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Full Length Research Paper

Effect of phenolic compounds on characteristics of strained yoghurts produced from sheep milk

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This study aims to investigate the effect of phenolic compounds extracted grape seed and pomegranate seed on characteristics of strained yoghurts produced from sheep milk. Firstly, phenolic compounds were extracted grape and pomegranate seed and their amounts for strained yoghurts were determined by estimating their phenolic activities. Also antimicrobial activity of seed extracts was determined. On 1st, 7th, and 14th day of storage, chemical analyses as dry matter, protein, acidity, pH, proteolysis and peroxide value and microbiological analyses such as enumeration of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* and sensory analysis were conducted on strained yoghurt samples. According to analysis results, while addition of phenolic compounds affects chemical and microbiological properties of strained yoghurt positively, sensory quality was affected negatively.

Key words: Natural antioxidants, phenolic compounds, sheep milk, strained yoghurt.

INTRODUCTION

In spite of modern improvements in food hygiene and food production techniques, food safety is an increasingly important public health issue. It has been estimated that as many as 30% of people in industrialized countries suffer from a foodborne diseases each year and at least two million people died from diarrheal diseases worldwide (Burt, 2004). Therefore there is still a need for new methods of reducing or inhibiting foodborne pathogen possibly in combination with existing methods (the hurdle principle, packaged under modified atmosphere, heating, refrigeration and addition of antimicrobial compound etc) (Burt, 2004; Valero and Francés, 2006; Celikel and Kavas, 2008). Also recently industrialized society appears to be experiencing a trend of green consumerism, desiring fewer synthetic food additives and products with a low impact on the environment (Burt, 2004; Sacchetti et al., 2005). For this reason, there is scope for new methods of producing food safe that have a natural and green image. The grape and pomegranate are the fruit crops most widely grown throughout the world (Baydar et al., 2004). The composition and properties of grapes and pomegranate have been extensively investigated and it was reported that fruit crops like these contain large amounts of phenolic compounds (Macheix et al., 1990; Baydar et al., 2004; Bajpai et al., 2005). These compounds have many favourable effects on human health like the lowering of human low-density protein, reduction of heart disease and cancer (Teissedre et al., 1996; Bingham et al., 2003; West, 2003; Baydar et al., 2004).

On the other hand, extracts obtained from wine and juice industries also have been used as natural anti-oxidants, because these extracts contain large quantities of monomeric phenolic compounds such as catechins and epicatechin-3-0 gallate and dimeric, trimeric and tetrameric procyanidins (Baydar et al., 2004).

Microorganisms have unfavourable effects on quality safety and shelf-life of foods. Use of synthetic additives is one of the procedures applied for preventing spoilage (Baydar et al., 2004). Whereas recently, in spite of these synthetic additives, interest in the application of plant extracts to prevent the spoilage of foods has increased. Especially, extracts from herbs and spices are the most commonly used plant materials for this aim (Sağdıç et al., 2002; Sağdıç and Özcan, 2003; Baydar et al., 2004). But, there is limited research on the inhibitory effects of fruit seed extracts (Jayaprakasha et al., 2003). Therefore the

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objectives of this research were to determine the antimicrobial activities and total phenolic activities of grape and pomegranate seed extracts and also to investigate adding of phenolic compounds in strained yoghurt characteristics made from sheep milk.

MATERIALS AND METHODS

Sheep milk used for the production of yoghurt was obtained from Yıldız Dairy Food Industry and Trade Limited Company (İzmir, Turkey) A commercial mixed strain of concentrated freeze-dried yoghurt starter culture was obtained from Ezal-Texel (France) TM08 2 type (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*). Grape and pomegranate seeds containing phenolic substances had been provided by Aksuvital Natural Products Food Industry (İstanbul, Turkey) and Enoat Food Industry (İstanbul, Turkey). Plastic containers (250 g) were purchased from Uzunyol Packaging and Chemical Industries and Trade Joint Stock Company (Gebze, Turkey).

Extraction of phenolic compounds

Dried grape and pomegranate seeds were ground to fine powder with a grinder. Then the powdered seeds (100 g) were extracted in a Soxhlet extractor with petroleum ether (60°C for 6 h) to remove the fatty compounds. The defatted grape and pomegranate seed powders were re-extracted in Soxhlet apparatus for 8 h with 200 ml acetone: water: acetic acid (90:95:0.5) at 60°C. The extracts were concentrated by rotary evaporation under vacuum at 70°C to get crude extracts. The extracts were stored in desiccators until using. The acetone: water: acetic acid extracts of seeds yielded 17.8% for grape and 18.24% for pomegranate, respectively (Baydar et al., 2004).

