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RESEARCH ARTICLE

Malondialdehyde and antioxidant content of pasteurized, semi-skimmed UHT and UHT milks

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Pastörize, yarım yağlı UHT ve UHT sütlerin malondialdehit ve antioksidan içeriği

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Öz

Amaç: Bu çalışmada pastörize, yarım yağlı UHT ve UHT sütlerin oksidan ve antioksidan parametrelerinin değerlendirilmesi için malondialdehit (MDA), vitamin (Vit) C, total protein (TP), redükte glutatyon (rGSH) ve katalaz (CAT) belirlenmesi ve bu parametreler arasındaki korelasyonların ortaya çıkarılması amaçlandı.

Gereç ve Yöntem: Hatay ilinde farklı marketlerden farklı markalara ait 30 adet süt örneği toplandı. Süt MDA, Vit C, TP, rGSH düzeyleri ve CAT aktiviteleri spektrofotometrik yöntemlerle belirlendi.

Bulgular: En düşük MDA düzeyi yarım yağlı UHT sütte belirlendi ($p < 0,05$). Pastörize sütün MDA düzeyi yarım yağlı UHT sütten daha yüksekti ($p < 0,05$). Pastörize sütlerde Vit C ($p < 0,001$) ve TP ($p < 0,01$) düzeyleri diğer sütlere göre daha yüksek bulundu. Pastörize ve UHT sütlerin rGSH düzeyleri, yarım yağlı UHT sütlere göre daha yüksekti ($p < 0,05$). UHT yarım yağlı süt CAT aktivitesi diğer sütlere göre oldukça yüksek bulundu ($p < 0,05$). Korelasyonlar açısından; pastörize süt MDA seviyesi, Vit C seviyesi ile pozitif korelasyon gösterdi ($r = 0,743$, $p < 0,01$). Yarım yağlı UHT süt TP seviyesi, CAT aktivitesi ile pozitif korelasyon gösterdi ($r=0,673$, $p < 0,05$).

Öneri: Sonuç olarak sunulan çalışmada, pastörize, yarım yağlı UHT ve UHT sütün oksidan (MDA), antioksidan parametreleri (Vit C, rGSH, and CAT), ve TP düzeyleri değerlendirildi ve tartışıldı. Bu çalışmada araştırılan değerler açısından, pastörize süt tüketiminin diğer ticari sütlere göre daha sağlıklı olabileceği önerilmektedir.

Anahtar kelimeler: Katalaz, malondialdehit, redükte glutatyon, vitamin C.

Abstract

Aim: In this study, to evaluate oxidant and antioxidant parameters of pasteurized, semi-skimmed UHT and UHT milks, the malondialdehyde (MDA), vitamin (Vit) C, total protein (TP), reduced glutathione (rGSH) levels and catalase (CAT) activity of them were determined and correlations between these parameters were revealed.

Materials and Methods: Thirty milk samples from different trademarks were collected from different markets in Hatay. Milk MDA, Vit C, TP, rGSH levels and CAT activity were determined by spectrophotometric methods.

Results: The lowest MDA level was determined in semi-skimmed UHT milk ($p < 0.05$). Pasteurized milk MDA level was higher than semi-skimmed UHT milk ($p < 0.05$). The levels of Vit C ($p < 0.001$) and TP ($p < 0.01$) in pasteurized milk were found to be higher than in the others. The rGSH levels of pasteurized and UHT milks were higher than semi-skimmed UHT milk ($p < 0.05$). UHT semi-skimmed milk CAT activity ($p < 0.05$) was found to be quite high compared to other milks. As regards correlations; pasteurized milk MDA level was positively correlated with Vit C level ($r = 0.743$, $p < 0.01$). Semi-skimmed UHT milk TP level was positively correlated with CAT activity ($r = 0.673$, $p < 0.05$).

Conclusion: As a result, the levels of oxidant (MDA), antioxidant parameters (Vit C, rGSH, and CAT), and TP in pasteurized, semi-skimmed UHT and UHT milk were evaluated and discussed in the present study. With this study, it is suggested that consumption of pasteurized milk may be healthier than semi-skimmed UHT and UHT milks.

