Polyphenols as Antioxidants in Bovine Milk Measured by the Superoxide Anion

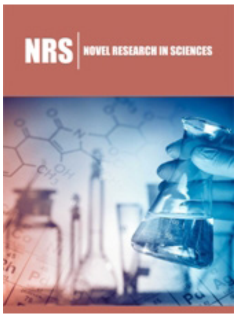
Galina MA[1]*, Higuera Piedrahita RI[1], Olmos J[2] and Pineda J[3]

[1]Faculty of Higher Studies Cuautitlán, National Autonomous University of Mexico, Mexico

[2]Faculty of Veterinary Medicine, Autonomous University of Querétaro, Mexico

[3]Faculty of Veterinary Medicine, University of Colima, Mexico

ISSN: 2688-836X



***Corresponding author:** Galina MA, Faculty of Higher Studies Cuautitlán, National Autonomous University of Mexico, Mexico

Submission: May 10, 2023

Published: May 24, 2023

Volume 14 - Issue 5

How to cite this article: Galina MA*, Higuera Piedrahita RI, Olmos J and Pineda J. Polyphenols as Antioxidants in Bovine Milk Measured by the Superoxide Anion. Nov Res Sci. 14(5). NRS.000849. 2023.
DOI: 10.31031/NRS.2023.14.000849

Abstract

Due to a high obesity incidence within the Mexican population (75% of individuals), Mexican residents are prone to higher chronic degenerative diseases On the other hand, it's known that milk can provide antioxidants that help prevent diseases associated with cellular oxidation (that could favor COVID infections) therefore, this paper's objective is to determine the degree of antioxidant protection in cow's milk, based on the superoxide contents including milk samples from zero grazing and grazing cows to study and identify tracing parameters correlated to Polyphenols in the feeding system. This parameter, expressed as a molar ratio between antioxidant compounds and an oxidation target, distinguishing products from grazing and zero-grazing animals, divided into three different production systems: (Grazing (G), Supplemented Grazing (SG) and Full Confinement (FC). In treatment G, cholesterol concentration was reduced during the month of May; unlike SG, where the amount of this metabolite increased during the same period; and in treatment FC, where it remained constant during months. The concentration of alpha- tocopherol in milk increased from 170 to 179.04µg/100g in the month of May for Grazing Animals (G), decreased from 115.25 to 105.45µg/100g and from 94.23 to 77.74µg/100g for SG and FC treatments respectively. The animals were kept in a humid tropical area, and the level of cholesterol in these cows' milk during the dry period was higher ($p<0.05$) than those animals fed with diets rich in concentrates (25.22 mg/100g), compared to those that consumed a greater amount of forage (G:15.63mg/100g, PS: 21.44mg/100g). The level of alpha-tocopherol in milk was higher ($p<0.05$) in grazing milk (173.02 µg/100g), compared to SG: 109.83µg/100g and FC: 83.95µg/100g. The Degree of Antioxidant Protection (DAP) increased significantly between treatments, with the highest value being observed in G (11.17), followed by SG (5.13) and FC (3.42) groups. It is concluded that there is an effect of the feeding system that directly influences the degree of antioxidant protection in milk, and this effect being greater in milk that comes from grazing cows.

Introduction

Oxidative stress

In milk, the intrinsic properties of the Reactive Oxygen Species (ROS) are the basis of the necessary mechanisms for biological development. They regulate many signal transduction pathways by directly reacting and modifying the structure of proteins, transcription factors and genes to modulate their functions. ROS are involved in regulating the activity of enzymes, mediating inflammation by stimulating cytokine production, signaling cell growth and differentiation, and eliminating pathogens and foreign particles. However ROS mainly including superoxide ions (O_2^-) , singles oxygen ($^1O_2$) hydrogen peroxide (H_2O_2) and peroxy nitrate ($ONOO^-$) are both inflammatory signaling molecule inflammatory mediators. A moderate increase in ROS can facilitate cell metabolism and differentiation, whereas the persistent generation of excess ROS might result in molecular oxidative damage, including lipid peroxidation, protein degradation, and DNA fragmentation, and over time cell and organism eventually causes various diseases, such neurodegenerative disease, atheroscleros.is, diabetes, cancer, and ageing [1,2].