Manufacturing of strained yoghurt

Pilot scale strained yoghurts were manufactured in duplicate, used 20 L of pasteurized (at 95°C for 5 min) whole sheep milk in the pilot plant of the Department of Dairy Technology (Ege University, İzmir, Turkey). After the milk samples were cooled to 43±2°C, starter culture (3% w/v 6.64×10^{10} cfu/ml) was added. Then the milk samples were incubated at 43°C. When the pH of yoghurt reached 4.70, samples were taken from the incubator, and transferred to a cold stored at 4°C–in a cold room for one night. Three extracts of seeds (Grape A, pomegranate B and commercial grape extract Enoat^{R} C) were added into yoghurt at the rate of 25 ppm (25 mg/L). The control yoghurt (D) vats were prepared without from phenolic compound. All yoghurts samples were transferred into cotton cloth bags. To prevent recontamination, the cloth bags were hung to drain the whey (~10°C) until about 23 to 24% total solids. The yoghurts were then packaged into plastic cups and stored at 4°C until analysis. The diagram of laboratory scale production of strained yoghurt with antioxidant was given in Figure 1.

Quantification of total phenolics

The concentration of phenolic compounds in the extracts was determined by the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965), using gallic acid as a standard analysis were carried out in triplicate and calculated from a calibration curve obtained with gallic acid. Total phenolic content was expressed as gallic acid equivalents in milligram per gram of extract (mg GAE/g extract).

Determination of antimicrobial activity

Antimicrobial activity of extracts was individually tested against some microorganisms, including *Escherichia coli* ATCC 25922*, Staphylococcus aureus* ATCC 6538 *and Salmonella typhimurium* (Public Health Laboratory source, İzmir, Turkey). Suspensions of the bacteria, adjusted to 10^{6} to 10^{7} cfu/ml final cell concentrations were containing 25 ml sterile Nutrient agar (Merck, Darmstad, Germany) at 43 to 45°C, and poured into petri plates (8 cm diameter). Agar was allowed solidify at 4°C for 1 h and the paper disc diffusion was used to detect the antibacterial activity of the extracts. Sterilized filter paper discs (6 mm) were soaked with 50 µl of 1, 2.5, 5, 7.5 and 20% solutions of each extract in absolute methanol and absolute methanol was used as control. The soaked discs were put on the plate, and incubated at 37°C for 18 to 24 h. At the end of the period, inhibition zones formed in the medium were measured in the millimeter (mm). All the tests were made in duplicate (Baydar et al., 2004).

Chemical analyses

Total solids, fat and protein contents of raw milk were determined according to Oysun (1996). Lactose content of milk was measured by polarimetric method using the BS polarimeter (Bellingham + Stanley Ltd., England) while pH was determined by the combined electrode Hanna pH 211 microprocessor pH meter. Ash content was determined by subtracting the total of protein, fat and lactose from total solids (Anonymous, 1981; AOAC, 1984).

Strained yoghurt samples were analyzed for total solids by over drying at 102°C (AOAC, 2003), fat by the method of Gerber-Van Gulik and titratable acidity by the titrimetric methods as describe in Turkish Standards (Anonymous, 2006). The pH was determined with a pH meter (Hanna pH 211 Microprocessor, Portugal). Peroxide value was determined by titration method (AOAC, 1990). The total nitrogen and water soluble nitrogen were determined by the Kjeldahl procedure, using the Gerhardt KB digestion and Vapodest distillation systems (C Gerhardt, Born, Germany) (International Dairy Federation, 1962). The tyrosine contents were determined spectrophotometrically (Analytikjena Spekol-1300) at 650 nm according to Hull method (Citti et al., 1963).

Microbiological analyses

The enumeration of *L. delbrueckii* ssp. *bulgaricus* (De Man, Ragosa, Sharpe Agar, Merck, Darmstadt) at 42°C for 48 h under anaerobic conditions, *S. thermophilus* (M 17 agar, Merck, Darmstadt) at 37°C for 48 h under aerobic condition were performed during the storage (Bracquart, 1981).

Sensory analyses

Six volunteer trained, of the Ege University, Faculty of Agriculture, Department of Dairy Technology evaluated yoghurt samples by the scoring test. Samples of about 200 g in a statistically balanced order were presented in plastic cups. Labeled with, two digit random numerical code. Subjects were instructed to rinse their mouths with water between samples. Panelists assigned scores to each yoghurt samples for taste (scale 1 to 9), texture (scale 1 to 9) and general acceptance (scale 1 to 9) as described by Bodyfelt et al. (1998).

