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Total phenolic compounds content and antioxidant activity in packed and bulk milk in different regions of Tehran, Iran

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ABSTRACT

Free radicals and active oxygen species are toxic as they oxidize biomolecules leading to cell death and tissue injury. Lipid oxidation is also a major cause of spoilage in foods. In this survey, antioxidant activity and total phenolic content in milk samples were aimed to investigate. Pasteurized and bulk milk samples were collected from market in Tehran. The total phenolic content was determined based on the Folin-Ciocalteu method. The FRAP (Ferric reducing antioxidant power) assay was used for antioxidant activity. Data were analyzed by SPSS. Brand 9 packed milk had the highest amount of antioxidant power and total phenolic content; 3.820±0.104 µm and 9.001±0.598 µm respectively. Brand 5 packed milk had the lowest amount of antioxidant power and total phenolic; 0.498±0.002 µm and 0.708±0.135 µm respectively. In bulk samples collected from different regions, region 9 had the highest amount of antioxidant capacity and total phenolic content; 8.611±0.002 µm and 8.200±0.760 µm respectively, while the samples from region 22 had the lowest amount of antioxidant capacity and total phenolic content of about 0.218 ±0.021 µm and 0.395±0.005 µm respectively. Based on the findings, there were significant amounts of antioxidant and total phenolic elements in both types of milk; however, some variations between results were measured. In bulk milk samples, antioxidant activity was higher than branded samples, whereas the total phenolic amounts were less than the branded ones. In the flavored milk samples, the antioxidant activity was higher than unflavored milk samples; however, the total phenolic amounts were lower than in unflavored samples.

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1. Introduction

The food Free radicals are extremely unstable and reactive compounds generated in the body during normal metabolic function or due to exposure to exogenous factors (1,2) Superoxide, hydroxyl and peroxide radicals, H_2O_2 and singlet oxygen which are categorized as reactive oxygen species (ROS) are known to cause oxidative damage (2,3). Oxidative damage plays a significantly pathological role in the initiation and/or progression of human diseases, such as atherosclerosis, myocardial and cerebral ischemia, inflammatory injury, diabetes, cancer, rheumatoid arthritis, cardiovascular diseases as well as in the aging process (4,5). Enough amounts of exogenous antioxidants are able to reduce the harm of ROS to the human body. These compounds can delay or inhibit the oxidative damage of proteins, nucleic acids and lipids caused by free radical- induced oxidative stress (6,8). Antioxidants are compounds that can eliminate free radical chain reactions by dissolving free radicals and prevent formation of reactive oxidants in the first place

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(9). Antioxidants help to protect the human body against damage by reactive oxygen species (ROS). Antioxidant defense protects both enzymatic and non-enzymatic reactions from oxidative damage. Antioxidants, when exposed to low concentrations in relation to oxidized acids, significantly inhibit oxidative processes, while they often oxidize themselves. Antioxidants are in possession of information about the reactivity of reactive oxygen species or free radicals (10,11). Cell damage caused by oxidative stress is associated with the disease (12). Lipid oxidation is a major cause of chemical spoilage in foods. It leads to many undesirable changes in flavor, texture and nutritional value in foods (15). Some proteins, especially milk proteins, were found to have the scavenging activity of active oxygen species (11-16). As they are flavorless, odorless and nutritive, they are used as natural antioxidants in foods. Phenolic compounds are widely present in dairy products. However, few studies have been conducted to check the presence of these compounds in dairy products such as milk. The aim of this study was to determine the total phenolic matter and to investigate the antioxidant effect in packed and unpacked milk samples in different regions of Tehran, Iran.

2. Materials and methods

In this study, 42 pasteurized milk samples were collected in three types of low-fat, semi-fat and high-fat, 12 chocolate milk samples, 8 coffee milk samples, 8 banana milk samples, 8 samples of honey milk from various brands and 42 local milk samples (Bulk) from areas 1 to 22 of Tehran. Samples were transferred to the Food Laboratory, Faculty of Pharmacy for measuring the total amount of total phenol compounds and antioxidant activity. All standards were of analytical grade and were purchased from Merck (Darmstadt, Germany).

