Changes in superoxide dismutase activity postpartum from Laoshan goat milk and factors influencing its stability during processing

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ABSTRACT
The aim of the study was to evaluate superoxide dismutase (SOD) activity from partum to 165 days postpartum of Laoshan dairy goat milk and factors affecting the stability of SOD by the autoxidation of pyrogallol method. The results indicated that the SOD activity in colostrum milk was significantly higher than that in mature milk; and it first decreased and then increased for mature milk. There was a significant decline in SOD activity from 98.54 U/mL at 65°C for 30 min treatment to 39.04 U/mL at 100°C for 5 min treatment. It indicated that SOD activity was not entirely lost, and SOD had strong resistance to high temperature. Maximal SOD activity was recorded at pH 6.5 whereas pH greater or lesser than this resulted in decreased activity. In addition, the change of SOD activity under alkaline conditions was less than that under acidic conditions. SOD activities were 105.12 and 67.08 U/mL when the rotational speeds were 45 and 400 g, respectively. Thus, SOD activity decreased as rotational speed increased. The SOD activity increased when the calcium ion concentration was increased, which ranged from 0.2 to 0.6 mg/mL. SOD activity was significantly increased by 26% in the first 4 days and decreased after 4 days in the process of fermentation. And the activity of SOD for the fifth day decreased 20% over that of the fourth day. These data comprehensively demonstrate a range of conditions change that significantly affects the integrity, activity and function of SOD in goat milk.

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Introduction
Goats are commonly raised by people in developing countries, many of whom are poor. Nowadays, goat milk is becoming increasingly popular. It is reputed to be a healthy food and is very rich in nutrients (Ribeiro and Ribeiro 2010; Sumarmono et al. 2015). Goat milk has been reported to be a functional food that can reduce different ailments, such as acidity, migraines, asthma, eczema, and colitis, in humans (Silanikove et al. 2010). It is rich in a variety of antioxidant enzymes, such as superoxide dismutase (SOD). SOD is an essential enzyme in human cells having vital antioxidant properties (Naif et al. 2015). It functions as the first line of defence, and can protect cells from oxidative damage caused by reactive oxygen species by converting the superoxide radical into oxygen and hydrogen peroxide, thereby protecting cells from oxidation (Arockiaraj et al. 2014; Lifeng et al. 2014). Moreover, SOD can also prevent the superoxide radical from integrating to some other molecules, such as superoxide can react with nitrogen oxide to peroxynitrite, initiating lipid peroxidation (Zhidkova et al. 2011).

SOD is classified into four major types: copper/zinc SOD (Cu/Zn-SOD), manganese SOD (Mn-SOD), iron SOD (Fe-SOD), and nickel SOD (Ni-SOD) (Zelko et al. 2002; Liochev and Fridovich 2010). As an antioxidant enzyme, SOD activity was analysed for the relation with milk somatic cell counts, such as macrophage, lymphocyte, and polymorphonuclear leukocyte (Hamed et al. 2008). Total SOD activity levels in serum were associated with lower intakes of most nutrients other than energy, carbohydrate, iron, copper, manganese, retinol equivalents, vitamin A, B₂, B₁₂, niacin, folic acid, vitamin C and fish fat (Maruyama et al. 2009). SOD is also as oxidative stress parameters in some special research (Chung et al. 2006; Schogor et al. 2013; Juma and Shen 2017). Peptides from natural sources such as milk are shown to have a wide spectrum of biological activities. Three peptides with antioxidant capacity were identified from camel milk protein hydrolysate and molecular analysis showed
that KQ-15 peptide was able to increase the expression of SOD gene (Homayouni-Tabrizi et al. 2016). The enzyme is also contained in bovine milk and has been suggested to diminish the oxidative problems associated with the superoxide anion. Kiyosawa et al. (2014) have reported that the SOD concentration of human milk is 2.0 to 2.3 times higher than that of bovine milk. Talukder et al. (2015) observed that cows with ovulatory oestrous cycles presented significantly greater SOD levels than cows that did not ovulate and postulated that the elevated level of milk SOD and the observed lower level of LPO, GSH-Px and GSH in ovulating cows may be an essential event preceding the ovulatory response.