Statistical analysis

Statistical analysis of the data was done using the analysis of

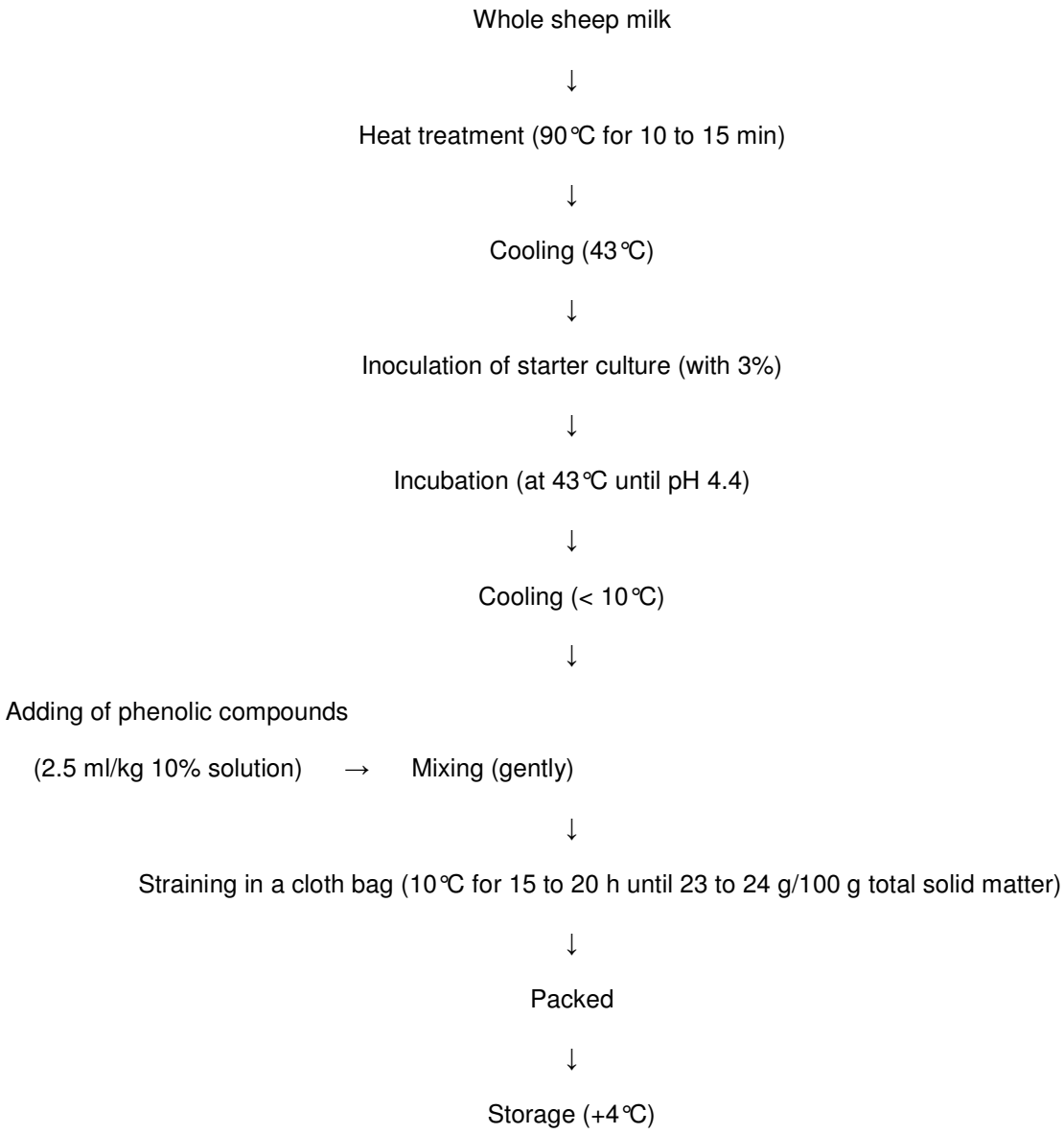

Figure 1. The flow diagram of laboratory scale production of strained yoghurt with antioxidant.

variance in SPSS© v.9.05 (SPSS Inc., Chicago, USA). Means with a significant difference were compared by Duncan's multiple range tests. All analyses were performed in duplicate.

RESULTS AND DISCUSSION

Phenolic activity

The contents of total phenolic in grape and pomegranate seed extracts are given in Table 1. The amount of total phenolics was of 121.80 and 224.53 mg GAE/g in grape seed extracts. The content of total phenolics in pomegranate seed extract was of 76.62 mg GAE/g. In the grape seed extracts the content of total phenolic was of 627.98 mg gallic acid equivalent GAE/g (Baydar et al., 2004). Turkmen and Velioglu (2007) reported that fresh tea leaves contain higher levels of poliphenolic compounds (523.7 to 680.2 mg GAE/g) than dry extract (481.8 to 560.8 mg GAE/g dry extract).