2.1. Total phenolic content

The total phenolic content was determined based on the Folin-Ciocalteu method with minor variations. An amount of 200 µl of milk sample was mixed with 1.5 ml of Folin-Kyokualte (diluted 10 times with two distilled water) and was placed at room temperature for 5 min. A solution of sodium bicarbonate 1.5 ml (60 g/l) was added to the mixture and placed at room temperature for 90 min, absorbance at 725 nm using visible UV spectrophotometer (GBC, Cintra 40) was evaluated. Total phenol was obtained by measuring the concentration of known standard solution of gallic acid (10 to 150 µg/ml in 80% methanol) by calibration curve (19). The results were calculated as the equivalent of gallic acid (GAE) per gram of powder and reported as mean ± standard deviation (SD).

2.2. Antioxidant activity

The FRAP (Ferric reducing antioxidant power) assay was initially performed by Benzie and Strain (20-22). The method is based on the reduction of the complex composition of triazine tridriryrite in the form of its color and the antioxidants presence. FRAP reagent was obtained by 5 ml TPTZ (2, 4, 6-tripyridyl-S-triazine, 10 mmol/l) solution in 40 mmol/l HCl plus 5 ml $FeCl_3$ (20 mol/l) and 50 ml of Acetate buffer (0.3 mol/l). It was prepared freshly and maintained at 37°C. Aliquots of sample (50 µl) supernatant were mixed with 1.5 ml FRAP reagent and the absorbance of reaction mixture was measured by spectrophotometer at 593 nm after incubation at 37°C for 10 min. The calibration curve was obtained by five concentrations of $FeSO_4 \bullet 7H_2O$ (100 - 1000 mmol/l) and the absorbance was measured as sample solution. The values were expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mmol/l $FeSO_4$ (23).

2.3. Statistical analysis

Three replicates of each sample were statistically analyzed and the values were reported as mean ± SD. The relationship between antioxidant activity and total phenolic content was analyzed by Pearson's correlation, using SPSS 22 statistical program. Data were also subjected to the analysis of variance and mean values compared by Tukey post-hoc multi-comparison test. Differences at p-value <0.001 were considered significant.

3. Results

Figure 1 shows amount antioxidant activity in the total fat, semi-fat and low fat packaged milk samples.

Table 1 shows the total amount of total phenolic compounds and antioxidant activity in the total sample of local milk (bulk) from different regions of Tehran.

Tables 2 to 5 show the total amount of phenolic compounds and antioxidant activity according to milk flavor in packed samples.

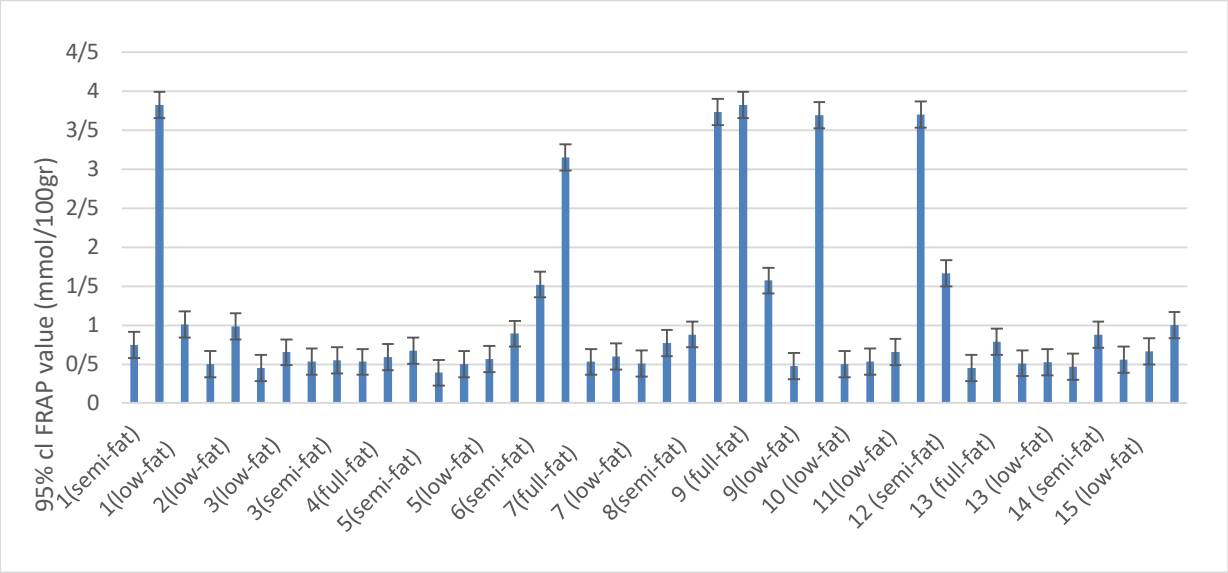

Figure 1. Antioxidant activity in packaged milk samples.