In dairy goats, the previous studies have focussed on the effects of the dietary supplementation on the activities of SOD, which showed that the dietary intake positively affected at least partially, some enzymatic and non-enzymatic antioxidant defences of blood and milk (Paraskevakis 2015; Sharifi et al. 2017; Tsiplakou et al. 2017). However, to date no studies have been published on SOD activity postpartum from goat milk and factors influencing its stability during processing. Changes in SOD activity per individual as well as days postpartum are expected because the material in goat milk change per individual and days postpartum. Additionally, since goat milk is also affected by conditions such as temperature, citric acid, rotational speed, calcium ion concentration, and fermentation time in the production process, it is hypothesised that these factors may affect the activity of SOD. Importantly, the SOD activity in goat milk is directly related to its nutritional value. The present study is an attempt to determine SOD activity in colostrum and mature milk of Laoshan goats by the autoxidation of pyrogallol method, which is simple and rapid, and is based on the ability of the enzyme to inhibit autoxidation.

Materials and methods

Ethical approval

Experiments on animals were performed in accordance with the ethical guidelines and regulations set forth by the Ethical Committee of Qilu University of Technology.

Collection and preparation of goat milk

The samples of mixed raw milk was collected from the Tai’an Three Xi Goat Farms in Shandong province (http://sxsyn.com/), milking by machine to investigate the factors influencing SOD stability during processing. In addition, 10 samples goats were randomly selected for collecting the colostrum and mature samples in order to investigate the variation of SOD activity with lactation period from partum to 135 days postpartum. All these 10 goats, aged of 30–35 months, were healthy, well-organised by utilising the free stall bar raising technique, feeding the TMR (total mixed rations) silage diet, clearing the faecal by labour, no acute mastitis and other clinical diseases and milking by manual milking at 10:00 am. The colostrum samples (n = 7 × 10) were collected from the first to seventh lactation day after parturition (the first 7 days of lactation and once a day individual). The samples of mature milk (n = 6 × 10) were collected on the 15th, 45th, 75th, 105th, 135th and 165th day after parturition individually. All samples were transported to the laboratory on ice and stored at −20 °C.

Determination of SOD activity in goat milk

No spoiled goat milk was selected, followed by melting at room temperature. The pH of goat milk samples was adjusted to 4.6 by 1 mol/L of phosphate, then the samples were centrifuged by refrigerated centrifuge at 4 °C and 550 g for 10 min according to Palmquist and Jenkins (2003), and separated into the upper fat, the middle whey and the bottom casein. Finally, whey was the only experimental sample to be collected, then the pH of whey was adjusted to 6.0 with sodium hydroxide. The total SOD activity in colostrum and mature milk were measured by the autoxidation of pyrogallol method (Gao et al. 1998; Lan et al. 2008). In the autoxidation of pyrogallol method, all experiments were performed at 25 °C ± 0.1 and A325 nm is measured every 30 s for 5 min. The rate of pyrogallol, which was determined by mixing 0.1 mol/L Tris–HCl, double dismutase water with pyrogallol. The rate of SOD activity, which was determined by mixing 0.1 mol/L Tris–HCl, double dismutase water, whey samples with pyrogallol. Moreover, the SOD active unit is defined as the amount of enzyme that inhibited 50% of pyrogallol per minute in each millilitre reaction solution.

Determination of SOD activity in goat milk with different temperature

Take the 100 mL goat milk samples in the test tube, and samples were heated at 65, 75, 85, 90, and 100 °C for 30, 20, 15, 10, and 5 min. Goat milk samples were stored in the refrigerator at 5 °C after the temperature of samples decreased to room temperature. According to the above method, SOD activity was measured every other day for a total of seven times.
**Determination of SOD activity in goat milk with different pH**

Take the 100 mL goat milk samples in the test tube, the pH of the samples was adjusted to pH 4.5, 5.5, 6.5, 7.5, and 8.5 and stored in the refrigerator at 5°C. According to the above method, SOD activity was measured every other day for a total of five times.

**Determination of SOD activity in goat milk with different rotational speed**

Take the 100 mL goat milk samples in the test tube, treated with rotational speeds of 45, 100, 180, 280, and 400 g, and stored in the refrigerator at 5°C. According to the above method, SOD activity was measured every other day for a total of seven times.