The difference from we have found with other authors can be attributed to the fact that phenols are a heterogeneous group of complete mixtures' organic substances the quality and quantity of which vary with growth stages ecological conditions, extraction conditions, extraction solutions used and other factors based on which the phenolics are extracted (Baydar et al., 2004; Bajpai et al., 2005; Celiktas et al., 2007; Farhoosh et al., 2007; Kouri et al., 2007; Lafka et al., 2007; Tawaha et al., 2007; Vundać et al., 2007).

Table 1. Total polyphenolic contein (mg GAE/g) of grape and pomegranate seeds.

Seeds	Polyphenolic total contein
Grape (A)	121.80 ± 2.05
Grape (B)	224.53 ± 4.07
Pomegranate (C)	76.62 ± 2.80

GAE = Gallic acid equivalents.

Table 2. AntImIcrobIal actIvIty of grape and pomegranate seed extracts.

		Zone diameter (mm)				
Microorganisms	**Extract**	**1%**	**2%**	**5%**	**7.5%**	**10%**
S. typhimurium $3.2x10^7$ CFU/mL	A	8.1±0.2^a	11.8±0.3^b	16±0.3^c	17.9±0.2^d	19.8±0.4^e
	B	7.2±0.1^a	9.0±0.2^b	11.2±0.3^{bc}	14.3±0.2^c	16.2±0.3^{cd}
	C	5.3±0.2^a	12.9±0.1^b	17.4±0.2^c	20.5±0.4^d	22.5±0.2^e
S. aureus $4.7x10^7$ CFU/mL	A	5.3±0.2^a	6.2±0.1^{ab}	7.2±0.1^{bc}	9.3±0.1	10.5±0.2
	B	3.2±0.4	4.5±0.3	5.1±0.1	6.0±0.2	7.0±0.1
	C	10.1±0.3^a	12.5±0.4^a	14.3±0.4a	20.0±0.2^b	24.6±0.1^c
E. coli $3.4x10^9$ CFU/mL	A	8.1±0.1	9.2±0.1	11.1±0.2	14±0.3	17.4±0.4
	B	5.6±0.1	7.6±0.2	8.7±0.1	9.8±0.2	10.7±0.1
	C	10.4±0.2	12.6±0.3	15.8±0.4	20.7±0.4	24.3±0.4

A= Grape A seed; B= grape B seed; C= pomegranate seed.

Antimicrobial activity

Table 2 summarizes the antimicrobial activity of the three phenolic compounds (2 grapes and 1 pomegranate). Bacteria susceptibility to different phenolic compounds as determined by the agar diffusion method showed that produced 6 mm in diameter inhibition holes turning it, into on phenolic compound with the highest inhibitory effects. In the dose response study, the inhibition zones increased with increasing concentration of phenolic compounds.

10 µl concentration (1%) of A, B, C inhibited weakly the development of bacteria. However, *S. typhimurium* was more sensitive than *S. aureus* and *E. coli* in medium containing C phenolic compound. At high concentration (10%) phenolic extract exhibited marked inhibition activity against pathogen bacteria and phenolic extracts of C compound was strongest effect (22.5 to 24.6 mm) than the others showing inhibition zones ranging from 7.0 to 19.8 mm. Comparatively, *E. coli* and *S. aureus* were less sensitive to inhibitory activity of A and B phenolic extracts than *S. typhimurium* which was more inhibited at the same concentrations of same phenolic extracts. On the other hand, all bacteria showed low susceptibility to B phenolic extracts with 7.0 and 16.2 mm diameter inhibition holes at same level/concentrations of B phenolic compound.

Recently, researchers in the phenolic compounds field had remarkably increased, mainly with regard to the antimicrobials used to control food pathogens and native flora, antioxidative potentials and the knowledge of the possible mechanism of action of these compounds. The results revealed the potential of some phenolic compounds such as grape, pomegranate, rosemary etc. as used natural preservatives in food technology. Also, in this study the results obtained were in agreement with those reported by several researchers (Baydar et al., 2004; Penney et al., 2004; Vundać et al., 2007).

The antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis* was examined by Celiktas et al. (2007). In the study, the results indicated that the tested bacteria were sensitive to the extract and antimicrobial activities of the extract against the tested bacteria differed, depending on location and seasonal variations. But in general, the antimicrobial activities of the methanolic extracts of phenolic compounds tested exhibited very low antimicrobial activity compared to the essential oils.