Keywords: Catalase, malondialdehyde, reduced glutathione, vitamin C

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Introduction

As an exceptional nutritional product, milk is a rich source of nutrients, including conjugated omega-3 fatty acid, linoleic acid, bioactive peptides, protein, enzymes, vitamins, calcium, and selenium (Khan et al 2019, Woźniak et al 2022). These nutrients are important for physiological processes and have antioxidant, antibacterial, anti-inflammatory, and anticancer properties (Khan et al 2019). Milk's antioxidant capacity is primarily composed of non-enzymatic antioxidants, including glutathione, vitamins, carotenoids, and antioxidant enzymes, including glutathione peroxidase, superoxide dismutase, and catalase (CAT) (Zivkovic et al 2015, Grażyna et al 2017).

Milk needs to be exposed to heat treatment for humans to consume it, ensure microbiological safety, and extend shelf life (Sucak et al 2020, Fatih et al 2021). However, heat treatment applications cause chemical and biochemical changes in the structure of milk constituents, causing some differences in its composition, especially proteins, carbohydrates, and vitamins, and hence can change the nutritional quality and sensory properties (Urgu et al 2017). For instance, lipid peroxidation, a complex network of oxidants and antioxidants, negatively affects the milk quality. End products as a biological lipid oxidation are reactive aldehydes, such as malondialdehyde (MDA) (Kapusta et al 2018). Antioxidants in milk can stop the action of free radicals by donating a proton, thus delaying the development of autoxidation (Khan et al 2019). Reduced glutathione (rGSH), a key component of the antioxidant system, converts dehydroascorbate to ascorbate and supports antioxidant defenses and detoxification (Szarka et al 2012). Vitamin (Vit) C leads to the reduction of compounds, including nitrate, molecular oxygen, cytochrome A, and C, and is capable of reacting with free radicals in aqueous environments (Khan et al 2019). Vitamin C is an essential water-soluble antioxidant in milk that is readily oxidized at the milk pH (Zivkovic et al 2015). It has a strong affinity to scavenge free radicals, including superoxide, nitric oxide, iron oxide, and alkoxyl radicals (Gutierrez et al 2018). One of the most active milk enzymes, CAT, a heme protein, converts hydrogen peroxide to water and oxygen. Synergistic interactions among antioxidants impart high antioxidant potential to milk and efficaciously protect milk fat against oxidation (Grażyna et al 2017). Briefly, milk includes plenty of enzymatic and non-enzymatic antioxidant compounds (Zivkovic et al 2015). In addition, milk protein is an essential indicator of milk quality (Khastayeva et al 2021).

To our knowledge, limited studies have been presented on the oxidant and antioxidant content of milk consumed and/or marketed in Turkey (Ertan et al 2017). Moreover, there was no study in the literature determining the levels of MDA, Vit C, rGSH, and CAT activity in commercial milk in Turkey. The oxidant-antioxidant potential of pasteurized, semi skimmed UHT and UHT milk by determining the levels of MDA, Vit C, total protein (TP), rGSH and activity of CAT has yet to be fully considered. Therefore, the main purpose of the present study was to evaluate the oxidant and antioxidant content of pasteurized, semi skimmed UHT, and UHT milk. For this aim, the levels of MDA, Vit C, TP, rGSH, and activity of CAT of them if pasteurized, semi skimmed UHT, and UHT milk was determined and correlations between these parameters were evaluated in this study.

Material and Methods

Thirty cow milk samples from different trademarks were collected from different markets in April 2022 in Hatay region (36° 21' 42,8" North, 36° 12' 49,1"East). Commercial pasteurized (8 samples), semi-skimmed UHT (12 samples), and UHT (10 samples) produced by twelve different companies in Turkey were purchased from national markets. Selected trademarks of milk samples constituted an important proportion of the Turkish pasteurized, semi-skimmed UHT, and UHT milk market. As the concentrations of oxidant and antioxidant compounds in milk usually affect milk storage conditions, the milk samples were collected on the same day by paying attention to the expiration date of the products. Approximate compositions of pasteurized, semi-skimmed UHT and UHT milk samples are presented in Table 1.

