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Comparison of antioxidant capacity of milk, defatted milk, whey, and deproteinized whey from cow, sheep, and goat, and effect of thermal treatments

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Abstract

Antioxidant potential of different milk types and thermal-treated milks may be of interest to milk processors, consumers, and nutritionists. The objectives of this study were comparison of the antioxidant potential of milk, defatted milk, whey, and deproteinized whey from cow, sheep, and goat, and also evaluation of the effect of thermal treatments (pasteurization and sterilization) on the antioxidant activity of the milk. The antioxidant potential of different milk samples and their fractions was examined using reducing power, 2,2-diphenyl-1-picrylhydrazyl, and 2,2-azinobis-3-ethylenzothiazoline-6-sulphonicacid methods. The results showed that the antioxidant potential of sheep raw milk was significantly higher than that of cow milk and goat milk. The results also indicated that thermal processing increased the reducing power and antioxidant potential of milk, and increasing heating temperature significantly increased reducing power and 2,2-diphenyl-1-picrylhydrazyl scavenging activity of milk, especially sheep milk and goat milk. Removing of whey proteins from whey of all animal species, particularly sheep, caused a significant decrease in the antioxidant potential of whey. The results of this study showed that sheep milk and its fractions are a good source of natural antioxidants, which may have higher health promotion effects on consumers from nutritional point of view.

Introduction

Food antioxidants not only retard the oxidation process in foods but also scavenge free radicals in human body and prevent from free radial-associated diseases.[1] Milk is one of the main sources of bioactive peptides with anti-cancer, anti-inflammatory, immunomodulatory, and antioxidant activities.[2] It contains a variety of antioxidants, including superoxide dismutase, catalase, glutathione peroxidase, biological peptides, and antioxidant amino acids, such as methionine and tryptophan.[3,4] Other milk components, including lactoferrin, vitamins (tocopherol and ascorbic acid), carotenoids, phenols, and selenium, have also antioxidant properties.[4-6] In addition, uric acid in cow milk acts as a water-soluble antioxidant.[7]

Milk antioxidants play a vitalrole in maintaining the quality of milk and its products, such as cheese.[8] Also, antioxidants in milk and dairy products play an essential role in preserving the nutritional value of proteins and lipids.[9] The antioxidants in milk and dairy products not only preserve the quality of the food but also have beneficial effects for consumers.[10] Some technological and processing methods like separation of milk fat (skimming) and thermal processing, may change the antioxidant capacity of milk and milk products. Therefore, in such products, the role of other stable antioxidants are crucial.[8]

Milk, as a rich nutrient source, provides an ideal environment for growth of pathogenic and spoilage micro-organisms.[11] Thermal processing (pasteurization and sterilization) is the most commonly used method to control microorganisms in milk, as well as preserve it for a long time. Pasteurization includes heating of milk at 63.00 ˚C for 30 min (low temperature for long time) or 72.00 ˚C for 15 sec (high temperature for short time). Ultra-high temperature sterilization process refers to heating of milk at 135 - 150 ˚C for 1 - 10 sec.[12] It has been shown that pasteurization processes cause minor damage to the flavor, color, bioactive compounds, and rheology of milk.[13] However, excessive heating conditions can result in adverse changes in milk, such as the denaturation of proteins, oxidation of lipids, and Maillard reactions, which further cause un-favorable flavors and colors in milk. Meanwhile, the loss of heat-sensitive bioactive compounds at higher temperatures results in a decrease in the nutritional value of milk.[14,15]

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Cow milk, sheep milk, and goat milk (especially in rural areas) are the most consumed dairy products. It has been shown that the milk of different species has many differences and similarities.[16] For example, goat milk differs in genetic polymorphisms, frequencies, and contents from cow milk,[17,18] and sheep milk has a higher viscosity than cow milk and it is very important in cheese making.[19] Goat milk has recently gained great popularity among researchers and consumers due to its bioactive peptides and proteins with antioxidant properties.[17]

In addition to cow milk, sheep milk and goat milk are consumed in some countries, such as Iran. Some studies have evaluated the antioxidant activity of cow milk, mainly using one or two methods. However, limited data are available regarding the antioxidant potential of sheep milk, goat milk, and their fractions. Therefore, the objectives of present study were evaluation and comparison of the antioxidant capacity of milk, defatted milk, whey, and deproteinized whey from cow, sheep, and goat, using different methods, and also examination of the effect of thermal treatments on the antioxidant activity of milk.