Also grape extracts at 1, 2.5, 5, 7.5 and 20% concentrations were tested for their antimicrobial effects by using the paper disc diffusion method against some food spoilage and pathogenic bacteria. The grape seed extracts at 20% concentration inhibited all bacteria except *Bacillus amyloliquefaciens* and whose extracts at 4% concentration were inactive against *Aeromonas hydrophila*, *B. subtilis*, *B. megatarium* and *B. amyloliquefaciens* (Baydar et al., 2004).

Generally, extent of the inhibitory effects of the extracts

Table 3. The changes during storage in the gross composition of strained yoghurt with several types of phenolic compound.

Parameter	Days	A	B	C	D
Total solids (%)	1	22.23 ± 0.59^{aA}	21.59 ± 0.11^{bA}	21.41 ± 0.33^{aA}	21.93 ± 0.52^{aA}
	7	21.82 ± 0.54^{aA}	21.33 ± 0.21^{bA}	20.89 ± 0.21^{aA}	20.60 ± 0.72^{aA}
	14	21.15 ± 0.20^{aB}	20.28 ± 0.09^{aA}	20.68 ± 0.16^{aA}	20.43 ± 0.19^{aA}
Titratable acidity (°SH)	1	2.14 ± 0.03^{aA}	2.14 ± 0.03^{aA}	2.18 ± 0.03^{aA}	2.14 ± 0.03^{aA}
	7	2.22 ± 0.03^{aA}	2.22 ± 0.03^{abA}	2.18 ± 0.08^{aA}	2.16 ± 0.06^{aA}
	14	2.36 ± 0.06^{bA}	2.32 ± 0.06^{bA}	2.32 ± 0.06^{aA}	2.28 ± 0.06^{aA}
pH	1	4.71 ± 0.01^{bC}	4.59 ± 0.01^{cAB}	4.62 ± 0.02^{bB}	4.55 ± 0.04^{bA}
	7	4.50 ± 0.01^{aA}	4.51 ± 0.01^{bA}	4.52 ± 0.02^{aA}	4.46 ± 0.03^{aA}
	14	4.45 ± 0.03^{aA}	4.47 ± 0.01^{aA}	4.48 ± 0.02^{aA}	4.46 ± 0.01^{aA}
Total nitrogen (%)	1	24.95 ± 2.05^{bC}	24.30 ± 0.99^{bB}	23.97 ± 0.81^{aA}	23.15 ± 0.42^{aD}
	7	25.57 ± 2.02^{aC}	24.70 ± 0.90^{abB}	24.67 ± 0.53^{abA}	23.26 ± 0.51^{aD}
	14	26.22 ± 1.73^{aC}	25.52 ± 1.17^{aB}	25.68 ± 0.78^{A}	23.43 ± 0.90^{aD}
Water soluble nitrogen (%)	1	0.33 ± 0.02	0.32 ± 0.04	0.30 ± 0.03	0.28 ± 0.01
	7	0.36 ± 0.01^{bc}	0.38 ± 0.03^{c}	0.32 ± 0.2^{ab}	0.30 ± 0.00^{a}
	14	0.40 ± 0.00^{a}	0.44 ± 0.02^{c}	0.35 ± 0.01^{a}	0.32 ± 0.01^{a}
Acetaldehyde (ppm/g)	1	0.81 ± 0.04^{C}	0.66 ± 0.00^{aB}	0.41 ± 0.01^{aA}	0.47 ± 0.01^{a}
	7	0.94 ± 0.06^{C}	0.79 ± 0.00^{aB}	0.50 ± 0.01^{bA}	0.57 ± 0.02^{aA}
	14	0.98 ± 0.06^{B}	0.89 ± 0.07^{bB}	0.63 ± 0.02^{cA}	0.66 ± 0.05^{bA}

A= Control sample (strainned yoghurt without phenolic compounds); B= strainned yoghurt sample containing grape A seed extracts; C= strainned yoghurt sample containing grape B seed extracts; D= strainned yoghurt sample containing pomegranate seed extracts.

could be attributed to their phenolic composition (Baydar et al. 2004). Similarly, Shoko et al. (1999) confirmed that phenolic were the most important compounds active against bacteria. They also identified gallic acid as the most active compound for inhibition of bacteria. Therefore our results indicated that the using of grape and pomegranate seed extracts may be exploitable as antimicrobial agents to prevent the spoilage of stored dairy products by bacteria (Baydar et al., 2004).

Chemical properties

The chemical composition of milk producing strained yoghurt included 12.8% total solids, 3.8% fat, 3.3% protein, 4.7% lactose, 0.85% ash and 6.74 pH.