Table 1. Total phenolic and antioxidant power content samples of bulk milk from different regions of Tehran

Region	Total phenolic Mean± SD (µmoll-1)	Antioxidant power (µmoll-1)
1	3.161±0.164	0.534±0.080
2	0.566±0.897	0.532±0.038
3	5.520±0.007	3.97±0.178
4	7.293±0.284	0.094±0.012
5	0.926±0.455	0.769±0.054
6	5.545±0.312	3.820±0.104
7	6.668±0.534	0.532±0.038
8	6.912±0.725	0.543±0.856
9	8.200±0.760	8.421±0.123
10	2.781±0.001	3.213±0.078
11	0.888±0.043	3.820±0.104
12	2.664±0.135	0.660±0.0028
13	7.725±0.574	0.434±0.132
14	6.356±0.001	0.510±0.031
15	7.448±0.016	0.529±0.021
16	1.841±0.308	0.470±0.014
17	2.685±0.005	0.500±0.000
18	1.582±0.134	0.489±0.004
19	2.997±0.361	3.691±0.196
20	3.303±0.008	0.770±0.055
21	1.582±0.134	0.880±0.068
22	0.395±0.005	0.218±0.021

Table 2. Total phenolic and antioxidant power content in the chocolate Milk samples

Brand code	Total phenolic Mean ± SD (µmoll-1)	antioxidant power (µmoll-1)
1	4.001±0.245	3.963±0.24
2	0.531±0.007	0.521±0.007
3	0.678±0.002	0.660±0.002
4	0.712±0.010	0.653±0.008
5	0.456±0.002	0.431±0.002
6	3.289±0.120	3.012±0.080
7	0.556±0.067	0.529±0.050
9	4.90±0.023	4.881±0.013
10	3.001±0.201	2.890±0.190
11	0.980±0.056	0.921±0.034
13	0.750±0.0501	0.783±0.049
14	0.579±0.0401	0.570±0.034

Table 3. Total phenolic and antioxidant power content in the coffee milk samples

Brand code	Total phenolic Mean ± SD(µmoll-1)	antioxidant power (µmoll-1)
1	0.542±0.002	0.568±0.002
2	1.780±0.002	1.762±0.003
5	0.312±0.011	0.301±0.011
6	1.999±0.150	2.009±0.104
7	0.971±0.020	0.914±0.019
9	4.812±0.061	4.710±0.055
10	1.001±0.003	1.045±0.003
11	0.991±0.003	0.981±0.004

Table 4. Total phenolic and antioxidant power content in the honey milk samples

Brand code	Total phenolic Mean ± SD (µmoll-1)	antioxidant power (µmoll-1)
1	0.689±0.002	0.670±0.002
2	1.603±0.003	1.566±0.003
5	0.551±0.029	0.451±0.021

6	1.998±0.020	1.932±0.024
7	0.911±0.020	0.915±0.021
9	3.812±0.201	3.687±0.102
10	1.209±0.003	1.109±0.003
11	1.017±0.004	1.006±0.004

Table 5. Total phenolic and antioxidant content power in the banana milk samples

Brand code	Total phenolic Mean ± SD(μmoll^{-1})	antioxidant power (μmoll^{-1})
1	0.861±0.002	0.670±0.002
2	1.761±0.003	1.566±0.003
5	0.566±0.002	0.451±0.021
6	1.998±0.021	1.932±0.024
7	1.012±0.022	0.915±0.021
9	3.881±0.141	3.687±0.102
10	1.235±0.003	1.109±0.003
11	1.222±0.003	1.006±0.004