**Determination of SOD activity in goat milk with different calcium ion concentration**

Take the 100 mL goat milk samples in the test tube, calcium chloride was added to reach concentrations of 0.2, 0.3, 0.4, 0.5, and 0.6 mg/mL and stored in the refrigerator at 5°C. According to the above method, SOD activity was measured every two days for a total of seven times.

**Determination of SOD activity in goat milk with different fermentation time**

Take the 100 mL goat milk samples in the conical flask. Samples were inoculated with lactic acid bacteria with a weight percentage of 18% and fermentation was conducted for 2 days at 37°C. According to the above method, SOD activity was measured every day for a total of 7 days.

**Statistical analysis**

The data of colostrum and mature milk were presented as mean±SD (standard deviation) from 10 goats, and each experiment was carried out in triplicate. Statistical evaluation was performed by using one-way analysis of variance (ANOVA) of the SPSS 19.0 Statistics Programme (SPSS Inc., Chicago, IL). Mean differences were established by Fisher’s least significant difference test for multiple comparison with a significance level $\alpha = 0.05$ and very significance level $\alpha = 0.01$.

**Results and discussion**

**Changes of SOD activity in the colostrum during postpartum lactation**

The SOD activity was the average of the colostrum from the Laoshan goats in Figure 1. As shown in Figure 1, SOD activity changed greatly according to the number of days postpartum. The SOD activity first increased and then decreased. The SOD activity increased from 102.89 to 126.31 U/mL in the first 4 days of sampling followed by a continuous decline. Statistically, the activity of SOD changed no significant for the first 6 days ($p > .05$), but it changed significantly compared to the 7th day ($p < .05$). Overall, the SOD activity in colostrum was the highest of the whole lactation period. In breast milk, the average concentrations of Cu/Zn- and Mn-SOD in colostrum were 0.14 and 1.11 µg/mL, respectively, and the percentages of Mn-SOD to total SOD in colostrum 88.8% (Kiyosawa et al. 2014). It is apparent that the SOD concentration of Laoshan goat milk is higher than the breast milk. The previous study showed that the SOD activity in sow colostrum increased until 12–18 h postpartum and remained at a similar level afterwards, which may indicate an adjustment of the antioxidant system to the current needs of the whole organism and the mammary gland in particular (Lipko-Przybylska and Kankofer 2012). The dynamic changes of the SOD activity in colostrum were related to the growth and development of newborn kids. Human milk exerts direct pharmacologic relaxation effects and provides better antioxidant protection compared with infant formulas because of the presence of specific...
enzymatic components, such as human SOD (Lugonja et al. 2013). The previous study showed that an important effect of lactation stage on milk γ-glutamyl transferase (GGT) activity, and a positive relationship between GGT activity and milk protein concentration was observed, which could influence protein synthesis, as well as protein composition, with possible implications for cheese-making properties related to the milk protein fractions (Calamari et al. 2015). The time-dependent alterations of IgA, IgG, IgM, the C3, C4 protein and CuZn-SOD in human breast milk collected in the diurnal and nocturnal colostrum were observed by the studies of França and Nicomedes (2010). Similar conclusions may arise from our study that is the correlations between protein concentrations, SOD activity with the lactation. It was found that the stage of lactation had a significant \( p < .01 \) influence on the protein content, which corresponds with the information provided by Pavić et al. (2002) and Kozaczynski et al. (2004). SOD could protect newborn kids from oxidative damage by maintaining redox homeostasis (Castillo et al. 2005). Meanwhile the high activity of SOD in colostrum could also affect the stability of other enzymes, and prevent lipid peroxidation in goat milk (Gaål et al. 2006; Lipko-Przybylska and Kankofer 2012). Antioxidative defence system in colostrum shows dynamic changes that allow for providing with necessary protection from oxidative stress conditions, which may appear after parturition (Albera and Kankofer 2010).

