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Research Article

Antioxidant Properties of Milk: Effect of Milk Species, Milk Fractions and Heat Treatments

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Abstract

Background and Objective: Oxidative stress is a term denoting an imbalance between the production of oxidants and the respective defense systems of an organism. Oxidants include reactive oxygen species (ROS), reactive nitrogen species (RNS) and others. Oxidative stress is deemed a causative factor of neurodegenerative disorders, cancer, liver injury, aging, diabetes, chronic pancreatitis and cardiovascular disease. In this study, the antioxidant activity of different milk species was investigated. Also, which milk components are responsible for antioxidant activity were also determined. In addition, the effect of pasteurization or sterilization on the antioxidant capacity of milk was studied. **Material and Methods:** The antioxidant activity of 14 different samples of cow, buffalo, goat, sheep and camel milk either raw or heat-treated by pasteurization or sterilization was investigated using DPPH radical scavenging activity, metal chelating activity and reducing power. **Results:** The results showed that sheep milk exhibited the strongest DPPH radical scavenging and metal chelating activities, while buffalo and sheep milk presented had the highest reducing power. Antioxidant activity of all milk fractions was lower than that of whole milk. Moreover, skim milk had the highest antioxidant capacity, while deproteinized milk was the lowest. Pasteurization did not affect the antioxidant activity of different types of milk. Sterilization led to increase the antioxidant activity of milk from different species. **Conclusion:** These findings indicated that sheep and buffalo milk showed the greatest antioxidant properties compared to other types of milk. Also, pasteurization did not affect the antioxidant activity of milk, while sterilization had positive effect on the antioxidant activity.

Key words: Antioxidant properties, different milk species, heat treatment, radical scavenging activity, pasteurization, dairy products

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Oxidative stress is a term denoting an imbalance between the production of oxidants and the respective defense systems of an organism. Oxidants include reactive oxygen species (ROS), reactive nitrogen (RNS) species, sulfur-centered radicals and others. Oxidative stress is deemed a causative factor of neurodegenerative disorders, cancer, liver injury, aging, diabetes, chronic pancreatitis and cardiovascular disease¹⁻⁵. Also, it is well-known that lipid oxidation occurring in food products causes deteriorations in food quality including rancid flavor and shortening of shelf life⁶. To prevent foods from deterioration and to protect the body from serious diseases, it is important to inhibit the oxidation of lipids and formation of oxidants occurring in the body cells and foodstuffs.

Antioxidants are molecules that scavenge or neutralize the free radicals and prevent the oxidation of other molecules or oxidative stress⁷. Synthetic antioxidants display strong antioxidant activity against several oxidation systems. However, because synthetic antioxidants pose toxic and carcinogenic effects, their use is restricted or prohibited in several countries^{8,9}. In recent years, consumers have more attentions and recommendations to use natural antioxidants from food sources rather than synthetic antioxidants. In this scenario, food antioxidants have been widely studied for their positive effects on human health, mainly due to prevent the oxidative stress.

Milk and dairy products are one of the most interesting and promising foods concerning its potential antioxidant capacity, due to the wide variety of antioxidant factors. Milk antioxidants, both lipophilic (carotenoids, α -tocopherol, vitamins A and D₃, phospholipids and coenzyme Q₁₀) and hydrophilic antioxidants (caseins, whey proteins, peptides, vitamins, minerals, low molecular weight thiols and trace elements) play a main role in maintaining pro-oxidant and antioxidant homeostasis in oxidation systems¹⁰. The antioxidant capacity of casein subunits (α -casein, β -casein and κ -casein) and whey proteins may be due to its ability to inhibit thiobarbituric reactive substances (TBARS) and lipid peroxide formation^{11,12}. Other antioxidant components can act as radical scavengers and metal ion binders¹⁰. Furthermore, milk includes various antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase as well as considerable amounts of phenolic compounds such as phenol, cresol, thymol and carvacrol^{13,14}.

To reduce the microbiological content and to extend shelf life, milk was usually undergone to different heat treatments. These treatments can be responsible for different effects on

the quality properties of milk including development of undesired color and flavor, enzyme inactivation, depletion of nutrient bioavailability and antioxidants¹⁵. Moreover, it has been reported that milk antioxidant capacity may increase as a consequence of heat treatments. Based on the intensity of the thermal treatment applied, pro-oxidant or antioxidant components are foreseeable to be generated¹⁶. It is likely that during milk heating, the formation of such components could be responsible for different and sometimes opposite, effects on the overall antioxidant activity¹².

There is a lack of studies that have examined the antioxidant capacity of milk from different dairy species and this study is the first dealing with the antioxidant properties of main different five milk species that are consumed by human and compare among them. As a consequence, the objective of this research was to compare the antioxidant activity among cow, buffalo, goat, sheep and camel milk and to study the influence of pasteurization and sterilization on the milk antioxidant activity. Also, which milk components are responsible for antioxidant activity were determined.

MATERIALS AND METHODS

Materials: The study was carried out from September, 2018 to February, 2019. The milk samples of cows ($n = 36$) and buffaloes ($n = 18$) were collected (1 L each) from open nucleus herd belonging to the Cattle Information System of Egypt (CISE) and Dairy Technology Unit, Faculty of Agriculture, Cairo University. Milk samples of goats ($n = 15$), sheep ($n = 15$) and camels ($n = 20$) were collected (250 mL each) from local farms. Animals were fed with green and concentrated fodders (16% protein) and straw. The lactation period of cows and buffaloes, goats, sheep and camels are 286, 180, 112 and 480 days, respectively. Immediately after sampling, each sample was transferred at 2-4°C to the laboratory of food additives department of the Regional Center for Food and Feed at the Agricultural Research Center, Egypt for analysis.