Table 3 shows the changes during storage in the gross composition of strained yoghurt with several types of phenolic compound. The total solid (TS) contents decreased slightly in all treatments during the storage period. Control group had the highest TS content, followed by D group strained yoghurt. The lowest TS were obtained for C group yoghurt. A similar trend was observed throughout the rest of storage period (7 and14 days). No significant differences ($p < 0.05$) were observed in TS content of different strained yoghurts either when fresh or during the storage period except C group yoghurts at the 14th day. Some researchers also reported that there were slightly observable differences in TS of Labneh, strained yoghurt by addition of different essential oils (Al.Otaibi and El.Demerdash, 2008; Ismail et al., 2006). The data observed in our study is also similar to those of other researchers (Tamime, 1978; Tamime and Robinson, 1985; Mehaia and Elkhadragy, 1999; Guizani et al., 2001; Al-Kadamany et al., 2003).

The change in total acidity (TA) is a very significant factor, since it affects the shelf life and acceptability of product (Al.Otaibi and El.Demerdash, 2008). Based on the results presented in Table 3, it is evident that acidity values of the experimental strained yoghurt increased significantly during the storage period. The highest values were determined with control group yoghurt when fresh and it increased up to the end of storage. It can be also said that the natural phenolic compounds had a negative effect on the starter culture and total viable counts (Abou-Dawood, 2002; Al.Otaibi and El.Demerdash, 2008). Also these results were in agreement with that obtained by Abbas and Osman (1998) who reported that the TA increased gradually during storage period.

The pH values found in the threated yoghurt samples were decreased significantly during storage period ($p < 0.05$). The highest pH values obtained with A group

Table 4. Peroxide value and tyrosine content of strained yoghurt samples during storage at 4°C.

Parameter	Days	A	B	C	D
Peroxide value *(MEG)*	1	2.10 ± 0.14^{aB}	1.15 ± 0.07^{aA}	1.15 ± 0.07^{aA}	1.10 ± 0.14^{aA}
	7	2.50 ± 0.14^{abB}	1.45 ± 0.07^{bA}	1.55 ± 0.07^{bA}	1.45 ± 0.21^{aA}
	14	2.75 ± 0.21^{bB}	1.85 ± 0.07^{cA}	1.85 ± 0.07^{cA}	1.65 ± 0.21^{aA}
Tyrosine (mg/5 ml)	1	0.114 ± 0.00^{aA}	0.123 ± 0.00^{aC}	0.117 ± 0.00^{aB}	0.128 ± 0.00^{aB}
	7	0.135 ± 0.01^{aA}	0.135 ± 0.01^{abA}	0.133 ± 0.01^{abA}	0.132 ± 0.00^{aA}
	14	0.174 ± 0.01^{bB}	0.149 ± 0.00^{bA}	0.142 ± 0.01^{bA}	0.147 ± 0.00^{bA}

A= Control sample (strainned yoghurt without phenolic compounds); B= strainned yoghurt sample containing grape A seed extracts; C= strainned yoghurt sample containing grape B seed extracts; D= strainned yoghurt sample containing pomegranate seed extracts; *MEG:* Miliekivalengram.

strained yoghurt containing phenolic compounds sourced grape when fresh and the end of storage. Also the results of pH were not significant ($p > 0.05$) at 7^{th} and 14^{th} day of storage and the results of acid production and decreased pH obtained agreed with Guizani et al. (2001) and Haj et al. (2007). But the pH values of different strained yoghurt samples can be changed by the starter culture type, specific incubation time and temperature.

Total nitrogen (TN) and soluble nitrogen of strained yoghurt made with the addition of the phenolic compounds showed a gradual, but significant decrease in all treatments during storage $p < 0.05$ (Table 3). The dates observed also started that strained yoghurt containing phenolic compounds had the highest TN content, followed by untreated control group. The lowest TN was from the strained yoghurt containing grape extracts. These results were in the same trend with other findings (Nergiz and Seçkin, 1998; Al-Kadamany et al., 2003; Al.Otaibi and El.Demerdash, 2008).

The changes in water-soluble nitrogen fraction (WSN) were given in Table 3. A highly important factor since it relates the shelf life, and the starter culture proteolytic activity. The percentage of soluble nitrogen of samples made with the addition of natural phenolic compounds showed a significant decrease in all treatments when fresh and during the storage period compared to the untreated control ($p < 0.05$). The lowest WSN was from grape extracts followed by yoghurt manufactured with pomegranate extracts and Enoate. The increases of soluble nitrogen content of yoghurts are ultimately assessed by starter culture, proteolytic enzyme systems of culture (Güzel-Seydim et al., 2005).

Glucose and amino acids are the main metabolites necessary for acetaldehyde production by yoghurt microorganisms. Threonine aldolase converts threonine to glycine and acetaldehyde. Both *L. bulgaricus* and *S. thermophilus* produce this enzyme (Güzel-Seydim et al., 2005). On the other hand, it is clear from Table 3 that the acetaldehyde values of all treatments and control sample was increased within the 14 days of storage. The highest value was obtained for strained yoghurt as control; while the lowest values were observed for strained yoghurt containing pomegranate seeds phenolic compounds.