Materials and Methods

Sample preparation. Dairy cow (Holstein), sheep (Ghezel), and goat (Mahabadi) milk samples were obtained from the animal farm of Faculty of Agriculture, Urmia University, Urmia, Iran. The milk samples were taken from five animals from each group of animals. The samples were immediately transferred to the laboratory under sterile and cold conditions (4.00 ˚C).

Measurement of physicochemical properties of milk samples. Fat and protein contents of milk samples were measured using Milkoscan device (Milkotronic Co., Nova Zagora, Bulgaria). Also, the acidity was measured using the Dornick method and the lactose level of the samples was determined using the polarimetric method.[20]

Preparation of defatted milk. The milk of each species was poured separately into 10.00 mL Falcon tubes and then, centrifuged at 3,000 rpm at 4.00 ˚C for 30 min. The separated fat was collected in the upper part of the tube completely.[10] The defatted milk was divided into two parts; one part was stored as defatted milk and the other part was used to prepare whey.

Preparation of whey. To obtain whey, caseins were precipitated by adjusting the pH to 4.60 using an acidic solution. Acetic acid (10.00%) was added to the defatted milk, shaken for 15 sec and then, placed in a water bath (42.00 ˚C) for 15 min. After cooling, the mixture was centrifuged at 4000 rpm for 10 min. The supernatant (whey) was collected, and its pH readjusted to 6.80 using NaOH (Merck, Darmstadt, Germany).[20] The whey was divided into two parts; one part was stored as whey and the other part was used to prepare deproteinized whey.

Preparation of deproteinized whey. Trichloroacetic acid (20.00%; Merck) was added to whey and mixed for 30 sec to precipitate whey proteins. The mixture was incubated at 42.00 ˚C for 10 min. After cooling and centrifuging (4,000 rpm for 10 min), the supernatant was collected and the pH was readjusted to 6.80.[21]

Thermal treatment of the milk. For pasteurization, milk samples were kept in a water bath at 63.00 ˚C for 30 min and then, immediately cooled in cold water. For sterilization, the samples were placed in an autoclave (121 ˚C) for 10 min (retort sterilization). Next, the samples were rapidly cooled on ice water and subjected to analysis.[22]

Reducing power determination. The sample (500 µL) was mixed with 2.50 mL of sodium phosphate buffer (pH: 6.60; Merck) and 2.50 mL of potassium ferricyanide (1.00%, Sigma-Aldrich, Steinheim, Germany) and then, placed in a water bath (50.00 ˚C) for 20 min. After that, 2.50 mL of trichloroacetic acid (10.00%) was added and it was centrifuged at 4,000 rpm for 10 min. Then, 2.50 mL of the supernatant was mixed with 2.50 mL of distilled water and 0.50 mL of ferric chloride (0.10%, Sigma-Aldrich). After 10 min, the absorbance was read at 700 nm using a spectrophotometer (Novaspec II; Pharmacia LKB, Uppsala, Sweden).[23]

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (RSA). The DPPH RSA was determined according to the method described by Bučević-Popović *et al*., and Aliakbarlu *et al.*[20,24] First, 100µL of the sample was mixed with 2.00 mL of DPPH solution (24.00 µg mL^{-1}; Sigma-Aldrich). Then, the mixture was incubated in the dark for 30 min. The discoloration of purple DPPH solution was measured at 517 nm using a spectro-photometer. The results were expressed as RSA percentages using the following equation:

$$RSA\ (\%) = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where, A_{blank} and A_{sample} were the absorbances of the blank and sample, respectively.

2,2-azinobis-3-ethylenzothiazoline-6-sulphonic acid (ABTS) RSA. The ABTS radical scavenging capacity of the samples was examined according to the method of Chen *et al.*[10] To prepare the ABTS stock solution, an equal volume of ABTS (7.00 mM; Sigma-Aldrich) and potassium persulfate (2.45 mM; Sigma-Aldrich) were mixed and incubated for 16 hr at room temperature. Then, the solution was diluted using ethanol to reach an absorbance of 0.70 ± 0.03 at 734 nm. After that, 100µL of the sample was added to 1.00 mL of ABTS solution and mixed. Next, the reaction mixture was allowed to stand in the dark for 6 min. Finally, the absorbance was recorded at 734 nm using a spectrophotometer. The ABTS RSA (%) was calculated using the formula described in the DPPH method.

