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Research Paper / Araştırma Makalesi

Total Antioxidant Capacity and Phenolic Content of Pasteurized and UHT-Treated Cow Milk Samples Marketed in Turkey

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ABSTRACT

In this study, UHT-treated (a total of 39 samples with 17 full-fat, 17 semi-fat and 5 skim milk) and pasteurized (5 full-fat) milk samples from different trademarks were obtained from national market chains, which constitute the majority of the Turkey's pasteurized and UHT milk market. Total antioxidant capacities and phenolic contents of milk samples were determined by the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and Folin-Ciocalteu (FC) methods, respectively. Mean total phenolic contents of milk samples ranged from 505.46±16.66 to 982.14±168.42 mg gallic acid equivalents (GAE)/L. Statistical results of the ABTS assay indicated that total antioxidant capacities in decreasing order were pasteurized [280.25±7.71 µM Trolox® Equivalent (TE)] > full-fat (240.30±15.71 µM TE) > skim (216.78±4.90 µM TE) > semi-fat (209.81±7.03 µM TE) milk samples. In general, total antioxidant capacity of milk samples determined by the Folin-Ciocalteu method increased with an increase in milk fat content. Antioxidant capacity of pasteurized milks determined by both methods was higher than UHT processed milks.

Keywords: Milk, Antioxidant, Phenolic compounds, Nutrition

Türkiye'de Satışa Sunulan UHT ve Pastörize İnek Sütü Örneklerinin Toplam Antioksidan Kapasitesi ve Fenolik Madde İçeriği

ÖZ

Bu çalışmada, Türkiye'deki pastörizasyon veya ultra yüksek sıcaklık (UHT) işlemi uygulanmış süt pazarının önemli bir bölümünü oluşturan farklı ticari markalardan UHT (17 tam yağlı, 17 yarım yağlı ve 5 yağsız olmak üzere toplam 39 numune) ve pastörize süt (5 tam yağlı) örnekleri ulusal market zincirlerinden toplanmıştır. Süt örneklerinin toplam antioksidan kapasiteleri ve fenolik içerikleri, sırasıyla 2,2'-azino-bis 3-etilbenzotiazol-6-sülfonik asit (ABTS) ve Folin-Ciocalteu (FC) yöntemleriyle belirlenmiştir. Süt örneklerinin toplam fenolik içeriği 505.46±16.66 ile 982.14±168.42 mg gallik asit eşdeğeri (GAE)/L arasında değişmiştir. ABTS yöntemiyle elde edilen verilerin istatistiksel analizi sonucunda örneklerin toplam antioksidan kapasitelerinin azalan sırayla pastörize (280.25±7.71 µM Trolox® eşdeğeri, TE)> tam yağlı (240.30±15.71 µM TE)> yağsız (216.78±4.90 µM TE)> yarım yağlı (209.81±7.03 µM TE) süt örnekleri şeklinde olduğu belirlenmiştir. Genel olarak, süt yağı içeriğinin artışı Folin-Ciocalteu yöntemi ile belirlenen toplam antioksidan kapasite değerinin de artmasıyla sonuçlanmıştır. Her iki yöntemle belirlenen sütlerin antioksidan kapasite değerleri pastörize sütlerde UHT ile işlenmiş sütlere göre daha yüksek bulunmuştur.

Anahtar Kelimeler: Süt, Antioksidan, Fenolik bileşikler, Beslenme

INTRODUCTION

An adequate daily intake of antioxidant compounds including phenolics is considered to be of great significance for controlling oxidative stress [1,2] and may be useful in the prevention of health problems like cardiovascular diseases and cancer and/or in the reduction of diseases like diabetes and neurodegenerative diseases (Parkinson's and Alzheimer's disease) [3]. Milk and dairy products are good sources of antioxidant compounds including proteins, enzymes, vitamins (vitamin E and C), phenolic compounds, carotenoids and organic acids [1, 2, 4-6]. Milk contains considerable amounts of phenolic compounds including a variety of compounds such as phenol, cresol, tymol and carvacrol [7] and antioxidant activity of dairy products could be also increased by the incorporation of phenolic constituents to these products [8]. Phenolic compounds may play a significant role in microbiological and organoleptic properties of milk and are both functional and nutritive ingredients for human health [9]. Concentrations of antioxidant compounds in milk are usually affected by dairy cattle feeding and milk storage conditions [5]. Since there are a variety of antioxidant components in milk and dairy products, measurement of total antioxidant capacity may be a useful method for detecting the cumulative role of antioxidant components in milk [10].

