC, Egypt

ABY-10 which contains *Streptococcus thermophilus*, *Lactobacillus delbrueckii subsp.bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium lactis* was obtained from Christian Hansen Laboratories (Denmark).

Methods: Yoghurt Manufacture: Yoghurt was manufactured according to the method of Tamime and Robinson [9]. Standardized milk cow`s with 3% fat, was treated thermally at 85°C for 10min. and then cooled to 42°C. After treated thermally, milk cow`s was divided to seven parts. The first part with no additives served as a control. To other treatments divided to six equal portion, then 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50% of each concentrated pomegranate then inoculated with 2% ABY-10 yoghurt starter culture, dispersed into plastic cups, 200g and incubated at 42°C until pH reaches 4.7. After complete coagulation, all treatments were stored in the refrigerator at 5°C for 12days and examined when fresh and after 3, 6, 9 and 12days of storage. All experiments were carried out in triplicate.

Chemical Analysis: Yoghurt treatments were analyzed for moisture, total protein, ash, pH values according to the procedure outlined by AOAC [10], Carbohydrates were calculated by difference. Iron and calcium in all samples were estimated by atomic absorption spectrophotometer (model 3300, Perkin-Elimer, Beaconsfield, UK) according to the procedure outlined by Perales *et al.* [11]. Fat and titratable acidity of yoghurt were determined according to Minstry and Hassan [12]. Diacetyl and acetyl methylcarbonyl were determined as described in Lees and Jago [13].

Rheological Properties: Syneresis was determined by measuring the volume of separated whey (ml whey/50ml yoghurt) collected after 30 min at room temperature [14]. Penetration was measured using a Koehler Pentetrometer as mentioned by El-Shabrawy *et al*. [15].

Microbiological Analysis: Lactic acid (LAB) bacteria were enumerated according Elliker *et al*. [16]. Coliforms were enumerated according to Harrigan and McCance [17] using Violt Red Bile agar media. Mould and yeast were determined according to Standard Methods for Examination of Dairy Products [18].

Sensory Evaluation: Yoghurt samples were assessed according to Nelsons and Trout [19] at zero time and after 3, 6, 9 and 12days of storage by ten panelists of staff members at Department of Dairy Science, Food Technology Institute, Giza, Egypt.

Statistical Analysis: All data were expressed as mean values ± standard deviation for three separate determinations. Statistical analysis was performed using one way analysis of variance (ANOVA). Differences among means were compared using the Duncan's multiple range test with a significant level of $P<0.05$. Relationship among measurement variables were studied using Pearson correlation, R being the correlation factor. Statistical analysis was conducted with the Statistical Analysis System [20].

RESULTS AND DISCUSSION

Table 1 shows the titratable acidity was the highest in yoghurt samples stored for 12 days in all treatments. Treatment with highly percent concentrated pomegranate gave highest titratable acidity values in fresh samples and through storage. Increase in acidity content during storage of yoghurt was also reported by El-Shibiny *et al.* [21]. It is obvious that the addition of concentrated pomegranate had a negligible effect on protein and fat percentage of the resultant yoghurt and slight decrease in moisture percentage but slight increase in carbohydrates and ash percentage, the slight differences may be due to the increase of the amount of pomegranate concentrated (Table 2). From the same table, the moisture content was lowest percent for control treatment in zero time and after storage for 12 days. There was slightly increase in ash content during the storage, due to the changes in total solids content [22]. Control yoghurt contained the lowest in acetyl methyl carbinol and was significantly different from other treatments (Table 3). This might be due to that pomegranate concentrated encourages the starter activity as a result of the high nutritive value. During cold storage period, acetyl methyl carbinol content of yoghurt samples increased. This might be due to the slow reduction of acetyl methyl carbinol [23].

The syneresis of yoghurt was affected by the concentrations of concentrated pomegranate used as shown in Table 4. Increased separation of whey from the yoghurt was observed in the higher level of concentrated pomegranate, Treatments revealed that yoghurt synersis decreased during the interval storage periods. Similar results were

Table 1: Effect of storage at 5±2°C on acidity of yoghurt with concentrated pomegranate