Preparation of milk samples

Milk samples were brought to the biochemistry laboratory in the cold chain. For the analysis, 50 ml of each milk sample was placed in a falcon tube and centrifuged at 5000 rpm for 20 minutes at 4oC. After centrifugation, supernatants (skim milk) were collected by removing the upper layer of fat with a scraper and used in the assays. Levels of MDA, Vit C, TP, and rGSH levels and activity of CAT in milks were determined in supernatants by spectrophotometric methods (UV 2100 UV–VIS Recording Spectrophotometer Shimadzu, Japan).

Measurement of malondialdehyde levels

Malondialdehyde levels were assessed spectrophotometrically according to the method recommended by Ohkawa et al. (1979). The methodology is based on spectrophotometric analysis at 532 nm of the pink complex generated by MDA with TBA, the second-generation byproduct of lipid peroxidation, as a result of incubation of sample in a boiling water bath for an hour at pH:3.5 and under aerobic circumstances. The values are given as nmol/ml.

Table 1. The composition of pasteurized, semi-skimmed UHT, and UHT milk samples

	Samples	Fat (%)	Carbohydrate (%)	Protein (%)
Pasteurized milk samples	P1	3.1	4.7	3
	P2	3	4.4	2.9
	P3	3	4.5	3
	P4	3.1	4.7	3
	P5	3.7	4.7	3.1
	P6	3	4.7	3
	P7	3	4.4	2.9
	P8	3.1	3.2	2.9
Semi skimmed UHT milk samples	S1	1.5	4.7	2.9
	S2	1.5	4.7	3
	S3	1.5	4.7	3
	S4	1.5	4.7	3
	S5	1.5	4.4	3
	S6	1.5	4.7	3
	S7	1.5	4.7	3
	S8	1	4.7	2.9
	S9	1.5	4.7	3
	S10	1.5	5.1	3.3
	S11	1.5	4.8	2.9
	S12	1.5	4.7	3
UHT milk samples	U1	2.5	4.6	3
	U2	3	4.7	3
	U3	3.3	4.8	2.9
	U4	3.1	4.7	3
	U5	3	4.7	3
	U6	3.3	4.5	3
	U7	3	4.5	3
	U8	3	4.8	2.9
	U9	3.2	4.5	3
	U10	3	5.6	3

Measurement of vitamin C levels

Vitamin C levels were determined using the manual Haag (1985) spectrophotometric method. Briefly, ascorbic acid is converted to dehydroascorbic acid with weak oxidizing agents. Then, dehydroascorbic acid gradually transforms into diketogulonic acid in weak acid solutions. Diketogulonic acid reacts with 2.4-dinitrophenylhydrazine (DNPH) and dehydroascorbic acid to produce bis 2.4-dinitrophenylhydrazone. The values are presented as µg/ml.

Measurement of total protein levels

Total protein levels were assayed by the Lowry's (1951) method spectrophotometrically by measuring the absorbance at 700 nm. Bovine serum albumin (Merck 112018, Germany) was used as a standard. The values are given as mg/ml.

Measurement of reduced glutathione levels

The Ellman (1959) method was used to evaluate the reduced glutathione levels. It is a kinetic procedure that relies on the principle of the reduction of 5.5'-dithiobis (2-nitrobenzoic) acid to trinitrobenzoat by glutathione. The optical density of the reduced disulphide compound by determining the absorbance at 412 nm. The values are presented as nmol/mg protein for milk cells and nmol/ml.

Measurement of catalase activities

Catalase activities were determined by using the method developed by Aebi (1984). Hydrogen peroxide is broken down into molecular oxygen and water by the enzyme CAT. The amount of hydrogen peroxide dissociation is often inversely correlated with CAT activity. The measurement of its activity was monitored by the decrease in absorption at 240 nm. The values are given as U/L.

Statistical analysis

The values obtained were evaluated by Windows Statistical Package for the Social Sciences program (IBM SPSS 22 version, USA). Analysis of variance (ANOVA) and the post hoc Duncan test were used to compare multiple groups, and also Spearsman's correlation was performed for correlation analysis. $p < 0.05$ was accepted as the significant level. Mean ± standard error (SE) was used to express the values.