Limited studies have been reported in the literature on antioxidant activity of milk and dairy products consumed and/or marketed in Turkey [11, 12]. Therefore, a comprehensive survey representing a major share of all commercial brands should be more helpful to present current status of the TAC and phenolic content of UHT-treated and pasteurized milk samples marketed in Turkey. The objective of this study is to determine the TAC and phenolic content of commercial UHT-treated and pasteurized milk samples in Turkey.

MATERIALS and METHODS

Materials

Commercial pasteurized (5 samples) and UHT milk samples (17 full-fat milk, 17 low-fat milk and 5 skim milk) produced by seventeen different companies in Turkey were purchased from national supermarkets. Selected trademarks of milk samples constitute a significant proportion of the Turkish pasteurized and UHT milk market.

Methods

Chemical Composition of Milk Samples

Total solids, milk fat, protein and lactose contents of milk samples were determined by the infrared spectrometric method [13], using a Bentley 150 instrument (Bentley Instruments Inc., Chaska, MN, USA).

ABTS Radical Scavenging Assay

Total antioxidant capacity of pasteurized and UHT milk samples was determined by ABTS method described by Re et al. [14] with slight modifications. Potassium persulfate (Merck, Darmstadt, Germany) (2.6 mM) was added into aqueous solution of ABTS (7 mM) (Merck, Darmstadt, Germany) to prepare the stock solution of ABTS radicals, and this mixture was stored at room temperature for 12–16 h in dark. Stock solution was diluted with chromatographic grade methanol (Sigma, St. Louis, MO, USA) to a final absorbance of about 1.1±0.02 at 734 nm in order to prepare the working solution. Then, milk samples (0.3 mL) were mixed with working solution (2.7 mL). Mixtures were incubated at room temperature for 30 min, and then they were centrifuged at 12,000*g* for 2 min at room temperature. Decreases in absorbance values were measured at 734 nm against methanol as a blank by using a spectrophotometer (Optizen Pop, Mecasys Co., Ltd., Korea). Results were expressed as 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) (Fluka, St. Louis, MO, USA) equivalent (TE) antioxidant capacity (TEAC).

Total Phenolic Content Analysis

Total phenolic contents of milk samples were spectrophotometrically measured by Folin-Ciocalteu method according to Singleton and Rossi [15] using gallic acid (Fluka, St. Louis, MO, USA) as a standard. Folin-Ciocalteu reagent (Merck, Darmstadt, Germany) was diluted with pure water (1:10) to prepare working solution. Sample or standard (1 mL) was mixed with FC working solution (5 mL) and incubated for 3 min, then 4 mL of sodium carbonate (Sigma, St. Louis, MO, USA) (75 g/L) was added into this mixture. After incubation for 2 hours at room temperature in dark, samples were centrifuged for 2 min at 12.000 g. Absorbance values of samples were measured at 760 nm against to distilled water by using a spectrophotometer. Results were expressed as gallic acid equivalents (GAE) per liter of milk samples.

Statistical Analyses

Analysis of variance (ANOVA) was used to determine statistically significant differences by means of the SAS software program (The SAS System for Windows 9.0, Chicago, USA). Separation of means for significant differences was conducted using the Duncan's multiple-range test at α=0.05 level. PROC CORR procedure of SAS was used to determine the Pearson's correlation coefficients among parameters.

RESULTS and DISCUSSION

Approximate composition of UHT and pasteurized milk samples are presented in Table 1 while total antioxidant capacity of UHT-treated full-fat, low-fat and skim milk samples is given in Tables 2 and 3. Mean TEAC values of heat-treated commercial milk samples with different fat contents are given Figure 1. Differences in TEAC values among UHT-treated milk samples with different

fat contents were statistically significant ($p<0.05$). TAC values of skim milk samples were higher than low-fat milk samples ($p<0.05$). Similar results were also obtained for total phenolic contents of milk samples (Tables 2 and 3).