		Storage periods (days)				
Properties	Treatments*	0	3	6	9	12
pH	Control	4.697^{A}±0.042	4.643^{AB}± 0.089	4.53^{A-E}±0.061	4.45^{B-G}±0.078	4.413^{C-I}± 0.071
	T1	4.647^{AB}±0.042	4.610^{ABC}±0.010	4.51^{A-E}±0.096	4.41^{D-I}± 0.147	4.240^{H-N}± .212
	T2	4.550^{A-D}±0.05	4.52^{A-E}± 0.082	4.47^{B-G}±0.063	4.39^{D-J}± 0.053	4.340^{E-K}± 0.060
	T3	4.51^{A-E}±0.085	4.49^{B-F} ±0.040	4.42^{C-H}±0.027	4.36^{D-J}± 0.070	4.29^{G-M}± 0.149
	T4	4.46^{B-G}±0.063	4.42^{C-H}± 0.098	4.37^{D-J}±0.099	4.31 $^{F-I}$± 0.201	4.25^{H-N}± 0.180
	T5	4.43^{C-H}±0.147	4.25 $^{H-N}$±0.180	4.20^{J-N}±0.050	4.15^{K-O}± 0.050	4.100^{M-O}± 0.100
	T6	4.220^{I-N}±0.131	4.12^{L-O}± 0.072	4.08^{NO}±0.072	4.000^{OP}±0.100	3.900 ^{P}± 0.100
Titratable acidity (%)	Control	0.88 ^{I}±0.010	0.92 ^{JK}±0.027	0.96 $^{G-K}$±0.036	0.99 $^{F-K}$±0.046	1.00 $^{F-J}$±0.100
	T1	0.89 ^{K}±0.036	0.94 ^{IJK}±0.036	0.99 $^{F-K}$±0.020	1.01 $^{E-J}$±0.020	1.03 $^{D-I}$±0.030
	T2	0.95 $^{H-K}$±0.044	0.98 $^{F-K}$±0.072	1.00 $^{F-J}$±0.100	1.04 $^{D-I}$±0.053	1.06 $^{B-H}$±0.121
	T3	0.99 $^{F-K}$±0.017	1.02 $^{D-I}$±0.020	1.03 $^{D-J}$±0.030	1.06 $^{B-H}$±0.053	1.08 $^{A-F}$±0.035
	T4	1.02 $^{D-J}$±0.044	1.03 $^{D-I}$±0.027	1.04 $^{D-I}$±0.052	1.09 $^{A-F}$±0.017	1.12 $^{A-D}$±0.070
	T5	1.03 $^{D-I}$±0.052	1.04 $^{D-I}$±0.036	1.05 $^{C-H}$±0.046	1.11 $^{A-E}$±0.027	1.16 ^{AB} ±0.053
	T6	1.05^{C-M}±0.050	1.06 $^{B-H}$±0.053	1.07 $^{B-G}$±0.052	1.15 ^{BC}±0.050	1.18 ^{A}±0.070

*Control: Cow milk without concentrated pomegranate T1: Cow milk with 0.25% concentrated pomegranate
T2: Cow milk with 0.50% concentrated pomegranate T3 Cow milk with 0.75% concentrated pomegranate
T4: Cow milk with 1% concentrated pomegranate T5: Cow milk with 1.25% concentrated pomegranate
T6: Cow milk with 1.5% concentrated pomegranate
Data are mean values ± standard deviation of three determinations.
A,B,C,…Mean values in the same row followed by different superscript letters are significantly different at P<0.05

Table 2: Effect of storage at 5±2°C on the chemical analysis of yoghurt with concentrated pomegranate.