**Changes of SOD activity in mature milk during postpartum lactation**

From Figures 1 and 2, it could be seen that the SOD activity of second day, third day and fourth day in colostrum was significantly higher than that of 15th, 45th and 105th day in mature milk \( p < .05 \). Compared with Figure 1, the activity of SOD in mature milk was smaller than that in colostrum, and it has significant difference \( p < .05 \). The SOD activity changed regularly with the progress of lactation, which was consistent with the results of study that the SOD activity of colostrum at one day after calving was the greatest and then decreased quickly with the coefficient of variation (CV) of 16.5% (Wang et al. 2000). Figure 2 showed that SOD activity in mature milk was first decreasing and then increasing generally, but there was little change with the extension of lactation period. The highest activity of SOD was 105 U/mL in 165th day (fifth lactation month), and the lowest was 82.52 U/mL in 105th day postpartum (sixth lactation month). Kiyosawa et al. (2014) have reported that human mature milk contained 0.43 and 0.99 µg/mL of the average concentrations of Cu/Zn- and Mn-SOD, respectively, which was much lower than that in Laoshan goat mature milk. There was no significant difference from 15th day to 165th day postpartum \( p > .05 \), and it showed that SOD activity was very stable during mature milk. Studies of Lipko-Przybylska and Kankofer (2012) showed that the observed differences in the activity of the enzyme in relation to lactation may be connected with the intensification of stress symptoms during labour or with the need to reach a certain level of maturity in order to create adequate antioxidant defence. In the previous research, no significant effect of breed or stage of lactation on SOD activity could be detected, but individual cows with high and low milk SOD levels during the entire lactation have been presented (Granelli et al. 2010). In this study, significantly differences of the activity of SOD were identified between individuals of the same lactation period, and the variation of SOD activity with lactation conformed to the previous results that for normal milk the SOD activity decreased in first and second lactation month, was the lowest in third and fourth lactation month, then increased in fifth and sixth lactation month, and remained stable in seventh and eighth lactation month (Wang et al. 2000).

**The effect of temperature on SOD activity in goat milk**

As shown in Figure 3, SOD had strong resistance to high temperature, and the activity of SOD was reduced by 58% at 100 °C for 30 min. The SOD activity was 98.54 and 45.28 U/mL at 65 and 95 °C for 30 min,
respectively, and the activity of SOD decreased significantly from 75 to 85 °C (p < .05). Heat treatments greater than 75 °C inactivated more than 20% of the SOD. Purified SOD fractionated from bovine milk was more sensitive to thermal processing than the SOD in bovine milk serum (Hicks et al. 1979). The present study showed that with the increase of storage time, the activity of SOD decreased gradually, but at the same temperature, there was no significant difference between the different preservation times (p > .05). Ma (2006) reported that the activity of SOD in fresh milk was 44.23 ± 4.06 U/mL, sterilised milk by 75 °C/15 s was 43.71 ± 6.56 U/mL, no notable change (p > .05), boiled milk by 95 °C/1 min was 37.15 ± 5.82 U/mL, going down ~15%, rising temperature had no notable change (p > .05). The Cu/Zn-SOD and Mn-SOD were kinds of metal enzymes, and the metal ion was stable with heat, so Cu/Zn-SOD and Mn-SOD were stable with heat. At the same time, SOD activity gradually decreased with the number of storage days, from the first day to the ninth day, which agreed with the previous study that the oxidative stability of milk was changed during the storage times (Marenjak et al. 2008). Meanwhile, with the extension of stored time, the change of SOD activity was statistically significant (p < .01) between different temperatures, which showed that the temperature and stored time had simultaneous effect on the activity of SOD. Kiyosawa et al. (2014) have reported that the SOD activity in milk during storage in the refrigerator (at < 5 °C) decreased rapidly with storage time and fell to 40% of that of fresh skim milk after 5 days. And, it is also reported that the SOD activity were significantly higher 14 h after the collection and cold storage in both breeds of dairy goats with no difference between the breeds (Marenjak et al. 2009). In the milk kept in the freezer (~80 °C) for 10 days, the SOD activity did not change in comparison with that of fresh skim milk (Nagasawa et al. 1972). The reduced SOD activity may have been caused by the effect of other components or by the attack of original enzymes such as protease.