Table 1. Approximate composition of UHT and pasteurized milk samples

Milk Samples*	Fat (%)	Protein (%)	Lactose (%)	Total Solids (%)	pH
FF1	3.11±0.00	3.22±0.01	4.51±0.02	11.59±0.04	6.68±0.00
FF2	3.32±0.00	3.00±0.01	4.50±0.01	11.63±0.00	6.73±0.00
FF3	3.28±0.01	2.90±0.01	4.51±0.01	11.52±0.00	6.75±0.00
FF4	3.29±0.00	2.97±0.00	4.61±0.01	11.66±0.01	6.68±0.00
FF5	3.15±0.00	3.20±0.00	4.55±0.01	11.72±0.01	6.72±0.00
FF6	3.30±0.00	3.02±0.01	4.58±0.01	11.71±0.01	6.77±0.00
FF7	3.61±0.01	3.16±0.00	4.66±0.01	12.25±0.00	6.74±0.00
FF8	3.14±0.00	3.16±0.00	4.58±0.00	11.69±0.01	6.68±0.00
FF9	3.25±0.01	3.07±0.01	4.52±0.01	11.62±0.01	6.73±0.00
FF10	3.70±0.01	3.23±0.00	4.63±0.01	12.33±0.01	6.69±0.00
FF11	3.19±0.00	2.99±0.01	4.55±0.01	11.54±0.01	6.72±0.00
FF12	3.39±0.01	3.25±0.01	4.77±0.01	12.22±0.00	6.74±0.00
FF13	3.23±0.00	3.10±0.00	4.73±0.01	11.88±0.03	6.81±0.00
FF14	3.17±0.01	2.94±0.01	4.03±0.00	10.94±0.02	6.57±0.00
FF15	3.77±0.01	3.06±0.01	4.55±0.01	12.17±0.01	6.50±0.00
FF16	3.56±0.00	2.76±0.00	4.09±0.01	11.18±0.02	6.46±0.00
FF17	3.49±0.01	3.20±0.01	4.85±0.00	12.37±0.02	6.41±0.01
LF1	1.47±0.02	2.61±0.04	4.27±0.06	9.22±0.12	6.41±0.01
LF2	1.95±0.01	2.98±0.00	4.59±0.00	10.36±0.02	6.44±0.00
LF3	1.79±0.00	3.16±0.01	4.69±0.01	10.48±0.00	6.38±0.00
LF4	1.59±0.00	3.02±0.01	4.64±0.01	10.12±0.02	6.42±0.00
LF5	1.77±0.01	3.01±0.01	4.58±0.01	10.21±0.00	6.44±0.01
LF6	1.94±0.00	2.90±0.00	4.36±0.01	10.04±0.01	6.48±0.01
LF7	1.79±0.00	2.81±0.01	4.49±0.01	9.94±0.01	6.47±0.00
LF8	1.79±0.00	2.79±0.00	4.50±0.01	9.94±0.01	6.46±0.00
LF9	1.88±0.01	2.93±0.01	4.42±0.01	10.07±0.00	6.48±0.00
LF10	1.95±0.00	2.78±0.01	5.67±0.01	11.48±0.01	6.49±0.00
LF11	1.82±0.01	2.72±0.01	4.62±0.01	10.03±0.02	6.45±0.00
LF12	1.74±0.01	3.23±0.01	4.89±0.00	10.76±0.01	6.49±0.00
LF13	2.18±0.02	3.06±0.01	4.66±0.01	10.73±0.01	6.45±0.00
LF14	1.74±0.01	3.39±0.01	5.03±0.00	11.04±0.02	6.43±0.01
LF15	1.74±0.01	3.04±0.01	4.70±0.01	10.33±0.01	6.44±0.00
LF16	1.57±0.00	3.30±0.01	4.85±0.01	10.57±0.02	6.48±0.00
LF17	1.63±0.00	3.11±0.01	4.81±0.00	10.42±0.02	6.47±0.00
SM1	0.29±0.00	3.11±0.01	4.69±0.01	8.93±0.01	6.42±0.01
SM2	0.12±0.00	3.25±0.01	4.86±0.01	9.11±0.01	6.46±0.01
SM3	0.12±0.01	3.10±0.01	4.64±0.01	8.70±0.01	6.40±0.00
SM4	0.15±0.00	3.09±0.01	4.96±0.01	9.10±0.01	6.50±0.00
SM5	0.12±0.00	3.17±0.00	4.91±0.01	9.08±0.01	6.46±0.00
PM1	3.23±0.01	3.01±0.01	4.65±0.04	11.74±0.06	6.67±0.00
PM2	3.45±0.01	3.15±0.00	4.76±0.02	12.19±0.01	6.61±0.00
PM3	3.29±0.00	3.01±0.01	4.86±0.01	12.00±0.01	6.62±0.00
PM4	3.24±0.01	3.25±0.01	4.84±0.00	12.13±0.01	6.58±0.00
PM5	3.17±0.01	3.08±0.01	4.73±0.00	11.86±0.01	6.59±0.00