		Storage periods (days)				
Properties	Treatments*	0	3	6	9	12
Moisture (%)	Control	80.95 ^{A}±0.063	80.91 ^{A}±0.115	80.52^{CD}±0.131	80.32^{D-G}±0.23	80.26 $^{E-H}$±0.23
	T1	80.93 ^{A}±0.052	80.90 ^{A}±0.050	80.47^{CDE}±0.061	80.21^{F-I}±0.102	80.14^{G-J}±0.10
	T2	80.92 ^{A}±0.030	80.74^{AB}±0.197	80.34^{C-G}±0.187	80.15^{G-J}±0.09	79.95^{JK}±0.087
	T3	80.79 ^{A}±0.102	80.56^{BC}±0.053	80.30^{D-H}±0.200	80.30^{D-H}±0.10	79.91 ^{K}±0.100
	T4	80.75^{AB}±0.05	80.32^{D-G}±0.07	80.24^{E-H}±0.151	79.98^{IJK}±0.080	79.54 ^{M}±0.080
	T5	80.41^{C-F}±0.09	80.13^{G-J}±0.15	80.12^{G-J}±0.106	79.94^{JK}±0.164	79.32 ^{N}±0.164
	T6	80.34^{C-G}±0.14	80.07^{H-K}±0.06	79.97^{JK}±0.061	79.88^{I}±0.07	79.11 ^{0}±0.072
Fat (%)	Control	3.10^{ABC}±0.100	3.20 ^{ABC}±0.10	3.20^{ABC}±0.010	3.30 ^{A}±0.050	3.300 ^{A}±0.589
	T1	3.10^{ABC}±0.140	3.20 ^{ABC}±0.10	3.20 ^{ABC}±0.132	3.30 ^{A}±0.131	3.300 ^{A}±0.090
	T2	3.10^{ABC}±0.050	3.15^{ABC}±0.05	3.20^{ABC}±0.100	3.30 ^{A}±0.100	3.300^{A}±0.036
	T3	3.10^{ABC}±0.010	3.15^{ABC}±0.05	3.20^{ABC}±0.090	3.21 ^{AB}±0.104	3.300^{A}±0.092
	T4	3.00 ^{BC}±0.050	3.10 ^{ABC}±0.10	3.15^{ABC}±0.056	3.20^{ABC}±0.036	3.250 ^{A}±0.036
	T5	3.00^{BC}±0.040	3.10^{ABC}±0.10	3.15^{ABC}±0.078	3.20^{ABC}±0.044	3.250 ^{A}±0.056
	T6	3.00^{BC}±0.010	3.00 ^{BC}±0.05	3.10^{ABC}±0.100	3.15^{ABC}±0.050	3.200^{ABC}±0.104
Protein (%)	Control	3.00^{A-D}±0.087	3.01^{A-D}±0.027	3.04 ^{AB}±0.053	3.05^{AB}±0.046	3.070 ^{A}±0.044
	T1	2.99^{A-E}±0.060	3.00^{A-D}±0.010	3.02^{ABC}±0.070	3.03^{AB}±0.027	3.050^{AB}±0.046
	T2	2.95^{A-I}±0.052	2.96^{A-H}±0.052	2.97^{A-G}±0.027	2.98^{AF}±0.010	3.000 $^{A-D}$±0.010
	T3	2.89^{D-I}±0.090	2.90^{C-I}±0.044	2.93^{B-I}±0.035	2.94^{B-I}±0.035	2.990^{A-E}±0.070
	T4	2.86^{F-I}±0.027	2.87^{E-I}±0.027	2.89^{D-I}±0.070	2.90^{C-I}±0.095	2.950^{A-I}±0.050
	T5	2.840^{HI}±0.053	2.85^{GHI}±0.087	2.87^{E-I}±0.079	2.89^{D-I}±0.053	2.930^{B-I}±0.113
	T6	2.830^{I}±0.0610	2.84^{HI}±0.036	2.85^{GHI}±0.050	2.87^{E-I}±0.079	2.900^{C-I}±0.056
Ash (%)	Control	0.87 ^{R}±0.027	0.89^{QR}±0.010	0.92^{O-R}±0.020	0.940^{M-Q}±0.01	0.94 $^{M-Q}$±0.017
	T1	0.91^{P-R}±0.010	0.93^{N-Q}±0.010	0.95^{L-Q}±0.010	0.957^{L-P}±0.02	0.98^{J-O}±0.010
	T2	0.93 $^{N-Q}$±0.01	0.94^{M-Q}±0.020	0.98^{J-O}±0.030	0.990 $^{J-N}$±0.01	0.99^{J-N}±0.017
	T3	0.95^{L-Q}±0.010	0.97^{K-P}±0.017	1.00^{J-M}±0.010	1.01^{H-I}±0.010	1.05^{F-I}±0.035
	T4	0.98^{J-O}±0.010	1.02^{G-K}±0.020	1.06^{E-H}±0.044	1.09^{C-F}±0.060	1.12^{BCD}±0.044
	T5	1.01^{H-I}±0.027	1.04^{F-J}±0.053	1.08^{C-F}±0.056	1.13^{BC}±0.052	1.15 ^{B}±0.020
	T6	1.04^{F-J}±0.040	1.07^{D-G}±0.027	1.11^{B-E}±0.010	1.250 ^{A}±0.053	1.27 ^{A}±0.027
	Control	12.08^{A}±0.072	11.99 ^{A} ±0.010	12.32 ^{A} ±0.231	12.40 ^{A} ±0.100	12.43 ^{A} ±0.099
	T1	12.07^{A}±0.020	11.97 ^{A} ±0.017	12.36 ^{A} ±0.144	12.50 ^{A} ±0.265	12.53 ^{A} ±0.256
	T2	12.10 ^{A}±0.100	12.21 ^{A} ±0.102	12.51 ^{A} ±0.235	12.58 ^{A} ±0.131	12.76 ^{A} ±0.115
	T3	12.27 ^{A}±0.157	12.42 ^{A} ±0.131	12.57 ^{A} ±0.324	12.54 ^{A} ±0.197	12.75 ^{A} ±0.155
	T4	12.40 ^{A}±0.100	12.69 ^{A} ±0.248	12.66 ^{A} ±0.232	12.83 ^{A} ±0.113	13.14 ^{A} ±0.251
	T5	12.74 ^{A}±0.053	12.89 ^{A} ±0.085	12.77 ^{A} ±0.270	12.84 ^{A} ±0.053	13.35 ^{A} ±0.150
	T6	12.79 ^{A}±0.115	13.02 ^{A} ±0.020	12.97 ^{A} ±0.027	12.85 ^{A} ±0.251	13.52 ^{A} ±0.231