Ferrous and ferric chloride, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), sodium 4-[3-pyridin-2-yl-5-(4-sulfophenyl)-1,2,4-triazin-6-yl] benzene sulfonate (Ferrozine), trichloroacetic acid (TCA) and potassium ferricyanide were purchased from Sigma-Aldrich (Egyptian International Centre for Import, Cairo, Egypt).

Milk fractions preparation: Milk fractions were prepared according to Zulueta *et al.*¹⁷. Whole milk samples were adjusted to room temperature and were diluted (1:500).

Skim milk was obtained by centrifuge the whole milk at 4500 rpm for 20 min and it was removed the upper layer of

cream. To obtain milk fat globule membrane (MFGM), cream was centrifugated with washing for 4 times. The obtained cream was churned to get MFGM. Skim milk and MFGM were diluted (1:500).

Whey was obtained by adding 0.5 mL acetic acid (10%) to 9.5 mL of skim milk placed in a tube with a screw cap. The mixture was shaken for 30 sec and then incubated at 42°C for 10 min, cooled at 4°C and centrifugated at 750 rpm for 15 min. About 1 mL of supernatant was transferred into a 50 mL volumetric flask and brought to volume with phosphate buffer solution (0.2 M, pH 6.6). About 1 mL of this solution was diluted 1/5 (v/v) with phosphate buffer to obtain a final dilution of 1/263.16.

To get deproteinized milk, 10 mL TCA (20%) was added to 10 mL skim milk placed in a test tube with a screw cap. After shaking for 30 sec, the mixture was incubated at 42°C for 10 min to remove all milk proteins. After cooling (4°C) and centrifuging at 750 rpm for 15 min, 1 mL of supernatant was transferred into a 25 mL volumetric flask and brought to volume with phosphate buffer solution. A successive 1/5 dilution was performed before sample analysis to obtain a final dilution of 1/250.

Heat treatments of different milk species: Milk samples from different dairy species were exposed to pasteurization at 63°C for 30 min or sterilization at 115°C for 20 min and stored at 2-4°C for analysis.

Physicochemical properties of milk: The total solids (TS), protein, fat and lactose contents of milk were determined according to the methods described in the Association of Official Analytical Chemists methods¹⁸. The pH values of milk were measured using a digital pH meter with a glass electrode (Jenway 3305, Jenway Limited, Essex, England).

Scavenging of DPPH free radical: The DPPH radical scavenging activity was measured using the method of Son and Lewis¹⁹. DPPH radical solution (0.004%, w/v) in 95% ethanol was prepared. A volume of 2 mL of this solution was added to 2 mL of sample, well vortexed and incubated for 30 min in dark room at room temperature. Absorbance of each sample at 517 nm was measured using spectrophotometer (T80 UV-Vis Spectrophotometer). Ethanol was used as a blank, while DPPH solution in ethanol served as control. The antioxidant activity was expressed as percentage of DPPH activity calculated as¹⁹:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_0 - A_s}{A_0} \times 100$$

where, A_0 is the absorbance at 517 nm of blank A_s is the absorbance at 517 nm of sample.

Metal chelating activity: The ability of different samples to chelate ferrous ions was assessed using the method of Decker and Welch²⁰. About 1 mL of diluted sample was first mixed with 3.7 mL of distilled water. A solution of 2 mM ferrous chloride (0.1 mL) was added and after 3 min the reaction was inhibited by the addition of 5 mM ferrozine (0.2 mL). The mixture was shaken vigorously and left at room temperature for 10 min. Optical density of the reaction mixture was measured at 562 nm. A blank without sample was prepared in a similar manner. The chelating capacity was calculated as a percentage using the following formula²¹:

$$\text{Fe}^{2+} \text{ chelating activity (\%)} = \frac{A_0 - A_s}{A_0} \times 100$$

where, A_0 is the absorbance at 526 nm of blank A_s is the absorbance at 562 nm of sample.

Reducing power: Reducing power of the milk samples was determined according to Yen and Chen²². A sample (2.5 mL) was mixed with 2.5 mL of potassium ferricyanide (1%), followed by incubation at 50°C for 20 min. After incubation, 2.5 mL trichloroacetic acid solution (10%) was added and centrifuged at 1000 rpm for 10 min. A supernatant (2.5 mL) was mixed with same volume of deionized water and 0.5 mL of ferric chloride (0.1%) was added. Absorbance was recorded at 700 nm on a double beam spectrophotometer.

Statistical analysis: Three replicates from each parameter were statistically analyzed and the data were recorded as the mean \pm standard deviation (SD). The Mstat-C software was used to carry out both randomize complete block design and the analysis of variance of factorial methods. The calculation of least significant differences (LSD) at $p \leq 0.05$ was used to compare the significant differences between the mean of different treatments.