Statistical analysis. All experiments were conducted in three replications. Data were analyzed using SPSS Software (version 18.0; IBM Corp., Armonk, USA). Two-way ANOVA and Turkey's *post hoc* tests ($p < 0.05$) were used to compare the means.

Results

Physicochemical characteristics of milk. As shown in Table 1, the percentages of fat (5.95%) and protein (6.00%) in sheep milk were higher than those in cow milk and goat milk. Also, cow milk had higher acidity than sheep milk and goat milk. The percentage of lactose (4.60%) in cow milk was higher than that in sheep milk and goat milk.

Antioxidant activity. Reducing powers of raw, pasteurized, and sterilized milk samples are shown in Table 2. Among the three species tested, sheep milk had the highest reducing power ($p < 0.05$). This may be due to the higher contents of fat and protein in sheep milk. Meanwhile, reducing power of raw goat milk was significantly ($p < 0.05$) higher than that of raw cow milk. Although sterilization significantly ($p < 0.05$) increased the reducing power of all milk samples, pasteurization had no significant effect on the reducing power. Table 2 also shows the reducing power of milk fractions. Similarly, sheep milk fractions (defatted milk and whey) had the highest reducing power. Whey of goat milk showed no reducing power; so, it can be concluded that casein is the main antioxidant component of goat milk. The DPPH radical scavenging activities of different milk samples and their fractions are presented in Table 3. It was found that raw sheep milk had the highest RSA, and sterilization significantly ($p < 0.05$) increased the anti-oxidant activity of all milk samples. However, pasteurization had no significant effect on DPPH RSA of milk. The fractions of sheep milk and goat milk showed the highest and the lowest RSA, respectively. The ABTS radical scavenging activities of milk samples and their fractions are given in Table 4. Among raw milk samples, the highest ABTS RSA was found in goat milk and sheep milk with no significant difference between them. Pasteurization significantly ($p < 0.05$) increased only RSA of sheep milk. Meanwhile, radical scavenging activities of sterilized milk of sheep and cow were significantly higher than those of pasteurized milk. Sterilization increased the radical scavenging activities of all three milk samples, but no significant difference was observed among radical scavenging activities of sterilized milk of the three species. It seems that fat content had no significant effect on ABTS RSA of the samples. As shown in Table 4, ABTS RSA of defatted milk of sheep and goat was equal. However, sheep milk whey showed the highest RSA. This is may be due to the bioactive peptides and amino acid contents of sheep whey proteins. Meanwhile, deproteinized whey samples showed a low level of RSA.

Table 2. Reducing power of the treated milks and milk fractions of different ruminants.

Animals	Treatments		
	Raw milk	Pasteurized milk	Sterilized milk
Cow	0.13 ± 0.02^{Ba}	0.14 ± 0.02^{Aa}	0.58 ± 0.02^{Ab}
Sheep	0.34 ± 0.03^{Cb}	0.21 ± 0.01^{Ba}	1.30 ± 0.09^{Bc}
Goat	0.08 ± 0.01^{Aa}	0.13 ± 0.02^{Aa}	1.31 ± 0.09^{Bb}
	Milk fractions		
	Defatted milk	**Whey**	**Deproteinized whey**
Cow	0.08 ± 0.03^{Ab}	0.08 ± 0.03^{Bb}	0.00^{Aa}
Sheep	0.22 ± 0.02^{Bc}	0.17 ± 0.02^{Cb}	0.04 ± 0.01^{Ba}
Goat	0.08 ± 0.02^{Ab}	0.00^{Aa}	0.00^{Aa}

^{ABC} Different uppercase letters in the same column for each treated milk or milk fraction show statistical differences ($p < 0.05$), and ^{abc} Different lowercase letters in the same row show statistical differences ($p < 0.05$).

Table 3. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (%) of treated milks and milk fractions of different ruminants.