*: FF: UHT-treated full-fat milk, LF: UHT-treated low-fat milk, SM: UHT-treated skim milk, PM: full-fat pasteurized (HTST) milk samples.

TAC values of commercial full-fat pasteurized milk samples are given Table 3. Differences in TEAC values among pasteurized and UHT-treated milk samples with different fat contents were statistically significant ($p<0.05$). Commercial full-fat pasteurized milk samples had higher mean TEAC values than UHT-treated milk samples (Figure 1).

For UHT-treated milk samples, TEAC values and total phenolic contents were highly correlated with total solid content of commercial milk samples with a Pearson's correlation coefficient (R) of 0.584 and 0.761, respectively ($p<0.0001$) (Table 4). Moreover, correlation of TEAC values with total phenolic contents of these milk samples with a coefficient of 0.936 was also significant ($p<0.0001$). On the other hand, correlation of TAC values or total phenolic contents with fat, protein or lactose contents of commercial UHT-treated milk samples was insignificant ($p>0.01$).

Table 2. Total antioxidant capacity of full-fat (FF) and low-fat (LF) UHT-treated milk samples (±standard deviation, SD)

Sample Code*	Assay		Sample Code	Assay	
	ABTS (μM TEAC)	FC (mg GAE/L)		ABTS (μM TEAC)	FC (mg GAE/L)
FF1	236.60±1.83	983.61±3.16	LF1	199.27±1.99	446.79±59.56
FF2	245.21±0.49	1021.40±4.22	LF2	205.38±0.59	534.41±16.22
FF3	242.48±2.00	1035.33±7.68	LF3	212.92±1.69	516.24±55.00
FF4	249.51±0.19	1064.06±38.68	LF4	215.44±1.72	492.83±31.90
FF5	245.18±0.33	1072.47±19.87	LF5	212.44±4.75	552.71±22.76
FF6	249.26±0.25	1155.26±7.83	LF6	210.68±2.12	529.37±14.73
FF7	248.10±0.40	990.43±1.35	LF7	211.90±0.67	522.10±14.31
FF8	243.97±1.68	1035.33±24.84	LF8	209.48±1.16	488.57±28.27
FF9	247.73±1.20	1062.25±16.26	LF9	208.20±1.59	467.06±28.33
FF10	246.31±0.09	1037.88±29.34	LF10	205.04±4.90	510.15±32.25
FF11	243.64±1.93	1088.10±32.36	LF11	200.12±1.11	452.84±4.31
FF12	242.71±0.40	1037.67±20.91	LF12	220.37±1.72	537.10±11.37
FF13	248.76±0.25	1031.72±0.76	LF13	206.27±4.17	514.99±18.99
FF14	266.86±0.43	1146.21±24.17	LF14	212.10±3.00	589.47±24.17
FF15	211.28±5.08	624.87±46.62	LF15	199.33±1.84	538.19±13.81
FF16	205.56±0.22	574.81±6.91	LF16	213.79±1.97	528.42±0.00
FF17	211.85±1.25	734.76±88.06	LF17	224.04±1.74	536.97±29.35
Mean±SD	240.30±1.06	982.14±21.94	Mean±SD	209.81±2.16	515.19±23.84