RESULTS AND DISCUSSION

Physicochemical properties of milk: Physicochemical properties of cow, buffalo, goat, sheep and camel milk are listed in Table 1. The pH of different types of milk was ranged from 6.52-6.72. Milk from sheep or buffaloes significantly had the highest content of fat, protein and total solids. However, goat and camel milk significantly exhibited lower content

Table 1: Physicochemical composition (%) of different types of milk

Milk species	pH	Fat	Protein	Lactose	Ash	Total solids
Cow	6.60±0.01 ^b	3.94±0.10 ^b	3.39±0.11 ^b	4.64±0.10 ^a	0.80±0.006 ^a	12.78±0.31 ^b
Buffalo	6.72±0.02 ^a	5.80±0.11 ^a	4.36±0.12 ^a	4.52±0.09 ^b	0.80±0.06 ^a	15.07±0.70 ^a
Goat	6.52±0.006 ^c	3.20±0.09 ^d	3.15±0.05 ^c	4.01±0.10 ^d	0.83±0.04 ^a	11.20±0.12 ^c
Sheep	6.65±0.01 ^b	5.71±0.21 ^a	4.50±0.10 ^a	4.20±0.08 ^c	0.85±0.005 ^a	15.27±0.51 ^a
Camel	6.55±0.02 ^c	3.44±0.02 ^c	2.99±0.09 ^c	4.18±0.02 ^c	0.71±0.015 ^b	11.26±0.22 ^c
LSD	0.0262	0.1331	0.1684	0.05954	0.05954	0.5489

Values are Means±Standard deviation, values in the same column with different superscript letters differ significantly (p<0.05)

Table 2: DPPH radical scavenging activity (%) of different types of milk and their fractions

Milk species	Milk fractions					LSD
	Whole milk	Skim milk	Whey	MFGM	Deproteinized milk	
Cow	18.89±0.078 ^{Ab}	9.05±0.49 ^{Bc}	9.60±0.55 ^{Bbc}	7.30±0.19 ^{Cbc}	3.40±0.4 ^{Db}	0.7022
Buffalo	20.11±0.99 ^{Ab}	10.95±1.55 ^{Bbc}	9.30±0.71 ^{Bc}	6.20±1.04 ^{Cc}	2.36±0.63 ^{Dc}	1.8878
Goat	18.17±1.02 ^{Ab}	12.55±1.05 ^{Bb}	11.45±1.55 ^{Bb}	9.25±1.01 ^{Ca}	3.40±0.36 ^{Db}	2.0474
Sheep	27.28±2.1 ^{Aa}	23.63±1.19 ^{Ba}	14.84±0.9 ^{Ca}	7.20±0.49 ^{Dbc}	4.30±0.03 ^{Ea}	2.1351
Camel	18.57±1.88 ^{Ab}	12.42±1.25 ^{Bb}	11.45±1.45 ^{Bb}	7.87±0.55 ^{Cab}	3.45±0.11 ^{Db}	3.1494
LSD	3.0817	2.6098	1.9079	1.5127	0.6999	

Values are Means±Standard deviation, MFGM: Milk fat globule membrane, different capital and small superscript letters represent significant differences in the row and column, respectively (p<0.05)

Table 3: Metal chelating activity (%) of different types of milk and their fractions

Milk species	Milk fractions					LSD
	Whole milk	Skim milk	Whey	MFGM	Deproteinized milk	
Cow	14.55±1.11 ^{Ac}	12.87±2.50 ^{ABab}	10.60±1.40 ^{Ba}	6.90±0.11 ^{Cab}	2.47±0.76 ^{Dabc}	2.5768
Buffalo	17.63±0.93 ^{Ab}	14.20±1.59 ^{Bab}	11.50±0.99 ^{Ca}	7.55±0.95 ^{Da}	2.40±1.25 ^{Ebc}	2.2159
Goat	12.83±0.89 ^{Ac}	11.30±1.01 ^{ABb}	10.23±1.50 ^{Ba}	6.30±0.10 ^{Cb}	3.06±0.11 ^{Da}	1.6405
Sheep	20.19±1.10 ^{Aa}	14.97±2.60 ^{Ba}	9.58±0.76 ^{Ca}	6.54±0.99 ^{Dab}	2.01±0.01 ^{Ec}	2.5140
Camel	12.95±1.10 ^{Ac}	11.17±1.76 ^{ABb}	10.63±1.82 ^{Ba}	6.60±0.1 ^{Cab}	2.90±0.01 ^{Dab}	2.2469
LSD	1.8747	3.6058	2.4522	1.1301	0.6163	

Values are Means±Standard deviation, MFGM: Milk fat globule membrane, different capital and small superscript letters represent significant differences in the row and column, respectively (p<0.05)

Table 4: Reducing power (A_{700nm}) of different types of milk and their fractions

Milk species	Milk fractions					LSD
	Whole milk	Skim milk	Whey	MFGM	Deproteinized milk	
Cow	0.0643±0.0025 ^{Ab}	0.0593±0.0091 ^{Aa}	0.0495±0.0095 ^{Aa}	0.0457±0.0005 ^{ABa}	0.0240±0.001 ^{Bb}	0.0230
Buffalo	0.0820±0.0023 ^{Aa}	0.0560±0.006 ^{Bab}	0.0520±0.0011 ^{BCa}	0.0465±0.0005 ^{BCa}	0.0335±0.0022 ^{Ca}	0.0208
Goat	0.0483±0.006 ^{Ab}	0.0460±0.0046 ^{ABb}	0.0403±0.0042 ^{BCab}	0.0350±0.0009 ^{Cc}	0.0253±0.0005 ^{Db}	0.0076
Sheep	0.0653±0.0068 ^{Ab}	0.0583±0.004 ^{ABa}	0.0513±0.0006 ^{Ba}	0.0390±0.0014 ^{Cb}	0.0223±0.0027 ^{Db}	0.0077
Camel	0.0373±0.0005 ^{Ab}	0.0330±0.0014 ^{Bc}	0.0310±0.001 ^{Bb}	0.0237±0.001 ^{Cd}	0.0133±0.001 ^{Dc}	0.0021
LSD	0.0283	0.0105	0.0125	0.0019	0.0034	