Animals	Treatments		
	Raw milk	Pasteurized	Sterilized
Cow	19.16 ± 0.08^{Ba}	21.81 ± 0.15^{Ba}	43.11 ± 1.56^{Ab}
Sheep	24.62 ± 1.21^{Ca}	25.82 ± 1.19^{Ca}	80.41 ± 2.54^{Cb}
Goat	13.34 ± 0.27^{Aa}	16.17 ± 0.30^{Ab}	73.12 ± 1.04^{Bc}
	Milk fractions		
	Defatted milk	**Whey**	**Deproteinized whey**
Cow	13.41 ± 0.17^{Ab}	13.35 ± 0.08^{Bb}	6.23 ± 0.92^{Ca}
Sheep	25.33 ± 0.35^{Bc}	18.89 ± 0.80^{Cb}	4.27 ± 0.46^{Ba}
Goat	11.40 ± 1.37^{Ac}	7.43 ± 0.19^{Ab}	0.17 ± 0.08^{Aa}

^{ABC} Different uppercase letters in the same column for each treated milk or milk fraction show statistical differences ($p < 0.05$), and ^{abc} Different lowercase letters in the same row show statistical differences ($p < 0.05$).

Table 4. The 2,2-azinobis-3-ethylenzothiazoline-6-sulphonicacid (ABTS) radical scavenging activity (%) of the treated milks and milk fractions of different ruminants.

Animals	Treatments		
	Raw milk	Pasteurized	Sterilized
Cow	48.66 ± 3.27^{Aa}	47.99 ± 2.81^{Aa}	68.46 ± 3.22^{Ab}
Sheep	59.57 ± 3.47^{Ba}	66.44 ± 3.14^{Bb}	74.35 ± 0.96^{Ac}
Goat	61.31 ± 3.22^{Ba}	65.60 ± 3.94^{Bab}	73.58 ± 4.20^{Ab}
	Milk fractions		
	Defatted milk	**Whey**	**Deproteinized whey**
Cow	60.34 ± 2.72^{Ac}	52.24 ± 3.59^{Ab}	9.41 ± 1.54^{Aa}
Sheep	64.60 ± 2.54^{ABb}	68.44 ± 1.67^{Cb}	7.97 ± 0.82^{Aa}
Goat	65.79 ± 2.99^{Bc}	59.52 ± 2.32^{Bb}	9.98 ± 1.40^{Aa}

^{ABC} Different uppercase letters in the same column for each treated milk or milk fraction show statistical differences ($p < 0.05$), and ^{abc} Different lowercase letters in the same row show statistical differences ($p < 0.05$).

Table 1. Physicochemical characteristics of milk samples from different ruminants.

Milk Sample	Fat (%)	Protein (%)	Lactose (%)	pH	Acidity (Dornic)
Cow	3.60 ± 0.70^{b}	2.90 ± 0.10^{c}	4.60 ± 0.30^{a}	6.30 ± 0.00^{a}	17.50 ± 0.70^{a}
Sheep	5.95 ± 0.10^{a}	6.00 ± 0.10^{a}	4.09 ± 0.40^{a}	6.67 ± 0.40^{a}	15.00 ± 0.00^{b}
Goat	3.85 ± 0.10^{b}	4.20 ± 0.00^{b}	4.02 ± 0.30^{a}	6.65 ± 0.10^{a}	15.50 ± 0.70^{b}

^{abc} Values for fat and protein are on a fresh weight basis. Different small letters in each column show statistical differences ($p < 0.05$).

Discussion

The results of chemical composition of the milk samples are consistent with the results reported in the previous works.[20,25] It was also reported that the fat and protein contents of sheep milk were higher than those of cow milk and goat milk.[26] In the present study, the geographic conditions, season, management, breed, and diet were the same for all animals. However, other factors, such as stage of lactation and individual animal genetics, affect milk composition.[20]

According to the results, sheep milk with higher fat content showed the highest reducing power. It was reported that the total antioxidant capacity of milk was increased with increasing fat content in milk, which might be due to the reactivity of lipid-soluble antioxidants (vitamins A and E and β-carotene) and fat globule membrane proteins.[10] However, in the case of goat milk, there was no significant difference between the reducing power of raw whole milk and defatted milk, indicating a slight contribution of lipid-soluble antioxidants in reducing power of goat milk. Another study showed that casein was the main contributor to the total antioxidant activity of whole milk.[21]