*See Table 1
Data are mean values ± standard deviation of three determinations.
A,B,C,…Mean values in the same row followed by different superscript letters are significantly different at P<0.05

Table 3: Effect of concentrated pomegranate addition on diacetyl and acetyl methyl carbonyl (µg/100g) content of yoghurt during cold storage

	Storage period (days)				
Treatments	0	3	6	9	12
Control	1.62 ^{B}±0.044	2.47 ^{B} ±0.171	2.84 ^{B} ±0.010	3.03 ^{B} ±0.010	3.04 ^{B} ±0.443
T1	1.13 ^{B} ±0.052	2.37 ^{B} ±0.061	2.68 ^{B} ±0.114	2.87 ^{B} ±0.061	2.92 ^{B} ±0.044
T2	0.98 ^{B} ±0.010	2.08 ^{B} ±0.082	2.49 ^{B} ±0.115	2.92 ^{B} ±0.131	2.93 ^{B} ±0.061
T3	2.23 ^{B} ±0.076	2.86 ^{B} ±0.040	3,04 ^{B} ±0.053	3.43 ^{B} ±0.214	3.46 ^{B} ±0.043
T4	3.34 ^{B} ±0.053	3.65 ^{B} ±0.132	3.96 ^{B} ±0.053	4.02 ^{B} ±0.020	4.04 ^{B} ±0.053
T5	2.86 ^{B} ±0.056	3.06 ^{B} ±0.053	3.38 ^{B} ±0.072	3.53 ^{B} ±0.100	3.64 ^{B} ±0.053
T6	2.85 ^{B} ±0.036	3.06 ^{B} ±0.053	3.36 ^{B} ±0.164	3.51 ^{B} ±0.035	3.62 ^{B} ±0.131

*See Table 1

Data are mean values ± standard deviation of three determinations.

A,B,C,…Mean values in the same row followed by different superscript letters are significantly different at P<0.05

Table 4: Effect of concentrated pomegranate addition on syneresis of yoghurt during cold storage.

	Storage period (days)				
Treatments	0	3	6	9	12
Control	46.91 ^{OP}±0.367	45.20 ^{Q}±0.321	42.50 ^{H}±0.529	37.65 ^{W}±0.304	36.40 ^{X}±0.529
T1	46.93 ^{OP}±0.399	43.90 ^{S}±0.557	43.13 ^{T}±0.147	42.15 ^{U}±0.150	41.10 ^{V}±0.100
T2	49.50 ^{L}±0.361	47.90 ^{N}±0.458	46.30 ^{P}±0.458	44.55 ^{R}±0.396	42.45 ^{U}±0.427
T3	51.65 ^{J}±0.676	50.25 ^{K}±0.250	48.40 ^{N}±0.458	45.55 ^{Q}±0.492	43.55 ST±0 .391
T4	57.15 ^{EF}±0.218	54.80 ^{H}±0.458	53.80 ^{L}±0.600	51.15 ^{J}±0.218	47.10 ^{O}±0.100
T5	62.95 ^{B}±0.427	62.35 ^{C}±0.522	58.40 ^{D}±0.400	56.70 ^{FG}±0.265	48.80 ^{H}±0.656
T6	63.60 ^{A} ±0.400	63.05 ^{B}±0.095	58.75 ^{D}±0.484	57.60 ^{E}±0.458	56.40 ^{G}±0.400

*See Table 1

Data are mean values ± standard deviation of three determinations.

A,B,C,…Mean values in the same row followed by different superscript letters are significantly different at P<0.05

Table 5: Effect of concentrated pomegranate addition on penetration (mm) of yoghurt during cold storage.

	Storage period (days)				
Treatments	0	3	6	9	12
Control	33.12 ^{P}±0.217	33.32^{OP}±0.193	34.00 ^{MN}±0.265	34.36 ^{M}±0.308	35.14 ^{L}±0.164
T1	33.66^{NO}±0.393	34.84^{I}±0.788	35.09 ^{I}±0.085	36.23 ^{IJ}±0.207	36.59 ^{L}±0.373
T2	34.24^{M}±0.122	35.56^{K}±0.406	36.13 ^{J}±0.113	37.31 ^{H}±0.149	37.98 ^{G}±0.475
T3	35.13 ^{L}±0.061	36.22^{IJ}±0.106	37.23 ^{H}±0.148	38.11 ^{FQ}±0.102	38.17 ^{FG}±0.061
T4	36.10^{I}±0.100	37.32^{H}±0.131	38.45 ^{F}±0.150	39.32^{E}±0 ^{A}.131	40.12 ^{D}±0.106
T5	37.14^{H}±0.342	38.12^{FG}±0.044	39.21 ^{E}±0.165	39.43^{E}±0.154	41.34 ^{B}±0.314
T6	38.43^{F}±0.225	39.31 ^{E}±0.248	40.56 ^{C}±406	41.28 ^{B}±0.231	42.27 ^{A} ±0.246

*See Table 1

Data are mean values ± standard deviation of three determinations.