Values are Means±Standard deviation, MFGM: Milk fat globule membrane, different capital and small superscript letters represent significant differences in the row and column, respectively (p<0.05)

of fat, protein, lactose and total solids than those of other milk types. These results are in consistent with the results of Niero *et al.*²³, who found that buffalo and sheep milk showed the highest protein, fat and casein percentages compared to cow and goat milk. Furthermore, Yoganandi *et al.*²⁴ reported that camel milk had lower total solids, fat, solids non-fat and protein contents as compared to cow and buffalo milk.

Antioxidant activity of different milk species: Antioxidants have different modes of action, thus it is preferable to utilize

a combination of assays in the evaluation of antioxidant activity. Hence, the present study evaluated the antioxidant capacity of milk from different species using DPPH radical scavenging activity, metal chelating activity and ferric reducing power and the results are shown in Table 2, 3 and 4.

Among the used types of milk, sheep milk significantly exhibited the highest DPPH radical scavenging activity (27.28%) and metal chelating activity (20.19%), followed by milk from buffaloes (20.11 and 17.63%, respectively). However, buffalo and sheep milk significantly showed the highest

reducing power values as it was 0.082 and 0.065, respectively. Moreover, obtained results revealed that no significant differences in the antioxidant properties among cow, goat and camel milk. Pihlanto⁶, Noziere *et al.*²⁵ and Chauveau-Duriot *et al.*²⁶ reported that total antioxidant activity (TAA) of milk depends on casein, whey proteins, milk fat fraction containing tocopherols, retinol and carotenoids as well as water-soluble compounds such as phenols, thiol groups and ascorbate. Thus, the observed variations in antioxidant activity may be due to the specific-specific milk chemical composition where sheep and buffalo milk had greater total solids, fat and protein percentage as shown in Table 1. Chen *et al.*²⁷ observed greater TAA in milk with greater fat percentage. In this respect, Niero *et al.*²³ found that sheep milk had the greatest TAA, averaging 7.78 mmol L⁻¹ of trolox equivalents (TE). Also, they observed that TAA of buffalo milk was lower (7.35 mmol L⁻¹ of TE) than that of sheep milk. Khan *et al.*²⁸ observed that TAA, DPPH radical scavenging activity and reducing power of buffalo milk was more than cow milk and they attributed that buffalo milk had higher concentration of vitamin C and E, selenium, zinc, tyrosine, cysteine and flavonoids which have antioxidant properties. Moreover, Niero *et al.*²³ noted that value of TAA for goat milk was similar to that of cow milk, having almost comparable chemical composition. The results of this study are in contrast with the results of Simos *et al.*²⁹, who reported that antioxidant potential of milk from Prisca goats was higher (66.70 mM α -tocopherol) than milk from cows (42.90 mM α -tocopherol).

Antioxidant activity of milk fractions: It would be valuable to highlight milk fractions which substantially contribute to the antioxidant capacity of milk. Hence, the antioxidant properties of milk fractions (skim milk, whey, MFGM and deproteinized milk) from the used types of milk were determined and are presented in Table 2, 3 and 4. The differences between whole and skim milk for the antioxidant activity values were statistically significant, suggesting that milk fat substantially influence antioxidant properties. Also, it was observed that with an increasing fat content in milk, antioxidant activity boosted significantly, which could be due to an involvement of lipids and the reactivity of lipid soluble antioxidants as well as the fat globule membrane proteins. Antioxidant activity of sheep skim milk was significantly the highest, whilst antioxidant activity of cow (DPPH radical scavenging activity) and camel skim milk (metal chelating activity and reducing power) was significantly the lowest. Chen *et al.*²⁷ illustrated that even if skim milk is deprived of several lipophilic antioxidants, milk soluble fraction containing other powerful antioxidant components may result in a more concentrated

ratio v/v as a result of the skimming process. Cervato *et al.*¹¹ and Zulueta *et al.*¹⁷ showed that the components of milk that supply the highest antioxidant activity are the casein fractions. It has been suggested that their free radical quenching is attributed to the oxidation of amino acid residues of the caseins themselves. This activity cannot be substituted by free amino acids because it is the primary structure of casein itself plays a determining role³⁰.