Results

Fat, carbohydrate, and protein contents on the labels of the pasteurized, semi-skimmed UHT, and UHT milk were reported as follows: Fat levels were 3-3.7%, 1-1.5%, and 3-3.3%, carbohydrate levels were 3.2-4.7%, 4.4-5.1%, 4.5-5.6%, and protein levels were 3-3.1%, 3-3.3%, 3-2.9% and were commonly in good agreement with each other (Table 1). The levels of MDA, Vit C, TP, and rGSH and the activity of CAT in pasteurized, semi-skimmed UHT, and UHT milk samples were presented (Table 2).

The lowest MDA level (7.82±0.52, $p < 0.05$) was determined in semi-skimmed UHT milk. Pasteurized milk MDA level (10.59±1.29) was higher than semi-skimmed UHT milk ($p < 0.05$). The levels of Vit C (11.82±1.53, $p < 0.001$) and TP (17.57±0.38, $p < 0.01$) in pasteurized milk were found to be higher than in the other milk types. The rGSH levels of pasteurized and UHT milks were higher than semi-skimmed UHT milk ($p < 0.05$).

Table 2. MDA and antioxidants levels in pasteurized, semi-skimmed UHT, and UHT milk samples

Groups	MDA (nmol/ml)	Vit C (µg/ml)	TP (mg/ml)	rGSH (nmol/ml)	CAT (U/L)
Pasteurized Milk	10.59±1.29^{a}	11.82±1.53^{a}	17.57±0.38^{a}	84.81±1.36^{a}	0.077±0.000^{b}
Semi-skimmed UHT Milk	7.82±0.52^{b}	6.28±0.35^{b}	15.66±0.38^{b}	77.28±2.93^{b}	0.089±0.005^{a}
UHT Milk	9.09±0.84^{ab}	5.58±0.81^{b}	16.07±0.42^{b}	85.29±1.83^{a}	0.079±0.000^{ab}
p	$p < 0.05$	$p < 0.001$	$p < 0.01$	$p < 0.05$	$p < 0.05$

^{a,b}Means within the same column with different superscripts are statistically significant. Data are given as Mean±SE. MDA: Malondialdehyde, Vit C: Vitamin C, TP: Total protein, rGSH: Reduced glutathione, CAT: Catalase

UHT semi-skimmed milk CAT activity (0.089±0.005, $p < 0.05$) was found to be quite higher compared to kinds of milk. Pasteurized and UHT milks were not different in terms of other parameters except Vit C and TP. UHT and semi-skimmed UHT milks were not different in terms of other parameters except rGSH. In addition, pasteurized milks had higher MDA, Vit C, TP, rGSH levels but lower CAT activity than semi-skimmed UHT milk.

The correlations between the parameters in milks were given in Table 3 (pasteurized milk), Table 4 (semi skimmed UHT milk) and Table 5 (UHT milk). As regards correlations: MDA levels in pasteurized milk were positively correlated with Vit C levels ($r = 0.743$, $p < 0.01$). TP levels in semi-skimmed UHT milk were positively correlated with CAT activities ($r = 0.673$, $p < 0.05$).

Table 3. Correlations between the parameters for pasteurized milk samples

	TP (mg/ml)	rGSH (nmol/ml)	CAT (U/L)	Vit C (µg/ml)
MDA (nmol/ml)	-0.228	-0.053	0.060	0.743**
TP (mg/ml)		0.046	-0.385	-0.469
rGSH (nmol/ml)			0.228	0.105
CAT (U/L)				0.238

MDA: Malondialdehyde, TP: Total protein, rGSH: Reduced glutathione, CAT: Catalase Vit C: Vitamin C.
**p < 0.01

Table 4. Correlations between the parameters for semi-skimmed UHT milk samples

	TP (mg/ml)	rGSH (nmol/ml)	CAT (U/L)	Vit C (µg/ml)
MDA (nmol/ml)	-0.036	0.305	-0.061	-0.049
TP (mg/ml)		0.316	0.673*	-0.212
rGSH (nmol/ml)			-0.243	-0.365
CAT (U/L)				0.212

MDA: Malondialdehyde, TP: Total protein, rGSH: Reduced glutathione, CAT: Catalase Vit C: Vitamin C
*p < 0.05.