**The effect of pH on the activity of SOD in goat milk**

At pH 6.5, the highest activity of SOD was at a concentration of 119.26 U/mL under (Figure 4). In the range of pH 4.5–6.5, with the increase of pH value, the activity of SOD increased gradually, and the activity of SOD decreased gradually when pH > 6.5. Statistical analysis found that the preservation time of the first day and the fifth day, there were no significant differences between the effects of pH on the activity of SOD in goat milk (p > .05), on the third day, compared with the condition of pH 6.5, the activity of SOD at pH 4.5 and pH 8.5 had significant difference (p < .05); and on seventh and ninth days, compared with pH 4.5, the SOD activity difference of the condition at pH 8.5, pH 7.5 and pH 6.5 reached significantly level (p < .01). At the same pH value, with prolonged storage time, the activity of SOD decreased gradually. Compared with pH 6.5, at pH 5.5, there was no significant

![Figure 3. The effect of temperature on the SOD activity in goat milk. The letter a, b, A and B indicate the significant difference in SOD activity. The same letter indicates no significant difference (p > .05) or no extremely significant difference (p > .01) in the SOD activity, different letters indicate significant difference (p < .05) or extremely significant difference (p < .01) in the SOD activity (p < .05). SOD: superoxide dismutase.](image-url)
difference \((p > .05)\), at the condition of pH 4.5 and pH 7.5, the difference of the SOD activity reached the extremely remarkable level \((p < .01)\), and significant difference \((p < .05)\) at pH 8.5.

The results showed that SOD belonged to acidic protease, which had the characteristics of tolerance to acidic conditions, and the activity of SOD was shortened in the pH condition of deviation from SOD activity stability. It can be seen that the pH value had a significant influence on the activity of SOD (Zhang et al. 2016; Klanian and Preciat 2017), acidic environment pH 6.5 was the most appropriate conditions of SOD activity, and the enzyme activity in the acidic environment was higher than in the alkaline condition. The loss of enzyme activity was caused by the loss of the centre metal ions of SOD in the pH acidic condition, and the conformation of the SOD was not reversible in pH alkaline (Wawrzykowski and Kankofer 2017).

**The effect of rotational speed on the activity of SOD in goat milk**

The SOD activity in milk is often affected by external factors in the dairy processing, such as shear, the process of centrifugation can also result in loss of SOD activity. The effect of rotational speed on the SOD activity was displayed in Figure 5. As the speed increased, the SOD activity decreased gradually, and the difference reached significant or extremely significant level \((p < .05, p < .01)\), which is due to the shear forces destroying the structure of the enzyme. On the first day of the storage time, the activities of SOD were 105.12, 94.86, 86.89, 77.64, and 67.08 U/mL at 45, 100, 180, 280, and 400g, respectively. The SOD activity still remained 52.4% on the ninth day, and the difference of the SOD activity was very significant \((p < .01)\) under different speed conditions. When rotational speeds were 280 and 400g, there were significant differences \((p < .05)\) for SOD activity in different preservation time. The loss of activity was about 38.2% in the whole process. In addition, the SOD activity decreased with the increase of storable days. Milk is a polyphasic secretion of the mammary gland with significant antioxidant activity and high calcium content bound by protein micelles. Filipovic et al. (2003) gave the structure of milk proteins and its relation with the SOD activity. Díaz-Castro et al. (2012) concluded that goat milk has positive effects on antioxidant defence, even in a situation of Fe overload, limiting lipid peroxidation. It can be seen that rotational speed was important to the activity of SOD in goat milk, it was a key factor and could not be ignored in the process of functional milk products.

**The effect of calcium ion concentration on the activity of SOD in goat milk**

Dairy products are an important source of calcium, and goat milk contains the amount of calcium needed by the human body (Vyas and Tong 2004). Nowadays, calcium was added to goat milk in order to increase calcium content. In this article, calcium chloride was added to milk samples at a concentration of 0.2–0.6 mg/mL. From Figure 6, the activity of SOD was
123.51 U/mL when the concentration of calcium ions was 0.6 mg/mL, and SOD activity increased by 31.9%. From the first day to the ninth day of the storage times, the activity of SOD decreased by 38%, in this process, the change of which was statistically significant ($p < .05$). In the same calcium ion condition, there were significant difference ($p < .05$) for different stored time. The disruption of casein micelles by Ca$^{2+}$ removal was followed by a significant decrease in SOD activity to 1.24–0.18 U mg$^{-1}$ protein. The loss of enzyme activity was ascribed to the changes in milk milieu induced by dissociation of casein micelles (Filipović et al. 2005). The inorganic Zn (ZnSO$_4$·7H$_2$O) also could cause a significant decline ($p < .05$) in the SOD activity compared to the both organic Zn (ZnMet) (Hassan et al. 2011). The breast milk iodine correlated negatively with SOD (Gutiérrezrepiso et al. 2014). Oxidised protein products can be easily found in meat.