Dairy products obtained from whey have received considerable interest for their nutritional, health-promoting and functional values. Whey proteins are widely used in various foods for their antioxidant activity and other functional properties. Thus, the antioxidant capacity of whey from different origins was studied. The DPPH radical scavenging, metal chelating and reducing power values of whey obtained from different types of milk are much lower than the respective milk values. This may be affirmed that the main contribution of antioxidant capacity results from caseins in milk. These results attributed to the higher content of antioxidant amino acids such as tryptophan, histidine, methionine, lysine and tyrosine. Also, the antioxidant activity of whey is derived from soluble proteins, represented mainly by β -lactoglobulin, α -lactalbumin, immunoglobulins and serum albumins³¹. Bertucci *et al.*³² and Cruz-Huerta *et al.*³³ identified several peptides from α -lactalbumin and β -lactoglobulin within different location hotspots exhibiting antioxidant properties. Hernandez-Ledesma *et al.*³⁴ found that the peptide sequence (Trp-Tyr-Ser-Leu-Ala-Met-Ala-Ala-Ser-Asp-Ile) in β -lactoglobulin has a free radical scavenging activity. From a GSH precursor perspective, β -lactoglobulin has 5 Cys residues, four of them involved in disulfide bonds with the remaining one having a free reactive thiol group³⁵. Also, whey contains lactoferrin, an iron-binding monomeric globular glycoprotein, that can bind 2 Fe³⁺ ions with a binding affinity of 10-20 M and thus it possess antioxidant capacity^{36,37}. These findings are in agreement with those obtained by Zulueta *et al.*¹⁷, who found that the total antioxidant capacity of whey was lower than the whole milk. Furthermore, whey origin may play a role in antioxidant capacity where obtained data indicated that sheep whey significantly had the highest DPPH radical scavenging activity and reducing power, while whey from cow and camel milk significantly exhibited the lowest compared to whey from other origins. On the other hand, no significant differences were observed in metal chelating activity among whey from different sources. The results of study correspond to Kerasioti *et al.*³⁸ reported that sheep whey protein had DPPH radical scavenging activity and iron-reducing power higher than that of bovine whey protein. Salami *et al.*³⁹ found that camel whey protein showed 40%

higher antioxidant activity by 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) than that of bovine whey protein.

Milk fat globule membrane (MFGM) has attracted attention as a source of high added-value ingredients⁴⁰. Comparing with whole milk from different sources, MFGM significantly exhibited low values of antioxidant properties (Table 2, 3 and 4). The MFGM obtained from goat milk significantly had the highest DPPH radical scavenging activity whereas MFGM obtained from buffalo was significantly the lowest. However, an opposite trend was observed in case of metal chelating activity. Reducing power of MFGM obtained from buffalo milk was significantly the highest, whilst camel-MFGM significantly showed the lowest. In this regard, Conway *et al.*⁴¹ reported that MFGM proteins are most likely responsible for oxygen radical absorbance and metal chelating capacities. Chen *et al.*²⁷ suggested the contribution of MFGM proteins to the antioxidant capacity of MFGM. Also, they found a significant increase in the free radical scavenging capacity of milk in proportion with the milk fat and thus MFGM content. Moreover, high content of polar lipids may too play a role in the superior antioxidant capacity found in MFGM. In addition, the protective action of gangliosides (glycol-sphingolipid) against reactive oxygen species (ORAC) and its ability to chelate iron have reported by Gavella *et al.*⁴². Phospholipids with long polyunsaturated fatty acids are recognized or their cation-binding activity⁴³.

As expected, deproteinized milk from different species significantly exhibited the lowest values of antioxidant activity compared to other milk fractions. Otherwise, DPPH radical scavenging activity of deproteinized sheep milk was significantly higher than others of deproteinized milk. Deproteinized goat milk significantly had higher metal chelating activity than that of others of deproteinized milk. Also, it was observed that deproteinized buffalo milk significantly presented high reducing power value as compared to deproteinized obtained from others milk. These differences could be related to the variations in deproteinized milk components among different milk species. These results are in consistent with those of Zulueta *et al.*¹⁷, who reported that ORAC values of deproteinized milk were lower than those obtained for milk and whey. They attributed that to complete milk protein precipitation with TCA, so that, the resulting solution contained small quantities of antioxidant compounds such as vitamin C and Uric acid.

Effect of heat treatments on milk antioxidant capacity:

Milk intended for human consumption was usually exposed to different heat treatments. However, these treatments may reinforce or inhibit the formation of antioxidants in heat-treated milk. Consequently, this research studied the influence of pasteurization and sterilization on the antioxidant capacity of milk from different dairy species and the results are shown in Fig. 1. The results illustrated that the antioxidant activity of pasteurized milk did not change significantly compared to raw different types milk. In agreement with the results of Khan *et al.*²⁸, pasteurization did not exhibit any effect on the antioxidant activity of buffalo and cow milk. Also, Zulueta *et al.*¹⁷ and Aloglu⁴⁴ found that pasteurization did not substantially influence total antioxidant activity of milk. Silvestre *et al.*⁴⁵ found that the long-time pasteurization of human milk (63°C/30 min) induced a significant loss of total antioxidant capacity, while total antioxidant capacity did not differ significantly in the milk samples exposed to high pasteurization (75°C/15 sec) compared the raw milk samples. Obtained results showed that the sterilization led to significant increase in the antioxidant capacity of milk except in case of the DPPH scavenging activity of cow and buffalo milk (Fig. 1a), metal chelating activity of buffalo, goat and camel milk (Fig. 1b) as well as reducing power of buffalo and sheep milk (Fig. 1c). In this respect, Taylor and Richardson⁴⁶ found that heat treatment of milk was associated with an increase in its antioxidant capacity. Also, Calligaris *et al.*⁴⁷ reported that severe heat treatment of milk led to increase the antioxidant properties. It has been reported from various studies that heat treatments may increase the antioxidant capacity of milk due to protein unfolding and exposure of thiol groups, potentially acting as hydrogen donors⁴⁸. They observed that heated milk at 120°C showed a progressive increase in antioxidant activity. Bressa *et al.*⁴⁹ reported the increase in antioxidant capacity during the heating of milk and that it can be considered for the formation of maillard reaction products, whose antioxidant properties are well documented. Calligaris *et al.*⁴⁷ found that the development of maillard reaction was occurred when milk was heat-treated at 120°C and slowly developed after 1.5-2 h at 80-90°C. During maillard reaction, melanoidins were generated which have a strong antioxidant activity. On the other hand, Aloglu⁴⁴ reported that sterilization technology did not affect total milk antioxidant capacity. This could be due to interactions between α -lactalbumin, β -lactoglobulin and casein micelles. During heating at temperatures lower than 90°C, whey proteins exhibit a very similar kinetic behavior and