Oxidative stress has been defined as the imbalance between the presence of reactive oxygen/nitrogen species (ROS/RNS), and the body's ability to counteract their actions through the antioxidant protection system [3-5]. In scientific literature, oxidative stress has been described as the product of an increase of ROS/RNS with a decrease in antioxidant protection ability, characterized by a reduction in the capacity of endogenous systems to combat oxidative attacks directed at biomolecular targets. Its severity is associated with several pathologies such as: cardiovascular diseases, cancer, diabetes., and aging [5,6]. It has been proven that oxidative stress is related to more than 100 diseases, either as a primary cause or as an associated factor, producing immune deficiency and facilitating COVID infections and/or death. [7-9]. It is an irreversible process of decay in the organism product of reactive oxygen species, which also expresses its negative influence on the physiology of aging, disabling defense functions, and promoting disease occurrence and reducing life span [4]. Oxidative stress is understood as the production of an excessive amount of ROS/RNS in the organism, which is the product of an imbalance between the generation and destruction of ROS/RNS. Therefore, oxidative stress is the repercussion of a production increase of free radicals, but also of a reduction in the antioxidant defense system [10]. Substances reactive to oxygen or nitrogen (ROS and RNS) should not only be seen as species that cause biomolecular damage affecting the enzyme system (chemical defense or detoxification), but also the cellular response system to molecular signals, and therefore modifying biosynthetic reactions [5].

Oxidative stress is due to free radical production such as: hydrogen peroxide (H_2O_2), superoxide (O_2), free oxygen (1/2 O_2) and hydroxyl radicals (OH); with some acquired exogenously, and others from metabolic processes such as: cellular respiration, exposure to microbial infections that produce phagocytic activation, during intense physical activity; or by the action of polluting substances such as cigarette smoke, alcohol, ultraviolet ionization, pesticides, coronavirus infection and ozone [10]. Previous works show how the substances most susceptible to oxidation are polyunsaturated fats-especially arachnoid acid and docosahexaenoic acid, which produce malondialdehyde and 4-hydroxy nonenal, recognized markers of lipid oxidation decline. [11]. The lipid oxidation also produces aldehydes that affect proteins and can impede their functions. [10]. Oxidative damage to lipid membrane components has been related to mechanisms of neurodegeneration, cancer, cardiovascular or inflammatory diseases. It has been confirmed that the excessive production of reactive oxygen species can lead to the overexpression of oncogenic genes, or the formation of mutagenic compounds that can cause proatherogenic activity and is related to the appearance of senile plaque or inflammation [12].

Antioxidants in milk

The concept of biological antioxidants has been defined as any compound that, when present at a lower concentration compared to that of an oxidizable substrate, is capable of delaying or preventing oxidation of the substrate [7,11]. Antioxidant functions involve reducing oxidative stress -DNA protection against malignant transformations- as well as other parameters of cell damage. Epidemiological studies have demonstrated the ability of antioxidants to contain the effects of reactive oxygen species activity and decrease the incidence of cancers and other degenerative diseases [13]. It has been confirmed that, under physiological conditions, the balance between oxidant and antioxidant compounds moderately favors the oxidants, thus producing a slight oxidative stress, overcoming the intervention of the organism's endogenous antioxidant systems [14]. This issue of oxidative stress becomes more acute with age when the endogenous antioxidants and repair systems cannot effectively counteract it. For this reason, supplementation of antioxidants into a diet is convenient, and it can be achieved in the form of alpha-tocopherol and beta-carotene, which can be obtained from grazing products such as milk [9,15].

Antioxidants, like vitamin D and E, carotenoids and phenolics (phenolic acids such as benzoic and hydroxybenzoic acids, cinnamic and hydroxycinnamic acid derivatives and flavonoids-flavanols, flavans, flavanones, flavanols, flavones, and anthocyanidins such as anthocyanin aglycones (flavil or skeleton of 2- phenyl chromenyl ions) are currently being considered as the main exogenous antioxidants. Clinical studies have shown a diet rich in fruits, vegetables, whole grains, and their abundant presence in plants and grass make food for grazing cows an important source of these beneficial compounds for health [16]. In recent research works, it has been shown that livestock production systems that are managed in grazing can have a positive impact on the health of the population, through the consumption of dairy products rich in omega 3, with an adequate balance of lower omega 6 at a ratio of 4:1 [17]. Its importance in relation to human health is documented, with a decrease in cholesterol [18]. On the other hand, its antioxidant capacity, containing alpha tocopherol, can be considered as functional foods and/or as a source of nutraceutical compounds [15]. It becomes especially relevant when people currently have health problems associated with cellular oxidative stress, diseases such as Parkinson's, Alzheimer's, Huntington's disease, emphysema, cardiovascular diseases, cancer, and diabetes [12].