*: FF: UHT-treated full-fat milk, LF: UHT-treated low-fat milk

Table 3. Total antioxidant capacity of UHT-treated skim milk (SM) and full-fat pasteurized (HTST) (PM) milk samples (±SD)

Sample*	ABTS (μM TEAC)	FC (mg GAE/L)	Sample*	ABTS (μM TEAC)	FC (mg GAE/L)
SM1	216.78±0.24	506.44±3.45	PM1	270.39±1.74	1138.88±89.79
SM2	224.84±3.13	489.35±10.36	PM2	285.86±1.20	1469.76±70.79
SM3	215.80±0.00	527.20±5.18	PM3	278.92±0.03	1289.06±18.99
SM4	214.08±0.31	501.56±27.63	PM4	290.45±4.45	1401.38±88.06
SM5	212.38±4.47	502.78±8.63	PM5	275.69±0.06	1168.19±82.88
Mean±SD	216.78±4.81	505.47±13.74	Mean±SD	280.26±1.50	1293.50±70.10

*: SM: UHT-treated skim milk, PM: full-fat pasteurized (HTST) milk samples.

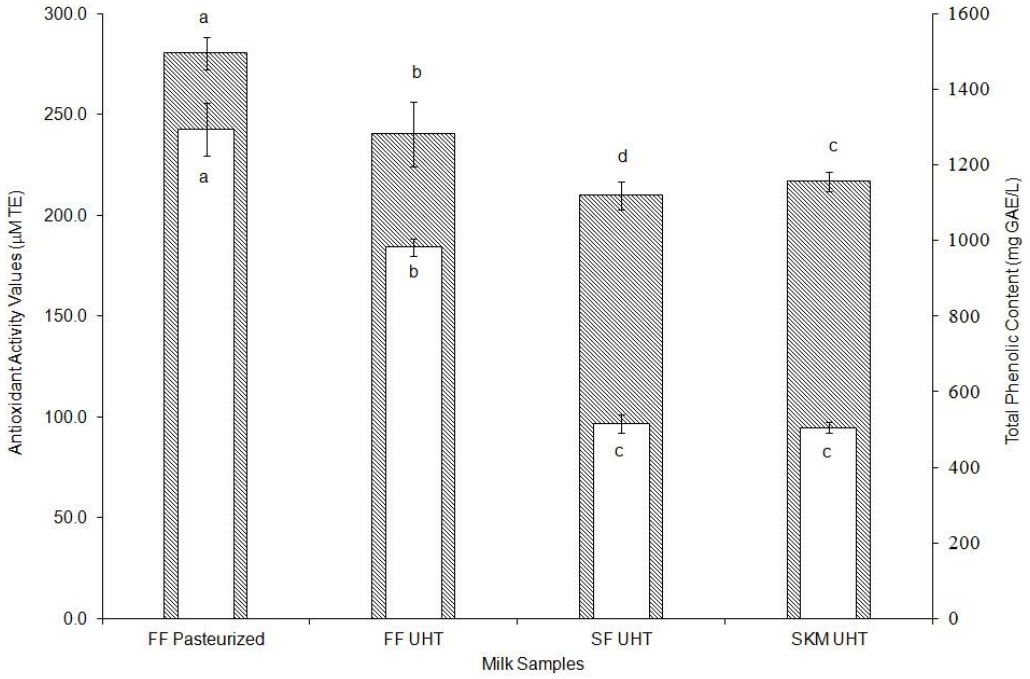

Figure 1. Changes in TEAC values (▨) and total phenolic contents (□) of milk samples depending on their fat contents and commercial heat treatments (FF: full-fat, LF: low-fat, SM: skim milk)

Table 4. Pearson correlation coefficients (R) among fat, protein, lactose, total solids contents, antioxidant activity and total phenolic contents of milks (n=39). Lower values in parentheses (p values) indicate that parameters are highly correlated with each other. Coefficients that are significantly different from zero are shown in bold.