This is presumably due to the suppressing ability of phenolic compound to produce acetaldehyde of LAB (Baydar et al., 2004; Celiktas et al., 2007). Also other scientists have stated that alcohol dehydrogenase produced by yoghurt cultures converts acetaldehyde to ethanol during storage as our study (Tamime and Death, 1980; Güzel-Seydim et al., 2005).

Table 4 also summarizes the effect of natural phenolic compounds on the proteolysis and peroxide value. Average tyrosine content of strained yoghurt samples made from different phenolic compound was changed between of 0.114 to 0.129 at first day of storage. Moreover, when the storage time was increased up to 14 days there was a subsequent increase for tyrosine value of all yoghurt samples. But, when comparing to the different treatments and storage time, it showed a significant difference between strained yoghurt samples ($p < 0.05$), especially containing different natural phenolic compounds. This observation agreed with the results reported by Dutta et al. (1971) and Rahman et al. (2009), who reported that yoghurt bacteria have slight proteolytic activity and also it can be said that the potential of phenolic compounds to control microbial spoilage may be attributed to a slight decrease in proteolytic activity (Penney et al., 2004). Also, when tyrosine content of yoghurt exceeds 0.5 mg/mL bitterness occurs (Asperger, 1977). In this research tyrosine contents of all treatments were lower than threshold level of bitterness.

The peroxide value (MEG) of strained yoghurt made with the respective phenolic compounds showed in Table 4. A gradual increase in MEG was observed during storage of strained yoghurt. The more pronounced changes were recorded with untreated control yoghurt, whereas the lowest values was recorded with treated B group strained yoghurt, followed by C group strained yoghurt containing natural phenolic compounds.

In fat deterioration, the first iniating step is the formation of free acids, which are susceptible to oxygen attack, resulting in the formation of many organic compounds and free fatty acids which are responsible for rancidity and off flavors in fatty food materials. Production of free

Table 5. Bacterial counts (CFU/g) of strained yoghurt samples during storage at 4°C.

Bacterial	Days	A	B	C	D
Lactobacillus bulgaricus	1	$4.0x10^7 \pm 0.4^{bA}$	$3.7x10^7 \pm 0.2^{bA}$	$4.8x10^7 \pm 0.2^{bB}$	$3.8x10^7 \pm 0.1^{bA}$
	7	$4.8x10^6 \pm 1.8^{aA}$	$3.2x10^6 \pm 0.1^{aA}$	$4.8x10^6 \pm 1.7^{aA}$	$4.6x10^6 \pm 1.9^{aA}$
	14	$7.3x10^6 \pm 0.1^{aA}$	$5.5x10^6 \pm 0.4^{aA}$	$6.4x10^6 \pm 1.1^{aA}$	$6.0x10^6 \pm 2.1^{aA}$
Streptococcus thermophilus	1	$9.4x10^7 \pm 0.2^{bA}$	$10.3x10^7 \pm 1.1^{bA}$	$6.9x10^7 \pm 0.8^{bA}$	$6.0x10^7 \pm 3.2^{bA}$
	7	$8.8x10^6 \pm 0.8^{aA}$	$9.3x10^6 \pm 2.1^{aA}$	$7.9x10^6 \pm 1.1^{aA}$	$7.2x10^6 \pm 1.1^{aA}$
	14	$8.4x10^6 \pm 0.7^{aA}$	$8.8x10^6 \pm 2.1^{aA}$	$6.9x10^6 \pm 1.1^{aA}$	$6.6x10^6 \pm 0.8^{aA}$

A= Control sample (strainned yoghurt without phenolic compounds); B= strainned yoghurt sample containing grape A seed extracts; C= Strainned yoghurt sample containing grape B seed extracts;D= strainned yoghurt sample containing pomegranate seed extracts.

fatty acids and increase in MEG are the best predictors of fat deterioration, which could be used to monitor the extent of spoilage (Rahman et al., 2009). Also a slight increase in MEG was observed as the type of phenolic compound used and may be due to the inhibitory effect of these compounds to moulds on lipolytic bacteria. These observation are in agreement with other findings (Al.Otaibi and El.Demerdash, 2008; Bandyopadhyay, 2008; Rehman and Salariya, 2006), who reported that the total volatile fatty acids of labneh were affected by the type of essential oil.

Statistically analysis of data revealed that MEG of strained yoghurt were significantly engaged during storage of the type of phenolic compounds ($p < 0.05$) except B group.