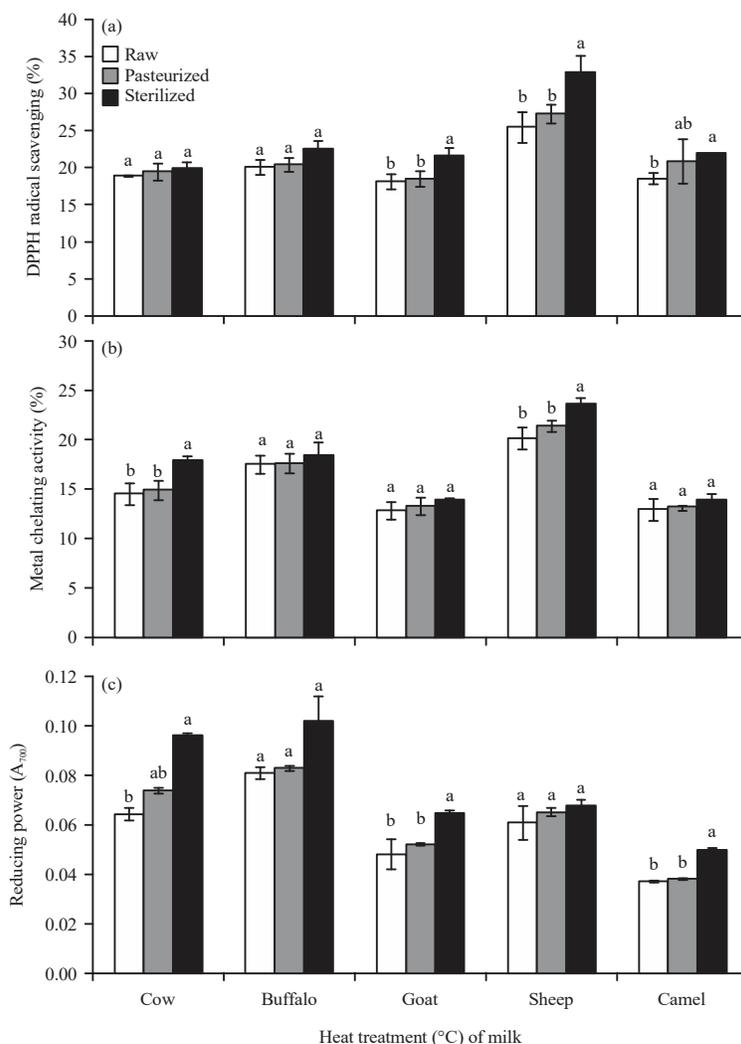


Fig. 1(a-c): Antioxidant properties of raw, pasteurized (63°C/30 min) and sterilized (115°C/20 min) milk from different dairy species (a) DPPH radical scavenging activity, (b) Metal chelating activity and (c) Reducing power. Different letters represent significant differences for each type of milk.

react with each other, form intermediate complexes and then interact with casein micelles⁵⁰. Consequently, these results obviously reveal that the antioxidant capacity of milk is greatly affected by not only heating temperature and time but also the interactions of milk proteins.

CONCLUSION

In conclusion, it can be reported that among the milk from 5 different mammals, sheep and buffalo milk exhibited the highest antioxidant activity compared to cow, goat and camel milk. This study confirmed that protein and fat are the major contributors to the antioxidant capacity of different milk species. Also, the results of this research indicated that

pasteurization did not appear any effect on antioxidant capacity of milk, whilst sterilization led to increase of antioxidant activity of milk. In this regard, the present study suggested that sheep and buffalo milk have more health benefits compared to other types of milk.

SIGNIFICANT STATEMENT

Numerous of health organizations all over the world recommend daily consumption of milk and dairy products for optimal health and wellness. In this regard, the present study contributes to characterize the antioxidant activity of milk for human health and suggests that sheep and buffalo milk have more health potentials compared to cow, goat and camel milk.

Also, this work illustrated that pasteurization had no effect on the antioxidant capacity of milk, while sterilization increased the antioxidant activity of milk.