Previously degree of antioxidant protection values greater than 7.0×10^{-3} were found in samples from grazing goats, and values lower than 7.0×10^{-3} were found in samples from zero-grazing goats, for both milk and cheese, meaning that cholesterol was highly protected against oxidative reactions, when herbage was the only feed or was dominant in the goat diet. The DAP reliability to measure the antioxidant protection of

cholesterol appeared more effective when the feeding system was based on grazing, or when cut herbage was utilized indoors by animals. The DAP index was able to distinguish dairy products when the grazed herbage in the goats' diet exceeded 15%. This has led to the development of techniques or equations to determine the degree of a food's antioxidant protection (GPA). This parameter's efficacy to distinguish milk and cheese from grazing and full confinement animals was discussed by Pizzoferrato et al. [16], proving to be a useful methodology to determine the origin of dairy products in relation to grazing.

Methods

Milk sampling was carried out in 30 production units located in the municipalities of Balancán and Tenosique in the state of Tabasco, which were divided into units that established their feeding based on Grazing (G), Grazing+ Concentrate (GS) and Full Confinement (FC); having 10 units with P, 10 units with PS and 10 with ES. Sampling was carried out during the dry season in Tabasco, which includes the months of March, April and May of 2022, taking samples every 28 days with three samples per unit. The animals were bovine crosses of Bos indicus x Bos Taurus, with different numbers of lactations, and in different periods of lactation. The animals were milked once a day. Milk samples (1.5 l/ranch) were taken from the general tank. These samples were transported in refrigerators with ice to later be frozen at -20 °C and stored until their saponification process. The determinate anion superoxide by spectrophotometry and incubation of 37°90µL of milk and 10µL of tetrazolium nitro blue in 1mg/L solution, over 30 minutes in a dark environment after 50µl dimethyl sulfoxide and 50µl of sodium hydroxide 2M were added. The sample absorption was measured at 600nm [19].

Result

The results of this study show that the cholesterol levels, alpha-tocopherol, and the degree of antioxidant protection were mostly constant among the farms sampled inside the same system (Table 1 & 2). In treatment G, the concentration of cholesterol was reduced in the month of May, unlike SG where the amount of this metabolite increased in the same period and in FC treatment where it remained constant for three months. Average levels of cholesterol in cow's milk in March, April, and May (mg/100g). Concentration of alpha-tocopherol in milk increased from 170 to 179.04µg/100g in the month of May for grazing animals (P), decreasing from 115.25 to 105.45µg/100g and from 94.23 to 77.74µg/100g for PS and E treatments respectively. Similarly, the cholesterol level in cow milk in the humid tropics during the dry period was higher ($p<0.05$) in those animals fed under stable systems with diets rich in starches (25.22 mg/100g), compared to with those who consumed a greater number of forages (Grazing (15.63mg/100g), Grazing + Supplement (21.44mg/100g). Regarding the results for the levels of alpha-tocopherol in milk was higher ($p<0.05$) in the milk whose origin was the grazing system (173.02µg/100g), compared to that which came from feeding that involved ingredients rich in starches (SG: 109.83µg/100g, E: 83.95µg/100g). In the present study, the Degree of Antioxidant Protection (DAP) had a statistically significant increase between treatments with the highest value being observed in Grazing (11.17), followed by the SG (5.13) and full confinement (3.42) groups.

Table 1: Levels of cholesterol, alpha-tocopherol and Degree Of Antioxidant Protection (DAP) in milk from cows from different feeding systems (P1, P2, etc.) under grazing systems in the municipalities of Balancán and Tenosique in the state of Tabasco.

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Cholesterol (mg/100g)	14.8	18.1	14.4	14.5	16.4	13.2	15	15.3	17.4	16.7
Alfa tocopherol (µg/100g)	179.2	171	163.7	170.5	175.1	176.7	161	185.3	177	170.9
DAP	12	9.4	11.3	11.7	10.6	13.3	10.7	12	10.1	10.1

Table 2: Levels of cholesterol, alpha-tocopherol and Degree of Antioxidant Protection (DAP) in milk from cows from different location (FC1, FC2, etc.) under Full Confinement (FC).