4. Discussion

The total phenolic contents of the samples showed large variations, 0.708 ± 0.135 μm in Brand 5 (semi-fat) milk and 9.001 ± 0.598 μm in Brand 9 (full-fat) milk. The antioxidant power of the samples showed large variations about FRAP values, 0.390 ± 0.001 μm in Brand 5 (semi-fat) milk and 3.820 ± 0.104 μm in Brand 9 (full-fat) milk. The total phenolic contents about regional samples, 0.395 ± 0.005 μm in region 22 and 8.200 ± 0.760 μm in region 9 was measured (Table 1). The antioxidant power about flavored milk, the total phenolic contents of chocolate, coffee, honey and banana milk were measured. The total phenolic content of the samples showed large variations, 0.456 ± 0.002 μm in Brand 5 chocolate milk and 4.9 ± 0.023 μm in Brand 9 chocolate milk (Table 2), 0.312 ± 0.011 μm in Brand 5 coffee milk and 4.812 ± 0.0607 μm in Brand 9 coffee milk (Table 3), 0.551 ± 0.029 μm in Brand 5 honey milk and 3.812 ± 0.201 μm in Brand 9 honey milk (Table 4) and about the last group, 0.566 ± 0.002 μm in Brand 5 banana milk and 3.881 ± 0.141 μm in Brand 9 banana milk (Table 5). The antioxidant power of the samples showed large variations, 0.456 ± 0.002 μm in Brand 5 chocolate milk and 4.9 ± 0.023 μm in Brand 9 chocolate milk (Table 2), 0.312 ± 0.011 in Brand 5 coffee milk and 4.812 ± 0.0607 μm in Brand 9 coffee milk (Table 3), 0.551 ± 0.029 μm in Brand 5 honey milk and 3.812 ± 0.201 μm in Brand 9 honey milk (Table 4), 0.566 ± 0.002 μm in Brand 5 banana milk and 3.881 ± 0.141 μm in Brand 9 banana milk (Table 5). Significant differences were observed in the total phenolic compounds in the brands 5 (semi-fat) and 9 (full-fat) with other brands ($p<0.001$) but there was no significant difference between other brands. Significant differences were observed in the total phenolic compounds in bulk lids in the regions of Tehran 9 and 22 with other areas ($p<0.001$), but there was no significant difference between the other regions. The antioxidant effect of these cultivars with other Brands ($p<0.001$) did not show any significant difference. The rest of the brands with each other had a significant difference. There was no significant difference between the rests of the brands with each other. There was a significant difference between Brand 5 and the rest of the brands. The sample of region 9 had the highest antioxidant effect at 8.611 ± 0.002 μm and was significantly different with other brands ($p <0.001$). According to a study by Oveisi, et al., it was found that breast milk provides higher levels of antioxidant compounds for the baby (24). In 2011, according to a study conducted on the effects of antioxidants in cancer prevention, it was concluded that the antioxidants present in milk are effective in preventing cancer (25). According to a study by Hamid Ezat, et al., on the oxidative stability of cow's milk and soy milk, no significant difference was observed between milk and soy milk. (26). In 2015, in a study by Ramos, et al., concluded that the antioxidant activity was moderate in a commercial fermented milk. (27). In a study by Ertan et al. (2017) on the antioxidant and total phenol of milk in Turkey it was found that increasing the fat in milk increases the antioxidant and total phenol levels (28). According to Calligaris et al. (2003) the overall antioxidant properties of milk can be effected and altered by heat treatments with different time temperature combinations. Also, the antioxidant activity of milk may increase during thermal treatments, because of exposure of thiol groups, which are potentially hydrogen donors, and inducing the formation of Maillard reaction products (29).

5. Conclusions

The results of the current study showed that among the packed Brand 1 and 9 full-fat milk samples and among the milk samples of the regions, the sample of district 9 had the most antioxidant and total phenol contents. A direct relation was observed between antioxidant and total phenolic in each milk samples but on average, the antioxidant activity properties of packed milk samples were higher than bulk milk samples and total phenolic compounds were lower respectively. In flavored milk samples, the antioxidant property was greater than, Non-flavored ones but content of total phenolic compounds was higher.

Conflict of interest

The authors have no conflict of interest.