A,,B,C,…Mean values in the same row followed by different superscript letters are significantly different at P<0.05

Table 6: Effect of concentrated pomegranate addition on iron (mg/100g) of yoghurt during cold storage.

	Storage period (days)				
Treatments	0	3	6	9	12
Control	0.20^{G}±0.010	0.21 ^{G} ±0.010	0.23 ^{G} ±0.010	0.23 ^{G} ±0.015	0.24 ^{G} ±0.010
T1	0.44 ^{H}±0.010	0.46 ^{H}±0.010	0.47^{H}±0.010	0.48 ^{H}±0.010	0.49 ^{H}±0.010
T2	0.68^{G}±0.010	0.68 ^{G}±0.010	0.69 ^{G}±0.017	0.71 ^{F}±0.010	0.73 ^{F}±0.010
T3	0.92 ^{E}±0.010	0.94 ^{E}±0.010	0.94^{E}±0.026	0.95 ^{E}±0.010	0.97 ^{E}±0.010
T4	1.16 ^{D}±0.053	1.19 ^{D}±0.065	1.21 ^{C}±0.020	1.22 ^{C}±0.010	1.22 ^{C}±0.608
T5	1.40 ^{B} ±0.026	1.42 ^{B} ±0.020	1.43 ^{B} ±0.010	1.43 ^{B} ±0.010	1.44 ^{B} ±0.017
T6	1.64 ^{A}±0.020	1.66 ^{A}±0.020	1.67 ^{A}±0.010	1.67 ^{A}±0.010	1.68 ^{A}±0.010

*See Table 1

Data are mean values ± standard deviation of three determinations.

A,,B,C,…Mean values in the same row followed by different superscript letters are significantly different at P<0.05

Table 7: Effect of concentrated pomegranate addition on calcium (mg/100g) of yoghurt during cold storage.

	Storage period (days)				
Treatments	0	3	6	9	12
Control	1.54^{M}±0.017	1.54^{M} ±0.010	1.55^{LM} ±0.050	1.55^{LM}±0.036	1.56^{KLM}±0.036
T1	1.56^{KLM}±0.010	1.56^{KLM}±0.020	1.57^{J-M}±0.010	1.57^{J-M}±0.026	1.58^{I-L}±0.010
T2	1.58^{I-L}±0.010	1.59^{H-K}±0.020	1.59^{H-K}±0.010	1.59^{H-K}±0.020	1.60^{G-J}±0.010
T3	1.60^{G-J}±0.010	1.60^{G-J}±0.017	1.61^{F-L}±0.010	1.61^{F-L}±0.000	1.62^{E-H}±0.010
T4	1.63^{D-G}±0.010	1.63^{D-G}±0.005	1.64^{C-F}±0.010	1.64^{C-F}±0.013	1.64^{C-F}±0.036
T5	1.64^{C-F}±0.005	1.65^{B-E}±0.004	1.65^{BCD}±0.010	1.66^{A-D}±0.010	1.66^{A-D}±0.006
T6	1.66^{A-D}±0.040	1.67^{ABC}±0.004	1.67^{ABC}±0.006	1.68^{AB}±0.016	1.69^{A}±0.016

*See Table 1

Data are mean values ± standard deviation of three determinations.

A,,B,C,…Mean values in the same row followed by different superscript letters are significantly different at P<0.05.

Table 8: Changes in organoleptic properties of concentrated pomegranate yoghurt during cold storage