	FC1	FC2	FC3	FC4	FC5	FC6	FC7	FC8	FC9	FC10
Cholesterol (mg/100g)	24.7	19.3	21.4	18.4	25.8	29.9	30.4	27.4	28.1	26.4
Alfa tocopherol (µg/100g)	82.9	82.3	84.6	84.7	83	82.6	83.4	84.6	85.9	85.2
DAP	3.3	4.2	3.9	4.6	3.2	2.7	2.7	3	3	3.2

Discussion

Herbage intake was estimated on the basis of herbage mass, measured on 2×2m of pasture before and after grazing. The real intake of grazed herbage for each group was 0 (control fed on commercial concentrate), 450, 600, and 1,100g of DM/d, and the contribution of grazing to the diet in each treatment was calculated as a percentage of the maximum actual herbage intake [from 0g/d (0% grazing) to 1,100g/d (100% grazing), [20]. Cholesterol and α-tocopherol [21] milk contents were determined, and, on this basis, the DAP values were calculated demonstrating the importance of grazing in antioxidant prevention. The linear equation ($y=a+bx$) fits the experimental points with a high correlation coefficient ($R2=0.97$) and can be utilized as an analytical calibration curve. Replacing the variable y with the DAP threshold value, able to differentiate "pasture" products from "stabled" ones (DAP=7.0 exp^{-3}), the x value can be calculated. This value (15%) can be considered the limit of DAP detection if the same amounts of freshly cut pasture herbage were ingested indoors, and not grazed by animals, the linear fitting of DAP values vs. the amount of cut herbage intake would show a lower correlation value (R2=cheese commercial quality), contributing to price setting and probably to animal well- being. Moreover, the DAP parameter was found to be related to oxidative reactions, the main determinant of quality loss in food: the higher the DAP value, the greater the product stability and safety, considering the risk related to the intake of cholesterol-oxides in humans as discussed by Pizzoferrato et al. [16], Esti M et al. [22].

In Tabasco México, grazing showed the highest DAP when compared to the other two management systems offered with different degrees of concentrate in the diet, DAP was inferior when concentrate was administrated [23]; results are in agreement with previous data obtained in another study carried out in goat cheese by Pizzoferrato et al. [16] in Italy in 2007, where they reported data with the same trends, having in their work four treatments in which the GPA of goat's milk and cheese was evaluated, obtaining higher values in the treatments where neither grains nor commercial concentrates were offered: DAP were 11.3 on full grazing, 9.8 supplemented grazing, supplemented grazing with concentrate, 10 cows in each observation were feed full commercial concentrates in confinement [24], Pizzoferrato et al. [16] in 2007 reported that no significant differences were found in their samples in cholesterol levels in goat milk, with cholesterol values of 14.7 for grazing, 11.8 for supplemented grazing, 12.8 for grazing with commercial supplement and 12.8 in full confinement. In the present study, the cholesterol levels in cow's milk were: 15.63 in grazing, 21.44 in grazing + supplement and 25.22 in confinement, showing a slight increase in cholesterol between the different treatments being higher in full confinement, this difference could be due to the milk source, since those researchers carried out his study on goat's milk. The data obtained from alpha-tocopherol in this work demonstrated significant differences in the treatments where there is supplementation, having an average of 173.03 in grazing, 109.83 in supplemented grazing and 83.96 in full confinement. Similar results to those reported by Pizzoferrato et al. [16] in goat milk where the highest value of alpha-tocopherol is 154.9 in grazing, 118.9 in supplemented grazing, 125.2 in grazing with concentrate and 77.1 in full confinement.

In the study by Pizzoferrato et al. [16] in 2007, samples were obtained for three years, and during these three years, the same trend was followed in the data for cholesterol, alpha-tocopherol and FPG. Similarly, in a study by Andrea Cabiddu et al. [20] in 2017 in Italy reported a higher amount of vitamin E, but also a higher amount of cholesterol in the milk of goats fed a diet high in forage. In this study, two feeding treatments were made: the first treatment determined a diet with low forage coverage and I call it LH (low herbage cover) and the second treatment HH (high herbage cover) was a diet with high forage coverage and obtained results in HH of 3.66 and LH of 3.52 in vitamin E, showing that the feeding of goats with high concentrations of forage contained greater amounts of this vitamin. In the case of cholesterol, higher amounts were obtained in the HH treatment, with values of 314, and in the LH treatment 294 were obtained. They also performed the GPA of their data, and a higher GPA was obtained in the second treatment 11.66 for HH and 11.97 for LH. This coincides with the similar parameters from this work since the cows were grazed with fresh forage, thus obtaining the best conditions to produce milk with high concentrations of alpha-tocopherol and therefore have a higher GPA (Degree of Antioxidant Protection) as discussed by Sies (1985).