REFERENCES

1. Waris, G. and H. Ahsan, 2006. Reactive oxygen species: Role in the development of cancer and various chronic conditions. *J. Carcinog.*, Vol. 5. 10.1186/1477-3163-5-14
2. Lonn, M.E., J.M. Dennis and R. Stocker, 2012. Actions of "antioxidants" in the protection against atherosclerosis. *Free Radic. Biol. Med.*, 53: 863-884.
3. Rochette, L., M. Zeller, Y. Cottin and C. Vergely, 2014. Diabetes, oxidative stress and therapeutic strategies. *Biochimica Biophysica Acta (BBA)-Gen. Subj.*, 1840: 2709-2729.
4. Li, S., H.Y. Tan, N. Wang, Z.J. Zhang, L. Lao, C.W. Wong and Y. Feng, 2015. The role of oxidative stress and antioxidants in liver diseases. *Int. J. Mol. Sci.*, 16: 26087-26124.
5. Zhou, D., W. Wang, X. Cheng, J. Wei and S. Zheng, 2015. Antioxidant therapy for patients with chronic pancreatitis: A systematic review and meta-analysis. *Clin. Nutr.*, 34: 627-634.
6. Pihlanto, A., 2006. Antioxidative peptides derived from milk proteins. *Int. Dairy J.*, 16: 1306-1314.
7. Lobo, V., A. Patil, A. Phatak and N. Chandra, 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn. Rev.*, 4: 118-126.
8. Zambonin, L., C. Caliceti, F.V.D. Sega, D. Fiorentini, S. Hrelia, L. Landi and C. Prata, 2012. Dietary phenolic acids act as effective antioxidants in membrane models and in cultured cells, exhibiting proapoptotic effects in leukaemia cells. *Oxid. Med. Cell. Longevity*, Vol. 2012. 10.1155/2012/839298.
9. Abdel-Hameed, E.S.S., M.A. Nagaty, M.S. Salman and S.A. Bazaid, 2014. Phytochemicals, nutritionals and antioxidant properties of two prickly pear cactus cultivars (*Opuntia ficus indica* Mill.) growing in Taif, KSA. *Food Chem.*, 160: 31-38.
10. Grazyna, C., C. Hanna, A. Adam and B.M. Magdalena, 2017. Natural antioxidants in milk and dairy products. *Int. J. Dairy Technol.*, 70: 165-178.
11. Cervato, G., R. Cazzola and B. Cestaro, 1999. Studies on the antioxidant activity of milk caseins. *Int. J. Food Sci. Nutr.*, 50: 291-296.
12. Tong, L.M., S. Sasaki, D.J. McClements and E.A. Decker, 2000. Mechanisms of the antioxidant activity of a high molecular weight fraction of whey. *J. Agric. Food Chem.*, 48: 1473-1478.
13. Lopez, V. and R.C. Lindsay, 1993. Metabolic conjugates as precursors for characterizing flavor compounds in ruminant milks. *J. Agric. Food Chem.*, 41: 446-454.
14. Albera, E. and M. Kankofer, 2009. Antioxidants in colostrum and milk of sows and cows. *Reprod. Domest. Anim.*, 44: 606-611.
15. Manzi, P. and A. Durazzo, 2017. Antioxidant properties of industrial heat-treated milk. *J. Food Meas. Character.*, 11: 1690-1698.
16. Nicoli, M.C., M. Anese and M. Parpinel, 1999. Influence of processing on the antioxidant properties of fruit and vegetables. *Trends Food Sci. Technol.*, 10: 94-100.
17. Zulueta, A., A. Maurizi, A. Frigola, M.J. Esteve, R. Coli and G. Burini, 2009. Antioxidant capacity of cow milk, whey and deproteinized milk. *Int. Dairy J.*, 19: 380-385.
18. AOAC., 2000. Official Methods of Analysis of AOAC International. 17th Edn., AOAC International, Gaithersburg, MD., USA., ISBN-13: 9780935584677, Pages: 2200.
19. Son, S. and B.A. Lewis, 2002. Free radical scavenging and antioxidative activity of caffeic acid amide and ester analogues: Structure-activity relationship. *J. Agric. Food Chem.*, 50: 468-472.
20. Decker, E.A. and B. Welch, 1990. Role of ferritin as a lipid oxidation catalyst in muscle food. *J. Agric. Food Chem.*, 38: 674-677.
21. Dorman, H.J.D., O. Bachmayer, M. Kosar and R. Hiltunen, 2004. Antioxidant properties of aqueous extracts from selected Lamiaceae species grown in Turkey. *J. Agric. Food Chem.*, 52: 762-770.
22. Yen, G.C. and H.Y. Chen, 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.*, 43: 27-32.
23. Niero, G., S. Curro, A. Costa, M. Penasa and M. Cassandro *et al.*, 2018. Phenotypic characterization of total antioxidant activity of buffalo, goat and sheep milk. *J. Dairy Sci.*, 101: 4864-4868.
24. Yoganandi, J., B.M. Mehta, K.N. Wadhvani, V.B. Darji and K.D. Aparnathi, 2014. Evaluation and comparison of camel milk with cow milk and buffalo milk for gross composition. *J. Camel Pract. Res.*, 21: 259-265.
25. Noziere, P., P. Grolier, D. Durand, A. Ferlay, P. Pradel and B. Martin, 2006. Variations in carotenoids, fat-soluble micronutrients and color in cows' plasma and milk following changes in forage and feeding level. *J. Dairy Sci.*, 89: 2634-2648.
26. Chauveau-Duriot, B., M. Doreau, P. Noziere and B. Graulet, 2010. Simultaneous quantification of carotenoids, retinol and tocopherols in forages, bovine plasma and milk: Validation of a novel UPLC method. *Anal. Bioanal. Chem.*, 397: 777-790.
27. Chen, J., H. Lindmark-Mansson, L. Gorton and B. Akesson, 2003. Antioxidant capacity of bovine milk as assayed by spectrophotometric and amperometric methods. *Int. Dairy J.*, 13: 927-935.
28. Khan, I.T., M. Nadeem, M. Imran, M. Ayaz, M. Ajmal, M.Y. Ellahi and A. Khaliq, 2017. Antioxidant capacity and fatty acids characterization of heat treated cow and buffalo milk. *Lipids Health Dis.*, Vol. 16, No. 1. 10.1186/s12944-017-0553-z.