	Body and Texture (30)				
Treatments	0	3	6	9	12
Control	29^{A}±0.978	28^{ABC}±1.034	26^{BCD} ±1.381	25^{CDE} ±0.842	23^{EFG} ±0.842
T1	28^{AB}±0.706	27^{ABC} ±1.003	25^{CDE} ±0.574	24^{DEF} ±0.776	23^{EFG}±0.947
T2	26^{BCD}±0.812	25^{CDE}±0.495	24^{DEF} ±0.947	23^{EFG} ±0.947	23^{EFG}± 1.381
T3	26^{BCD}±0.574	25^{CDE}±0.697	23^{EFG} ±0.776	22^{FGH} ±1.381	22^{FGH}±0.842
T4	25^{CDE}±0.605	24^{DEF} ±0.591	22^{FGH} ±0.706	21^{GHI} ±0.933	20^{HIJ}±0.605
T5	24^{DEF}±0.600	23^{EFG} ±0.776	21^{GHI}±0.600	20^{HIJ} ±0.842	18^{J}±0.600
T6	23^{EFG}±0.521	22^{FGH} ±0.697	20^{HIJ}±0.873	19^{IJ} ±0.822	15^{K}±0.697
	Appearance and colour (15)				
Control	14^{A}±1.381	13^{AB}±1.361	12^{BC}±0.873	11^{CD}±0.842	10DE±0.539
T1	13^{AB}±0.613	13^{AB}±0.841	12^{CD}±1.381	11^{CD}±0.946	10DE±0.565
T2	12^{BC}±0.873	12^{BC}±0.947	11^{CD}±1.034	10DE±0.574	9^{EF}±0.521
T3	11^{CD}±0.841	11^{CD}±0.776	10DE±0.706	9^{EF}±0.605	8^{FG}±0.491
T4	10DE±0.605	10DE±0.605	9^{EF}±0.818	8^{EF}±0.591	7^{GH}±0.697
T5	9^{EF}±0.591	9^{EF}±0.491	8^{FG}±0.812	7^{GH}±0.491	6^{HI}±0.401
T6	8^{FG}±0.776	8^{FG}±0.491	7^{GH}±0.762	6^{HI}±0.591	5^{I}±0.600
	Acidity (10)				
Control	8^{AB}±0.482	8^{AB}±0.812	8^{AB}±1.381	7^{BC}±0.495	6^{CD}±0.776
T1	8^{AB}±0.495	8^{AB}±0.573	8^{AB}±1.381	7^{BC}±0.459	6^{CD}±0.574
T2	9^{A}±0.539	9^{A}±0.392	9^{A}±1.381	8^{AB}±0.401	7^{BC}±0.423
T3	9^{A}±0.565	9^{A}±0.600	9^{A}±1.381	8^{AB}±0.482	7^{BC}±0.459
T4	7^{BC}±0.423	7^{BC}±0.491	7^{BC}±1.381	6^{CD}±0.539	5DE±0.512
T5	7^{BC}±0.574	7^{BC}±0.605	7^{BC}±1.381	6^{CD}±0.565	4^{E}±0.502
T6	7^{BC}±0.392	7^{BC}±0.591	7^{BC}±0.776	6^{CD}±0.425	4^{E}±0.512
	Flavour (45)				
Control	43^{B}±1.406	43^{B}±1.354	41^{CD}±1.659	39^{E}±1.602	25^{H}±1.003
T1	44^{A}±1.406	42^{BC}±1.454	42^{BC}±1.439	40DE±1.453	25^{H}±1.034
T2	43^{B}±1.381	42^{BC}±1.454	41^{BC}±1.859	40DE±1.433	24^{I}±0.818
T3	42^{BC}±2.273	43^{B}±1.454	41^{CD}±1.533	40DE±1.363	24^{I}±0.706
T4	42^{BC}±1.602	43^{B}±1.391	41^{CD}±1.607	39DE±1.533	23^{J}±0.812
T5	40DE±1.581	41^{CD}±1.419	39^{E}±1.363	35^{F}±1.391	19^{K}±0.762
T6	39^{E}±1.546	40DE±1.433	35^{F}±1.586	30^{G}±1.419	10^{L}±0.292
	Total (100)				
Control	94^{A}±0.577	92^{C}±0.577	87^{G}±0.577	82^{J}±0.577	64^{S}±0.577
T1	93^{B}±0.577	91^{D}±0.577	87^{G}±0.577	82^{J}±0.577	64^{S}±0.577
T2	90^{E}±0.577	88^{F}±0.577	87^{G}±0.577	81^{K}±0.577	63^{T}±0.577
T3	88^{F}±0.577	88^{F}±0.577	83^{L}±0.577	79^{M}±0.577	61^{U}±0.577
T4	84^{H}±0.577	84^{H}±0.577	79^{M}±0.577	74^{P}±0.577	55^{V}±0.577
T5	80^{L}±0.577	80^{L}±0.577	75^{O}±0.577	68^{R}±0.577	47^{W}±0.577
T6	77^{N}±0.577	77^{N}±0.577	69^{Q}±0.577	61^{U}±0.577	34^{X}±0.577

*See Table 1

Data are mean values ± standard deviation of three determinations.

A,B,,C,…Mean values in the same row followed by different superscript letters are significantly different at P<0.05

Table 9: Effect of concentrated pomegranate on lactic acid bacteria, coliform bacteria and yeast & mould of yoghurt during storage periods.