Another study conducted by Ruiz-Nuñez et al. [25] studied the degree of antioxidant protection of organic milk, reporting that their indoor feeding data showed the lowest values compared to grass-fed groups, and the groups fed with grass + corn grain in the concentration of vitamin E, A and β-carotene [26-34]. The Degree of Antioxidant Protection showed an increasing trend in organic milk. Grazing + maize feeding was associated with the highest level of FPG (9% higher than grass-only feeding and 79% higher than indoor feeding) demonstrating a very similar trend to that found by Cabbidu et al. [20] in his study in Italy, and that we attribute to feeding the animals with hay forage; however, the prevalence follows in all reported cases where diets based on fresh forage denote a higher GPA. It is worth mentioning that, in the study by Ruiz-Nuñez et al. [25], their forage + corn feeding treatment was only supplemented with 4 kg. of corn to balance the diet to the cow's energy needs, and no commercial concentration was offered.

Conclusion

In this research we successfully developed the DAP parameter, which was used to differentiate products from grazing and zero-grazing feeding systems, provided that grazed herbage exceeded 15% in the animals that were in the total diet group. In fact, the grazing action, not just the herbage intake cut from pasture, is a quality determinant for commercial milk and cheese, contributing to price setting and probably to animal well-being. Moreover, the DAP parameter was found to be related to oxidative reactions, the main determinant of quality loss in food: the higher the DAP value, the greater the product stability and safety, considering the risk related to the intake of cholesterol-oxides in humans. The present observation showed a direct effect of the feeding system on the cholesterol and alpha- tocopherol concentration in cow's milk, influencing the degree of antioxidant protection of the product, this effect was significant higher when animals were feed only in pasture where not supplementation was added, particularly when concentrates are offered in high volumes to the diet.

References

1. Liochev SJ (2003) Reactive oxygen species and the free radical theory of aging. Free Radic Biol Med 60: 1-4.
2. Waris G, Ashan H (2006) Reactive oxygen species role in the development of cancer and various chronic conditions. J Carcinogenic 5: 14.
3. Pearson T, Popescu BO, Cedazo-Imguez A (2014) Oxidative stress in Alzheimer's disease: Why did antioxidative therapy fail. Oxid Med Cell Lomgev 2014: 427318.
4. Maulik N, Mcfadden D, Otan H, Thirunavukkarasu M, Parinandi NL (2013) Antioxidants in longevity and medicine. Oxid Med Cell Longev 2013: 820679.
5. López-Alarcona E, Nicola A (2103) Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. Anal Chim Acta 763: 1-10.
6. Sies H (1985) Oxidative stress: From basic research to clinical applications. Am J Med 91(3): 31-38.
7. Halliwell B, Gutteridge JMC, Cross CE (1992) Free radicals, antioxidants and human disease: Where are we now? J Lab Clin Med 119(6): 598-620.
8. Gutteridge JM (1993) Free radicals in disease processes: A compilation of cause and consequence. Free Radic Res Com 19(3): 141-158.
9. Galina MA, Ortíz-Rubio MA, Guerrero CM, Vazquez P, Pineda LJ (2016) Effect of feeding management on the nutritional composition of artisan soft cheese made with ewe's milk. Journal of Nutritional Ecology and Food Research 3(1): 25-31.