Table 5. Correlations between the parameters for UHT milk samples

	TP (mg/ml)	rGSH (nmol/ml)	CAT (U/L)	Vit C (µg/ml)
MDA (nmol/ml)	0.108	-0.576	0.551	0.108
TP (mg/ml)		-0.060	-0.071	-0.286
rGSH (nmol/ml)			-0.699	0.518
CAT (U/L)				0.048

MDA: Malondialdehyde, TP: Total protein, rGSH: Reduced glutathione, CAT: Catalase Vit C: Vitamin C

Discussion

Drinking milk is subjected to heat treatment applications in order to make raw milk safe from a microbiological point of view (Urgu et al 2017). Concurrently, heat treatment, commonly required for milk safety and stability, maybe in charge of quality alterations in milk, such as their oxidant and antioxidant properties (Ertan et al 2017). In the literature, it has been presented that the antioxidant properties of milk may be changed by heat treatment with different time-temperature combinations (Calligaris et al 2004). In the present study, the levels of MDA, Vit C, TP, rGSH, and CAT activity of pasteurized, semi- skimmed UHT, and UHT milk were determined, and correlations between these parameters were investigated.

Malondialdehyde results from the peroxidation of fatty acids with multiple bonds present in cell and organelle membranes. Increased peroxidation of lipids by free radicals in cell membranes leads to an increase in MDA level. Malondialdehyde and other lipid peroxides can interact with DNA or proteins, causing structural changes (Gaweł et al 2004, Kapusta et al 2018). Lipid peroxidation negatively affects milk quality (Kapusta et al 2018). In the current study, the lowest MDA level was determined in semi-skimmed UHT milk, the highest MDA level was determined in pasteurized milk, and pasteurized milk MDA level was higher than UHT milk. Higher MDA levels of pasteurized milk samples than UHT and semi-skimmed UHT milk samples might be associated with the highest fat content of pasteurized milk samples. It is thought that the same reason may be in terms of MDA levels of the differences between the UHT, and semi-skimmed UHT milk samples. Moreover, Calligaris et al. (2004) mentioned that the thermal treatment increases pro-oxidative activity in milk through both losses of natural antioxidants and generation of new oxidizing molecules in the early stages of the Maillard reaction, which occurred after 1.5- and 2-h heating at 80°C and 90°C, respectively. Thus, the other reason that maybe related to the increase in the MDA levels of pasteurized milk in the present study can be attributed to the Maillard reaction. Additionally, it is known that the reaction continues during storage, although the Maillard reaction accelerates with heat treatment (Urgu et al 2017).

Changes occur in the quality and chemical content of milk exposed to high temperatures. It has been reported that lactose, protein, vitamin, enzyme, lipid, and calcium/ phosphorus balance, which constitutes the chemical structure of milk, is disrupted in high heat treatments (Sakkas et al 2014, Sucak et al 2020). Vitamin C reacts with hydroxyl radicals and superoxide and forms the first antioxidant defense against oxidant agents (Khan et al 2019). In this study, Vit C levels in pasteurized milk were higher than in the other milk types, and semi-skimmed UHT and UHT milks were not different in terms of Vit C levels. The literature reviewed that in the case of pasteurized milk, when the storage time is from a few days to twenty days at a temperature ranging from 1°C to 8 °C, vitamin loss is insignificant despite UHT milk where the loss of vitamin C is significant. These amounts were reported for loss of Vit C in pasteurized and UHT milk 0-10% and <15-25%, respectively (Woźniak et al 2022).