Unlike pasteurization, sterilization enhanced the reducing power of all milk samples. This finding is in agreement with the results of other researchers reported that the antioxidant potential of milk did not alter after mild heating (< 100 ˚C for 1 min), but more severe heat processing increased the antioxidant activity due to the Maillard reaction and formation of brown melanoidins possessing antioxidant capacity.[21,27] It was shown that Maillard reaction products had antioxidant activity and could retard peroxide formation.[28]

Our results suggest that casein is the major antioxidant component of goat milk. In line with that, a previous study reported that casein had major contribution in the total antioxidant capacity of milk.[21] Similarly, it was shown that the difference in the antioxidant activity between defatted milk and whey was due to the reactivity of caseins.[10] Removing whey proteins from whey decreases the reducing power. Whey contains several proteins, such as β-lactoglobulin, and α-lactalbumin, making up approximately 70.00 - 80.00% of the total protein, as well as proteoso-peptones, immunoglobulins, and serum albumin.[21] It was reported that albumin had major role in the antioxidant activity of whey.[21] Meanwhile, it was claimed that the antioxidant capacity of deproteinized whey was due to the water-soluble antioxidant compounds, such as vitamin C and uric acid.[21]

Sheep milk whey showed higher DPPH radical scavenging activity. The higher ability of sheep milk whey to scavenge DPPH radicals could be due to the higher content of some amino acids.[29] Another study reported that DPPH RSA of sheep whey protein was higher than that of cow whey protein. Sheep whey protein also showed greater iron-reducing power than cow whey protein.[30]

It was also reported that the sterilized milk of different species did not have different ABTS RSA, being in accordance with our finding.[31] The heat caused by pasteurization has been shown to have an important role in increasing the antioxidant potential of dairy products.[32]

Milk is a diverse source of molecules and antioxidant compounds, including tocopherols, carotenoids, ascorbate, low-weight thiols, and phenols.[8,26] The antioxidant compounds of milk not only play a crucial role in the preservation of milk and dairy products but also have a significant role in the health promotion of the consumers.[9] Due to the fact that milk and dairy products are one of the most important foods in human diet, the study of their antioxidant potential is very important both in terms of nutritional importance and effects on shelf life. Therefore, various studies have been conducted on the antioxidant activity of milk and milk products. The results of a study showed that the type of milk (cow or ewe) had a significant effect on the antioxidant potential of obtained kefir.[33] A part of the antioxidant potential of milk is related to the whey proteins, such as β-Lactoglobulin found in different types of milk, especially sheep milk.[34] The antioxidant properties of whey proteins can be due to the peptides and sulfur-containing amino acids, like methionine and cysteine.[35] However, it was shown that whey had lower antioxidant potential, but heating the whey may increase its antioxidant activity by 20.00 - 50.00%.[10]

The results of our study showed that, in general, sheep milk had higher antioxidant potential than goat milk and cow milk. Meanwhile, the fat content of milk might have an effect on the antioxidant capacity of milk due to the fat-soluble vitamins (A, D, E, and β-carotene).[36] According to the results of present study, the protein and fat content of sheep milk was higher than others. Regarding the antioxidant potential of goat milk, it was reported that the hydrolysates of casein and whey proteins of goat milk showed potent RSA.[17]

This study investigated the antioxidant activity of milk, defatted milk, whey, and deproteinized whey from cow, sheep, and goat, and evaluated the effect of thermal treatments on the antioxidant capacity of milk. The results showed that the sterilized milk had the highest antioxidant activity among treated milk samples. This may be due to the Maillard reaction products (melanoidin) with antioxidant capacity. It was also found that the defatted milk had higher activity than whey and deproteinized whey. This indicates the role of casein in the antioxidant capacity of milk. Whey proteins had also a significant role in the antioxidant activity; so, the deproteinized whey showed the lowest antioxidant activity. In general, sheep milk and its fractions exhibited the highest antioxidant potential, which may be related to their higher fat and protein

contents. Thus, sheep milk and its products might have a suitable health-promoting effect on consumers from an antioxidant perspective. Sheep milk can also be considered to develop new functional foods.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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