		Storage periods (days)				
Properties	Treatments*	0	3	6	9	12
LAB	Control	$52x10^9$	$41x10^9$	$36x10^9$	$29x10^9$	$11x10^9$
	T1	$40x10^9$	$32x10^9$	$29x10^9$	$21x10^9$	$7x10^9$
	T2	$34x10^9$	$24x10^9$	$15x10^9$	$9x10^9$	$79x10^8$
	T3	$98x10^8$	$90x10^8$	$87x10^8$	$79x10^8$	$65x10^8$
	T4	$87x10^8$	$83x10^8$	$80x10^8$	$76x10^8$	$55x10^8$
	T5	$82x10^8$	$80x10^8$	$78x10^8$	$71x10^8$	$41x10^8$
	T6	$79x10^8$	$76x10^8$	$72x10^8$	$69x10^8$	$33x10^8$
Coliform	Control	ND	ND	ND	ND	ND
	T1	ND	ND	ND	ND	ND
	T2	ND	ND	ND	ND	ND
	T3	ND	ND	ND	ND	ND
	T4	ND	ND	ND	ND	ND
	T5	ND	ND	ND	ND	ND
	T6	ND	ND	ND	ND	ND
Yeast & Mould	Control	ND	ND	ND	ND	$2x10^1$
	T1	ND	ND	ND	ND	$6x10^1$
	T2	ND	ND	ND	ND	$8x10^1$
	T3	ND	ND	ND	ND	$9x10^1$
	T4	ND	ND	ND	ND	$11x10^1$
	T5	ND	ND	ND	ND	$13x10^1$
	T6	ND	ND	ND	ND	$15x10^1$

*See Table 1

reported by El-Nagar and Shenana [22]. On the other hand, the susceptibility to synersis decreased with adding fibers. Also, the syneresis decreased by increasing the fibers level [24]. Cerning *et al*. [25] reported that the expolysaccharides reduced syneresis when used in yoghurt. The penetration readings were inversely related to firmness. In Table 5 shown increasing concentrations of pomegranate in yoghurt treatments lead to increase penetration values. Upon storage, the penetration values generally decreased indicating firmness increase. Paseephol *et al*. [26] reported similar observation it was as suggested that the protein content in the most important factor influencing textural properties of gel network resulting in a firmer gel structure. Addition of concentrated pomegranate had a slight increase on iron and calcium content of the resultant yoghurt, the slight differences may be due to the increase of the amount of concentrated pomegranate concentration (Table 6 and 7).

Sensory properties of yoghurt samples are shown in Table 8. The highest scores were obtained in control group followed by treatment containing 0.25% concentrated pomegranate. Increasing the levels of concentrated pomegranate after 1% negatively affected the flavour scores. Similarly, the addition of concentrated pomegranate influenced the body and texture of the yoghurt samples. No significant differences were found in the appearance score of samples. With respect to general acceptability of the yoghurt samples, control treatment showed the highest score, followed by T1, T2 and T3 yoghurt samples containing 0.25, 0.5, 0.75 and 1% of concentrated pomegranate, respectively. Throughout the storage period, slight decrease of scores in all treatments until the end of this period (12days). This may be due to increase in the acidity which affect the rheological properties [24, 27]. Data presented in Table 9 illustrated that the count of lactic acid bacteria of all treatments with concentrated pomegranate had higher count compared to the control at fresh time and through the storage period and according to codex standard (243-2003). The same table demonstrates that all samples were free of coliform bacteria, while moulds and yeasts were detected in low number at the end of storage as a result of high hygienic condition during the preparation and storage period.

CONCLUSION

It could be concluded that probiotic yoghurt product was developed by incorporating concentrated pomegranate as a supplement 1% was the most acceptable yoghurt and was not different from the control. Therefore, it is possible to make good quality yoghurt from cow's milk by adding concentrated pomegranate till to 1%.