It is known that heat processes subjected to milk proteins with antioxidant potential affect the milk's total antioxidant capacity (Stobiecka et al 2022). Reduced glutathione protects cells against free radicals, reactive oxygen species, and endogenous and exogenous toxic compounds (Meister and Anderson 1983). In the present study, rGSH levels in pasteurized and UHT milks were higher than in semi-skimmed UHT milk. Similar to the previous study (Ertan et al 2017), it is thought that the thermal process and fat separation may influence the total antioxidant capacity of commercial milks. One of the most active milk enzymes CAT has been reported to play a central role in milk redox control (Silanikove et al 2005). Catalase is a heme protein and converts hydrogen peroxide to water and oxygen (Grażyna et al 2017). In this study, UHT semi-skimmed milk CAT activity was found to be quite higher compared to other milk types. Stobiecka et al. (2022) informed that heat treatment (temperature and/or time) of milk could inhibit or reinforce the formation of antioxidative compounds in the ultimate product. Moreover, Calligaris et al. (2004) presented that milk antioxidant values may increase during thermal treatments, by the reason of the exposure of thiol groups, which are potentially acting as hydrogen donors, and the formation of Maillard reaction products. Furthermore, Mehta and Deeth (2016) reported that an increase in milk antioxidant activity could be attributed to the Maillard reaction (a chemical reaction between amino and carbonyl groups), essentially between lysine residues in milk proteins and lactose.

Ertan et al. (2017) reported that the total antioxidant capacity of pasteurized milk samples is higher than UHT and the total antioxidant capacity of UHT is higher than semi-skimmed UHT milk samples. Similarly, in the present study, pasteurized milks had higher Vit C and rGSH levels than semi-skimmed UHT milk, and also pasteurized, and UHT milks was not different in terms of rGSH levels and CAT activity. In the literature, milk's total antioxidant capacity was reported to increase with milk fat content (Stobiecka et al 2022). In line with the literature, pasteurized and UHT milks have fat higher than semi- skimmed UHT, thus they contain higher antioxidants than semi-skimmed milk in the present study may be considered.

Milk protein is an important indicator of milk quality (Khastayeva et al 2021). The current study showed that TP levels in pasteurized milk were higher than in the other milk types. Semi-skimmed UHT and UHT milks were not different in terms of TP levels. Similar to the current study, Sucak et al. (2020) reported no difference in TP levels of semi-skimmed UHT and UHT milks. In addition, the fact in this study that the TP level in UHT milk was lower than in pasteurized milk may suggest that pasteurized milk exhibits higher quality than UHT milk.

To our knowledge, no study on the correlation among the mentioned parameters levels in pasteurized, semi-skimmed UHT, and UHT milk was found in the literature review. Ertan et al. (2017) reported no significant correlation between total antioxidant capacity values with protein levels of UHT milk samples. The correlation results of this study were in good agreement with the previous study (Ertan et al 2017). In the present study, pasteurized milk MDA levels were positively correlated with Vit C levels, and semi-skimmed UHT milk TP levels were positively correlated with CAT activities.

Conclusion

The current study evaluated the levels of oxidant (MDA), antioxidant parameters (Vit C, rGSH, and CAT), and TP in pasteurized, semi-skimmed UHT and UHT milk and discussed. This study presented that pasteurized milk had higher Vit C, TP, and rGSH levels than semi-skimmed UHT milk, and also pasteurized milk had higher Vit C and TP levels than UHT milk. As a result, this study suggests that the consumption of pasteurized milk may be healthier than semi-skimmed UHT and UHT milk.

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Conflict of Interest

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During this study, any pharmaceutical company which has a direct connection with the research subject, a company that provides and / or manufactures medical instruments, equipment and materials or any commercial company may have a negative impact on the decision to be made during the evaluation process of the study. or no moral support.

References

Aebi H, 1984. Catalase in vitro. Methods Enzymol, 105, 121-126.

Calligaris S, Manzocco L, Anese M, Nicoli, MC, 2004. Effect of heat-treatment on the antioxidant and pro-oxidant activity of milk. Int Dairy J, 14(5), 421-427.

Ellman G, 1959. Tissue sulphydryl groups. Arch Biochem Biophys, 82(1), 70-77.