Microbiological count

Strained yoghurt samples by adding three different phenolic compounds were subjected to microbiological analyses. Results obtained for *L. bulgaricus* and *S. thermophilus* counts are indicated in Table 5.

Regarding enumeration of the starter culture bacteria, the results obtained by *S. thermophilus* indicated that the highest count was obtained for strained yoghurt manufactured with grape A at the first day of storage (10.3×10^7 cfu/g), while the lowest counts (6.6×10^6 cfu/g) were obtained for strained yoghurt made with pomegranate seed extract at the 14th day of storage. In the case of *L. bulgaricus*, the highest count was found from C group strained yoghurt compound at the first day of storage (4.8×10^7 cfu/g) and the lowest count was observed in B group strained yoghurt at the 7th day of storage (3.2×10^6 cfu/g).

The obtained results suggest that the bacterial populations were not surpressed by the natural phenolic compounds during fermentation, and at the first day of fermentation. Whereas the storage period lead to the decreased in bacterial counts. It has previously been stated that addition of some essential oils to yoghurt, labneh and some fresh cheese during its production had a stimulatory effect on lactic acid bacteria by enhancing their growth and acid production (El-Nawawy et al., 1998; Al.Otaibi and El.Demerdash, 2008). But it has been reported that the presence of some phenolic compounds including grape, pomegranate extracts, in the manufacture of yoghurt decreased the yoghurt bacteria compared to untreated controls during storage. This observation was encouraged by Al.Otabi and El.Demerdash, (2008), Baydar et al. (2004), and Schelz et al. (2006) that similarly the phenolic extracts of grape and pomegranate at the similar concentrations inhibited the test microorganisms.

Sensory analysis

The sensory properties of different strained yoghurt were also observed and the dates are presented in Table 6. There were considerable and significant differences in the flavour of treated samples as compared with the control. The control samples when fresh and 14 days of storage were preferred compared to the treated samples. The general acceptance of strained yoghurt samples containing phenolic compounds significantly decreased. Also in all cases the general acceptance of sensory evaluation decreased gradually as result of storage. Nevertheless, it can be concluded that the phenolic compounds of grape extracts could be used in order to have acceptable flavour and consistency without any sign of spoilage.

Conclusion

In this research, of the effects of phenolic compounds extracted from different materials like grape and pomegranate seed on characteristics of strained yoghurts produced from sheep milk were investigated. The fruits like grape and pomegranate containing phenolic compounds have a potential to inhibit and become inactive in food matrix at different concentrations for bacteria. The inhibitory effect of compounds was increasing by the concentration factors. Also, it is suggested to investigate different fruits containing higher

Table 6. Storage time and addition of grape and pomegranate effect on sensory properties of strained yoghurt .

Parameter	Days	A	B	C	D
Flavour	1	7.5 ± 0.7^{aB}	6.9 ± 0.2^{bB}	6.5 ± 0.4^{aB}	4.3 ± 0.8^{aA}
	7	6.5 ± 0.4^{aA}	5.1 ± 0.4^{aA}	5.3 ± 1.0^{aA}	5.1 ± 0.7^{aA}
	14	6.9 ± 0.6^{aC}	6.1 ± 0.3^{abBC}	5.6 ± 0.4^{aB}	4.4 ± 0.1^{aA}
Consistence	1	7.0 ± 0.0^{aA}	6.8 ± 0.7^{aA}	6.9 ± 1.6^{aA}	6.0 ± 0.4^{bA}
	7	6.6 ± 0.8^{aA}	5.9 ± 0.1^{aA}	5.9 ± 1.3^{aA}	5.8 ± 0.2^{abA}
	14	7.5 ± 0.1^{aC}	6.6 ± 0.4^{aB}	6.1 ± 0.3^{aB}	5.0 ± 0.0^{aA}
General acceptance	1	7.0 ± 1.0^{aA}	6.4 ± 0.5^{aA}	6.1 ± 0.8^{aA}	4.7 ± 0.9^{bA}
	7	6.7 ± 0.8^{aA}	5.1 ± 0.6^{aA}	5.5 ± 1.5^{aA}	4.8 ± 0.1^{aA}
	14	6.9 ± 0.6^{aB}	5.7 ± 0.4^{aA}	5.7 ± 0.2^{aA}	4.6 ± 0.4^{aA}

A= Control sample (strainned yoghurt without phenolic compounds); B= strainned yoghurt sample containing grape A seed extracts; C= strainned yoghurt sample containing grape B seed extracts; D= strainned yoghurt sample containing pomegranate seed extracts.

amount phenolic compounds than used in research and study the effects over a longer time period in yoghurt medium. Furthermore, this study shows that phenolic extracts had effects on survival of yoghurt bacteria, physiochemical and sensory properties of strained yoghurts.

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