REFERENCES

1. Buttriss, 1997. Nutritional properties of fermented milk products. Int. J. Dairy Technol., 50: 21-27.
2. Coisson, J.D., F. Travaglia, G. Piana, M. Capasso and M. Arlorio, 2005. Euterpeoleracea juice as a functional pigment for yogurt. Food Research International, 38: 893-897.
3. Schubert, S.Y., E.P. Lansky and I. Neeman, 1999. Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. J. Ethnopharmacol., 66(1): 11-17.
4. Seeram, N.P., Y. Zhang, J.D. Reed, C.G. Krueger and J. Vaya, 2006. Pomegranate Phytochemicals. In N.P. Seeram, R.N. Schulman & D. Heber, Pomegranates: Ancient Roots to Modern Medicine. New York: CRC Press, pp: 3-46.
5. Gad, A.S. and M.H. Abd El-Salam, 2010. The antioxidant properties of skim milk supplemented with rosemary and green tea extracts in response to pasteurisation, homogenisation and the addition of salts. International Journal of Dairy Technology, 63: 349-355.
6. Hoffman, P.C.H., K.H. VanHet Hof, L.B.M. Tijburg and M.B. Atan, 2001. Addition of milk does not affect the absorption of flavonoids from tea in man. Free Radical Research, 34: 297-300.
7. Serafini, M., M.F. Testa, D. Villano, M. Pecorari, K.V. Wieren, E. Azzini, A. Brambilla and G. Maiani, 2009. Antioxidant activity of blueberry fruit is impaired by association with milk. Free Radical Biology and Medicine, 46: 769-774.
8. Seeram, N.P., W.J. Aronson and Y. Zhang, 2007. Pomegranate ellagitannin-derived metabolites inhibit prostate cancer growth and localize to the mouse prostate gland". J. Agric. Food Chem., 55(19): 7732-7.
9. Tamime, A.Y. and R.K. Robinson, 1985. A general Treatment of Yogurt and its Manufacture; Science and Technology, Pergamon Press, Oxford.
10. AOAC, 2005. Official Methods of Analysis. 16th Ed, Washington, DC: Association of official Analytical Chemists. Inc. Arlington. Virginia. USA.
11. Perales, S., R. Barberá, M.J. Logarda and R. Farre, 2005. Biovability of calcium from milk-based formulas and fruit suices containing milk and cereals estimated by *in vitro* methods (Solubility, dialysability and uptake and transport by CaCO-2 cells). J. Agric. and Food Chemistry, 53(9): 3721-3728.
12. Mistry, V.V. and H.N. Hassan 1992. Manufacture of nonfat yoghurt from high milk protein powder. J. Dairy Sci., 75: 947-957.
13. Lees, G.J. and G.R. Jago, 1970. The estimation of diacetyl in presence of other carbonal compounds. J. Dairy Res., 37: 129-132.
14. Abd El-Salam, M.H., S. El-Shibiniy, M.B. Mahfuz, H.F. El-Dein, H. El-Atriby and V. Antila, 1991. Preparation of whey protein concentrate from salted whey and its use in yoghurt. J. Dairy Research, 58: 503-510.
15. El-Shabrawy, S.A., R.A. Awad and S.A. Saad, 2002. Manufacture and properties of flavored processed cheese spread with different fruit flavor. J. Annal. Agric. Sci., Ain Shams Univ., Cairo, 10: 641-657.
16. Elliker, P.R., A.W. Anderson and G. Hannesson, 1956. An agar medium for lactic acid streptococci and lactobacilli. J. Dairy Sci., 39: 1611-1617.
17. Harrigan, W.F. and M.E. McCance, 1996. Laboratory Methods in Microbiology. Academic Press, London & New York, pp: 292-293.
18. APHA, 1992. Methods for the Microbial Examination of Food. 3rd Ed. Carl Vanderzant and Don F. Splitt Solesser (Eds.) American Public Health Association Washington D.C. 2000S.
19. Nelson, J. A. and G.M. Trout, 1981. Judging of Dairy Products. (4th Ed.). AVI Publishing Company, INC, Westport, Connecticut, pp: 295-313.
20. SAS, 1996. Statistical Analysis System for windows. 39(2): 195-203. In: SAS/STAT User's Guide, Version 4.10, Release 30. Kaur, H. and U. Bajwa, 1999. Effect of incorporation 6.12, SAS Institute Inc. Cary, NC, US.
21. El-Shibiny, S., H. El-Dein and A.A. Hofi, 1979. Effect of storage on the chemical composition of zabady. Egyptian J. Dairy Sci., 7: 1-7.
22. El-Nagar, G.F. and M.E. Shenana, 1998. Production and acceptability of Bio-yoghurt. Proc. 7th Egyptian Conf.
23. Driessen, F.M. and Z. Puhan, 1988. Technology of mesophilic fermented milk. IDF Bull., 227: 75-81.
24. El-Nagar, G.F. and C.S. Brennan, 2001. The influence of fiber addition on the texture and quality of stirred yoghurt. Proc. 8th Egyptian Conf. Dairy Sci. And Techn., pp: 505-523.
25. Cerning, J., C. Bouillanne, M.J. Desmazeaud and M. Landon, 1990. Exocellular polysaccharides produced by lactic acid bacteria. FEMS Microbiology Reviews, 87: 113-130.

26. Paseephol, T., D.M. Small and F. Sherkat, 2008. Rheology and texture of set yoghurt as affected by inulin addition. Journal of Texture Studies, 39: 617-634.

27. Ibrahim, G.A., M.I. Kobeasy, N.Sh. Mehanna and D.A. Gad El-Rab, 2003. Production of novel types of functional fermented milk products. Egypt. J. Nutrition, XVIII(2): 1-32.