Ertan K, Bayana D, Gokce O, Alatossava T, et al., 2017. Total antioxidant capacity and phenolic content of pasteurized and UHT-treated cow milk samples marketed in Turkey. Academic Food Journal, 15(2), 103-108.

Fatih M, Barnett MPG, Gillies NA, Milan AM, 2021. Heat treatment of milk: a rapid review of the impacts on postprandial protein and lipid kinetics in human adults. Front Nutr, 8, 643350.

Gaweł S, Wardas M, Niedworok E, Wardas P, 2004. Malondialdehyde (MDA) as a lipid peroxidation marker. Wiadomości Lek, 57(9-10), 453-455.

Grażyna C, Hanna C, Adam A, Magdalena BM, 2017. Natural antioxidants in milk and dairy products. Int J Dairy Technol, 70(2), 165-178.

Gutierrez AM, Boylston TD, Clark S, 2018. Effects of pro-oxidants and antioxidants on the total antioxidant capacity and lipid oxidation products of milk during refrigerated storage. J Food Sci, 83(2), 275-283.

Haag W, 1985. Zur methodik und praktischen Bedeutung der vitamin C - best immung be im Rind in Vergangenheit und Gegenwart. Inaugural Dissertation. Justus Liebig Universitaet, Giessen.

Kapusta A, Kuczyńska B, Puppel K, 2018. Relationship between the degree of antioxidant protection and the level of malondialdehyde in high-performance Polish Holstein-Friesian cows in peak of lactation. PLoS One, 13(3), e0193512.

Khan IT, Bule M, Ullah R, Nadeem M, et al., 2019. The antioxidant components of milk and their role in processing, ripening, and storage: Functional food. Vet World, 12(1), 12-33.

Khastayeva AZ, Zhamurova VS, Mamayeva LA, Kozhabergenov AT, et al., 2021. Qualitative indicators of milk of Simmental and Holstein cows in different seasons of lactation. Vet World, 14(4), 956-963.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, 1951. Protein measurement with pholin phenol reagent. J Biol Chem, 193(1), 265-275.

Mehta BM, Deeth HC, 2016. Blocked lysine in dairy products: formation, occurrence, analysis, and nutritional implications. Compr Rev Food Sci, 15(1), 206-218.

Meister A, Anderson ME, 1983. Glutathione. Annu Rev Biochem, 52, 711-760.

Ohkawa H, Ohishi N, Yagi K, 1979. Assay for lipit peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95(2), 351-358.

Sakkas L, Moutafi A, Moschopoulou E, Moatsou G, 2014. Assessment of heat treatment of various types of milk. Food Chem, 159, 293-301.

Silanikove N, Shapiro F, Shamay A, Leitner G, 2005. Role of xanthine oxidase, lactoperoxidase, and NO in the innate immune system of mammary secretion during active involution in dairy cows: manipulation with casein hydrolyzates. Free Radic Biol Med, 38(9), 1139-1151.

Stobiecka M, Król J, Brodziak, A, 2022. Antioxidant activity of milk and dairy products. Animals, 12, 245.

Sucak MG, Ağyar O, Özkaya A, Yılmaz E, 2020. Determination of chemical parameter changes in raw and UHT milks. Bitlis Eren Univ J Sci & Technol, 9(1), 1-6.

Szarka A, Tomasskovics B, Bánhegyi G, 2012. The ascorbate-glutathione-α-tocopherol triad in abiotic stress response. Int J Mol Sci, 13(4), 4458-4483.

Urgu M, Saatli TE, Türk A, Koca N, 2017. Determination of hydroxymethylfurfural content of heat-treated milk (pasteurized, UHT and lactose-hydrolised UHT milk). Academic Food Journal, 15(3), 249-255.

Woźniak D, Cichy W, Dobrzyńska M, Przysławski J, et al. 2022. Reasonableness of enriching cow's milk with vitamins and minerals. Foods, 11, 1079.

Zivkovic JV, Sunaric S, Trutic N, Denić M, et al. 2015. Antioxidants and antioxidant capacity of human milk. Acta Fac Med Naissensis, 32(2): 115-125.

Author Contributions

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Data Collection and / or Processing: Filiz Kazak
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Ethical Approval

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