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References

1. Zino S, Skeaff M, Williams S, et al. Randomised controlled trial of effect of fruit and vegetable consumption on plasma concentrations of lipids and antioxidants. British Med J 1997; 314:1787-91.
2. Kikuzaki H, Nakatani N. Antioxidant effects of some ginger constituents. J Food Sci 1993; 58:1407-10.
3. Halliwell B, Gutteridge JMC. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 1984; 219:1-14.
4. Coyle JT, Puttfarcken P. Oxidative stress, glutamate & neurodegenerative disorders. Sci 1993; 262:689-95.
5. Halliwell B, Zentella A, Gomez EO. Antioxidants and human disease: a general introduction. Nutr Rev 1997; 55:44-9.
6. Baardseth P. Effect of selected antioxidants on the stability of dehydrated mashed potatoes. Food Addit & Contamin 1989; 6:201-7.
7. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agri & Food Chem 2001; 49:5165-70.
8. Norshazila S, Syed Zahir I, Mustapha Suleiman K, et al. Antioxidant levels and activities of selected seeds of Malaysian tropical fruits. Malay J Nut 2010; 16:149-59.
9. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agri & Food Chem 2005; 53:1841-1856.
10. Kumar SH. Free Radicals & Antioxidants: Human and Food System. Advance in Appl Sci Res, 2011; 2:129-135.
11. Karakaya S, EI SN, Tas AA. Antioxidant activity of some foods containing phenolic compounds. Int J Food Sci & Nutr 2001; 52:501-8.
12. 12. Hambraeus L, Lönnerdal B. The Physiological role of lactoferrin. Medical / Nutritional Aspects: International Dairy Federation, 2003. Chap 18.
13. Lindmark-Månsson H, Åkesson B. Antioxidative factors in milk. British J Nutr 2000; 84:103-10.
14. Furst P. The role of antioxidants in nutritional support. Proceed of Nutr Soci 1996; 55:945-61.
15. Wang YC, Yu RC, Chou CC. Antioxidative activities of soymilk fermented with lactic acid bacteria and bifidobacteria. Food Microbiol 2006; 23:128-35.
16. Suetsuna K, Ukeda H, Ochi H. Isolation and characterization of free radical scavenging activities peptides derived from casein. J Nutr Biochem 2000; 11:128-31.
17. Tong LM, Sasaki S, McClements DJ. Mechanisms of the antioxidant activity of a high molecular weight fraction of whey. J Agri & Food Chem 2000; 48:1473-8.
18. Karaman Ş, Tütem E, Başkan KS, Apak R. Comparison of total antioxidant capacity and phenolic composition of some apple juices with combined HPLC–CUPRAC assay. Food Chem 2010; 120:1201-9.
19. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Method in Enzym 1999; 299:152-78.
20. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agri & Food Chem 2005; 53: 1841-56.
21. Ardekani MRS, Khanavi M, Hajimahmoodi M, et al. Comparison of antioxidant activity and total phenol contents of some date seed varieties from Iran. Iran J Pharma Res 2010; 9:141-6.
22. Hernández-Ledesma B, Miralles B, Amigo L, et al. Identification of antioxidant and ACE-inhibitory peptides in fermented milk. J Sci Food & Agri 2005; 85: 1041-8.
23. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analyt Biochem 1996; 239:70-6.
24. Oveisi MR, Sadeghi N, Jannat B, et al. Human breast milk provides better antioxidant capacity than infant formula. Iran J Pharma Res: 2010; 9:445-49.
25. University Oberta, Barcelona, Revista Espanola do nutrition human dietary, accepted 8th March 2012, vol 161.
26. Torkibaghadorani S, Ehsani MR, Mirlohi M, et al. Oxidative stability of milk and soy milk fatty compound induced by Lactobacillus plantarum fermentation during refrigerated period. Biolog J Microorgan, 2015; 4:37-48.
27. Hernàndez-Ledesma B, Miralles B, Amigo L, et al. Identification of antioxidant and ACE-inhibitory peptides in fermented milk. J Sci Food & Agri 2005; 85:1041-8.
28. Ertan K, Bayana D, Gokce O, et al. Total antioxidant capacity and phenolic content of pasteurized and UHT-treated cow milk samples marketed in Turkey. Akademik Gıda 2017; 15:103-8
29. Calligaris, S, Manzocco L, Anese, M, et al. Effect of heat-treatment on the antioxidant and pro-oxidant activity of milk. Int Dairy J 2003; 14:421-7