Parameters	Fat (%)	Protein (%)	Lactose (%)	Total Solids (%)	ABTS (µM TE)	Total Phenolics (mg GAE/L)
Fat (%)	1.000	-0.018 (0.9133)	-0.338 (0.0351)	0.935 (<0.0001)	0.584 (<0.0001)	0.761 (<0.0001)
Protein (%)		1.000	0.371 (0.0199)	0.229 (0.1610)	0.296 (0.0675)	0.217 (0.1852)
Lactose (%)			1.000	-0.005 (0.9762)	-0.229 (0.1617)	-0.254 (0.1181)
Total Solids (%)				1.000	0.580 (0.0001)	0.743 (<0.0001)
ABTS (µM TE)					1.000	0.936 (<0.0001)
Total Phenolics (mg GAE/L)						1.000

According to the Turkish Food Codex (TFC) Notification on Raw and Heat Treated Milks [16], fat content (w/v) of heat treated whole milk, full-fat, semi-fat and skim milks should be ≥3.5%, ≥3%, ≥1.5% and ≤0.15%, respectively. Two samples (FF7 and FF15) from full-fat UHT group had more than 3.5% fat content, and these samples should be classified as whole milk (Table 1). A sample of UHT-treated skim milk (SKM1) had a fat content of 0.29%, which is higher than the permitted value of ≤0.15%. In the TFC Notification, minimum protein content of milks should be 2.8% (m/v). Only two low-fat UHT milk samples (LF1 and LF11) had slightly lower protein contents (2.61±0.04 and 2.72±0.01%) than 2.8%. In general, protein and fat contents of 39 milk samples were in good agreement with the TFC with an exception of 5 UHT-treated milk samples.

Higher TEAC values of full-fat UHT-treated milk samples than UHT-treated low-fat and skim milk samples might be related to lipid soluble antioxidants such as vitamin A and E and β-carotene and fat globule membrane proteins [2,5]. TEAC values and total phenolic contents of skim milk samples were higher than low-fat milk samples, and the fact that milk samples were of commercial type, and variations in raw material composition (e.g. different protein and lipid soluble antioxidant contents) and unequal number of analyzed samples (i.e. 17 low-fat versus 5 skim milk samples) could be responsible for this contradictory result. Unlike the results of this present study, correlation between fat content and antioxidant capacity of milk samples was reported by Chen et al. [17] and Zulueta et al. [2]. Studying the antioxidant capacities of milk samples by the oxygen radical absorbance capacity (ORAC) method, Zulueta et al. [2] reported the ORAC values of whole, low-fat and skimmed milk samples as 14.044, 13.104 and 12.697 mM TE, respectively. Similarly, TEAC values of milk samples with 3, 1.5, 0.5 and 0.1% fat contents were reported as 2.241, 1.852, 1.561 and 1.246 mM TE by Chen et al. [17]. In these studies, milk samples with different fat contents were obtained from the same batch of raw milk in order to avoid matrix variations. The results of this present study, on the contrary, better reflect the current antioxidant status of commercially available heat-treated UHT milk samples.

Heat treatment, generally required for milk safety and stability, can be responsible for quality changes in milks like their antioxidant properties. Calligaris et al. [18] reported that overall antioxidant properties of milk can be changed by heat treatments with different time–temperature combinations. They also reported that antioxidant activity of milk may increase during thermal treatments, due to exposure of thiol groups, which are potentially acting as hydrogen donors, and the formation of Maillard reaction products. This present study is focused on the determination of current antioxidant statues of commercial milk samples, and matrix differences, differences in several processing parameters like heat treatment conditions and the number of available samples could be responsible for this contradictory result. Moreover, although antioxidant activity of milk samples has been previously reported in several studies [2,12,17,19-21], results of these studies are scarcely comparable with each other because of a wide range of assays [22] and variations in a single assay [23] for determining antioxidant activity of foods.

CONCLUSIONS

Results of this study indicated that TACs of commercial milk samples are highly variable depending on the fat content of milk and heat treatment used in processing. Mean TAC values of full-fat milk samples were higher than those of skim or low-fat UHT-treated milk samples. In this study, the number of available pasteurized full-fat milk samples marketed in Turkey was lower than the number of UHT-treated full-fat milk samples, and mean TAC values of pasteurized milk samples were higher than those of UHT-treated full-fat milk samples. In conclusion, total antioxidant capacity and total phenolic content of commercial milk samples may be influenced by thermal process and fat separation.

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