29. Simos, Y., A. Metsios, I. Verginadis, A.G. D'Alessandro and P. Louiudice *et al.*, 2011. Antioxidant and anti-platelet properties of milk from goat, donkey and cow: An *in vitro*, *ex vivo* and *in vivo* study. *Int. Dairy J.*, 21: 901-906.
30. Laakso, S., 1984. Inhibition of lipid peroxidation by casein evidence of molecular encapsulation of 1,4-pentadiene fatty acids. *Biochim. Biophys. Acta (BBA)-Lipids Lipid Metab.*, 792: 11-15.
31. Rival, S.G., C.G. Boeriu and H.J. Wichers, 2001. Caseins and casein hydrolysates. 2. Antioxidative properties and relevance to lipooxygenase inhibition. *J. Agric. Food Chem.*, 49: 295-302.
32. Bertucci, J.I., C.S. Liggieri, M.L. Colombo, S.E.V. Cavalli and M.A. Bruno, 2015. Application of peptidases from *Maclura pomifera* fruit for the production of active biopeptides from whey protein. *LWT-Food Sci. Technol.*, 64: 157-163.
33. Cruz-Huerta, E., D.M. Maqueda, L. de la Hoz, V.S.N. da Silva, M.T.B. Pacheco, L. Amigo and I. Recio, 2016. Identification of iron-binding peptides from whey protein hydrolysates using iron (III)-immobilized metal ion affinity chromatography and reversed phase-HPLC-tandem mass spectrometry. *J. Dairy Sci.*, 99: 77-82.
34. Hernandez-Ledesma, B., A. Davalos, B. Bartolome and L. Amigo, 2005. Preparation of antioxidant enzymatic hydrolysates from α -lactalbumin and β -lactoglobulin. Identification of active peptides by HPLC-MS/MS. *J. Agric. Food Chem.*, 53: 588-593.
35. Le Maux, S., S. Bouhallab, L. Giblin, A. Brodkorb and T. Croguennec, 2014. Bovine β -lactoglobulin/fatty acid complexes: Binding, structural and biological properties. *Dairy Sci. Technol.*, 94: 409-426.
36. Baker, H.M. and E.N. Baker, 2004. Lactoferrin and iron: Structural and dynamic aspects of binding and release. *Biometals*, 17: 209-216.
37. Kim, J., H.D. Paik, Y.C. Yoon and E. Park, 2013. Whey protein inhibits iron overload-induced oxidative stress in rats. *J. Nutr. Sci. Vitaminol.*, 59: 198-205.
38. Kerasioti, E., D. Stagos, A. Priftis, S. Aivazidis, A.M. Tsatsakis, A.W. Hayes and D. Kouretas, 2014. Antioxidant effects of whey protein on muscle C2C12 cells. *Food Chem.*, 155: 271-278.
39. Salami, M., A.A. Moosavi-Movahedi, M.R. Ehsani, R. Yousefi and T. Haertle *et al.*, 2010. Improvement of the antimicrobial and antioxidant activities of camel and bovine whey proteins by limited proteolysis. *J. Agric. Food Chem.*, 58: 3297-3302.
40. Dewettinck, K., R. Rombaut, N. Thienpont, T.T. Le, K. Messens and J. van Camp, 2008. Nutritional and technological aspects of milk fat globule membrane material. *Int. Dairy J.*, 18: 436-457.
41. Conway, V., S.F. Gauthier and Y. Pouliot, 2012. Antioxidant activities of buttermilk proteins, whey proteins and their enzymatic hydrolysates. *J. Agric. Food Chem.*, 61: 364-372.
42. Gavella, M., M. Kveder, V. Lipovac, D. Jurasin and N. Filipovi-Vincekovic, 2007. Antioxidant properties of ganglioside micelles. *Free Radic. Res.*, 41: 1143-1150.
43. Cui, L. and E.A. Decker, 2016. Phospholipids in foods: Prooxidants or antioxidants? *J. Sci. Food Agric.*, 96: 18-31.
44. Aloglu, H.S., 2013. The effect of various heat treatments on the antioxidant capacity of milk before and after simulated gastrointestinal digestion. *Int. J. Dairy Technol.*, 66: 170-174.
45. Silvestre, D., M. Miranda, M. Muriach, I. Almansa, E. Jareno and F.J. Romero, 2008. Antioxidant capacity of human milk: Effect of thermal conditions for the pasteurization. *Acta Paediatr.*, 97: 1070-1074.
46. Taylor, M.J. and T. Richardson, 1980. Antioxidant activity of skim milk: Effect of heat and resultant sulfhydryl groups. *J. Dairy Sci.*, 63: 1783-1795.
47. Calligaris, S., L. Manzocco, M. Anese and M.C. Nicoli, 2004. Effect of heat-treatment on the antioxidant and pro-oxidant activity of milk. *Int. Dairy J.*, 14: 421-427.
48. Shukla, P. and U. Bajwa, 2013. Effect of heat treatments on antioxidant activity in sucrose-milk protein model systems. *Int. J. Comput. Eng. Res.*, 3: 20-24.
49. Bressa, F., N. Tesson, M.D. Rosa, A. Sensidoni and F. Tubaro, 1996. Antioxidant effect of Maillard reaction products: Application to a butter cookie of a competition kinetics analysis. *J. Agric. Food Chem.*, 44: 692-695.
50. Corredig, M. and D.G. Dalgleish, 1999. The mechanisms of the heat-induced interaction of whey proteins with casein micelles in milk. *Int. Dairy J.*, 9: 233-236.