10. Poljsak B, Suput B, Milisav D (2013) Achieving the balance between ROS and antioxidants: When to use the synthetic antioxidants. Oxid Med Cell Longev 2013: 956792.
11. Godic A, Poljsak B, Adamic M, Dahmane R (2014) The role of antioxidants in skin cancer prevention and treatment. Oxid Med Cell Longev 2014: 860479.
12. Pisoschi AM, Pop A (2015) The role of antioxidants in the chemistry of oxidative stress: A review. European Journal of Medicinal Chemistry (97): 55-74.
13. Cheeseman H, Slater TF (1993) An introduction to free radical biochemistry. Br Med Bull 49(3): 48-493.
14. Dröge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82(1): 47-95.
15. Galina MA, Puga DC, Pineda J, Hummel J, Ortíz RM (2017) Effect of Lactobacilli symbiotic on rumen, blood, urinary parameters and milk production of Jersey cattle during late pregnancy and early lactation and grazing regarding antioxidant activity. International Journal of Applied Science and Technology, p. 11917.
16. Pizzoferrato L, Manzi P, Marconi S, Fedele V, Claps S, et al. (2007) Degree of antioxidant protection: A parameter to trace the origin and quality of goat's milk and cheese. J Dairy Sci 90(10): 4569-4574.
17. Claps S, Galina MA, Rubino R, Pizzillo M, Morone G, et al. (2014) Effect of grazing into the omega 3 and aromatic profile of bovine cheese. Journal of Nutritional Ecology and Food Research 2(3): 245-250.
18. Leiber F, Holy EW, Forestier M, Richter EK, Akhmedov A, et al. (2011) Dietary α-linolenic acid inhibits arterial thrombus formation, tissue factor expression and platelet activation. Arteriosclerosis Thrombosis and Vascular Biology 31(8): 1772-1780.
19. Marchesiono M, Cortéz M, Albrechts C, Abelly L, Soria E (2017) Modifications of the superoxide anion level in breast milk by the intake of flavonoids and carotenoids. Public Health in Mexico 59(5): 526-531.
20. Cabiddu A, Addis M, Fiori M, Spada S, Decandia M, et al. (2017) Pros and cons of the supplementation on with oilseed enriched concentrates on milk fatty acid profile of dairy sheep grazing Mediterranean pastures. Small Ruminant Research 147: 63-72.
21. Claves E, Vossen E, Smet S De (2016) Determination of α -tocopherol by reversed-phase HPLC in feed and animal-derived foods without saponification. J Sci Food Agric 96(2): 522-529.
22. Esti M, Cinquanta L, Panfili G, Manzi P, Marconi S, et al. (2004) Effects of variety and olive ripeness on nutritional quality and oxidative stability of extra virgin olive oils. Food Agric Environ 2: 129-134.
23. Priolo A, Cornu A, Prache S, Krogmann M, Kondjoyan N, et al. (2004) Fat volatiles tracers of grass feeding in sheep. Meat Science 66(2): 475-481.
24. Shingfi eld KJ, Bonnet M, Scollan ND (2013) Recent developments in altering the fatty acid composition of ruminant-derived foods. Animal 7(1): 132-162.
25. Ruiz-Núñez B, Janneke Dijck-Brouwer DA, Muskiet Frits AJ (2016) The relation of saturated fatty acids with low-grade inflammation and cardiovascular disease. J Nutr Biochem 36: 1-20.
26. Brown AJ, Jessup W (1999) Oxysterols and atherosclerosis. Atherosclerosis 142(1): 1-28.
27. Caboni MF, Hrelia S, Bordoni A, Lercker G, Capella P, et al. (1994) *In vitro* effects of 5α cholestane 35α, 5,6α triol on cultured rat cardiomyocytes. J Agric Food Chem 42(11): 2367-2371.
28. Galina MA, Pineda J, Higuera-Piedrhita R, Vázques P, Haenlein G, (2019) Effect of Grazing on the fatty acid composition of goat's milk or cheese. Adv Dairy Res 7(3): 227.
29. Panfili G, Manzi P, Pizzoferrato L (1994) HPLC simultaneous determination of tocopherol, carotenes, retinol and its geometric isomers in Italian cheeses. Analyst 119(6): 1161-1165.
30. Pizzoferrato L, Manzi P, Rubino R, Fedele V, Pizzillo M (2000) Degree of antioxidant protection in goat milk and cheese: The effect of the feeding system. International Conference on Goats, Tours, France, pp. 580-582.
31. Pizzoferrato L, Manzi P (1999) Effect of the antioxidant potential of goat cheeses: Preliminary results. Caseus 4: 28-30.
32. Róg T, Gierula MP, Vattulainen I, Karttunen M (2007) What happens if cholesterol is made smoother: Importance of methyl substituents in cholesterol ring structure on phosphatidylcholine-sterol interaction. Biophysical Journal 92(10): 3346-3357.
33. Tornambe G, Ferlay A, Farruggia A, Chilliard Y, Loiseau P, et al. (2010) Influence of botanical diversity and development stage of mountain pastures on milk fatty acid composition on carotenoids, fat-soluble vitamins and sensory properties. Grassland in a Changing World, Grassland Science in Europe, Kiel, Germany 15: 589-591.
34. Zhishun J, Jid L, Mingong S, Ye Y, Heifang Y (2022) Detection strategies for superoxide anion. A Review Talanta 236: 122892.