**Figure 5.** The effect of rotational speed on the activity of SOD in goat milk. The letter a, b, A and B indicate the significant difference in SOD activity. The same letter indicates no significant difference ($p > .05$) or no extremely significant difference ($p > .01$) in the SOD activity, different letters indicate significant difference ($p < .05$) or extremely significant difference ($p < .01$) in the SOD activity ($p < .05$). SOD: superoxide dismutase.

**Figure 6.** The effect of calcium ion concentration on the activity of SOD in goat milk. The letter a, b and c indicate the significant difference in SOD activity. The same letter indicates no significant difference in the SOD activity ($p > .05$), different letters indicate significant differences in the SOD activity ($p < .05$). SOD: superoxide dismutase.
and milk during processing and storage (Li et al. 2014). Thus, the effect of calcium ion concentration on the activity of SOD was important for our study of dairy products and the concentration of calcium ion was the key factor to maintain milk products quality (Sasaki et al. 2010; Kamal et al. 2017).

The effect of fermentation time on the activity of SOD in goat milk

Fermented dairy products are one of the most common dairy products. It could be seen from Figure 7 that the SOD activity was 94.13 U/mL before ferment, it was 128.36 U/mL in the first 4 days after ferment, and significantly of increasing by 26%. The SOD activity decreased after four days, for the fifth day decreased 20% over that of the fourth day, and the SOD activity of the eighth day in goat milk decreased by nearly 19.6% compared to the second day (p < .05). There were no statistically significant differences (p > .05) between the fourth day and the second day for SOD activity. However, the SOD activity was relatively higher than other fermented milks, by more than 80 U/mL. The previous research showed that the SOD activity was risen up to 60% after ferment (Ma 2007) and the retention of SOD activity in soy-milk was ~86% kept for 3 days and there’s no significant difference for the days (p > .05) (Gao and Jia 2010). Zhang et al. (2006) reported that the sequences of SOD gene from L4 is highly homology with the sequences of Mn-SOD gene of Escherichia coli (98%) and the milk fermented by L4 demonstrated better Fe²⁺ chelating ability and reducing activity than unfermented milk. The fermented milk-soymilk had an anti-atherosclerotic activity by increasing SOD and total antioxidant status (TAS) activity of the blood and relieving the degree of thiobarbituric acid reactive substances (TBARS) compared to the other treatments, and so the fermented milk were effective in preventing and retarding the hyperlipidemia-induced oxidative stress and atherosclerosis (Tsai et al. 2009).

Conclusions

In short, there was a significant difference for SOD activity between the first 6 days and the 7th day postpartum (p < .05), and no significant difference for the following 6 months after the 7th day postpartum in dairy goat SOD (p > .05), that the SOD activity of mature milk was very stable. In order to get highly active SOD products, colostrum should be chosen. And during to its stability, mature milk is suitable for producing most of products. The SOD activity was easily affected by temperature, pH, rotational speed, calcium ion concentration, and fermentation time. With the extension of storage time, heating temperature and the speed increasing, and with the decrease of Ca²⁺ concentration, the SOD activity decreased gradually. At the same storage time, the SOD activity difference of different temperature, speed and Ca²⁺ concentration have reached significant or extremely significant level (p < .05, p < .01). The most suitable pH for SOD was 6.5, enzyme activity in acidic environment was more than alkaline environment. The first 4 days of fermentation of goat milk, the SOD activity was significantly increased, but after 4 days of fermentation, the activity of SOD in goat milk began to gradually reduce. This showed that the SOD in goat milk belongs to acidic protease, has the characteristics of tolerance to acidic conditions, and under the condition of the deviation of pH, the activity of SOD retention time will be shortened. So please try to keep pH at neutral levels in SOD products.

The temperature, pH value, speed and concentration of Ca²⁺ were of extreme importance for SOD of goat milk. SOD in milk belongs to sensitive component, the treatment time and temperature are all the key factors that could not be ignored in the process of functional milk products. In the deep processing of health care function of dairy goat, if the development of high SOD activity of milk products, in order to obtain the best activity of products, when the processing conditions were determined, all aspects of SOD activity, microbial and product quality were of the need for consideration. In order to achieve SOD activity up to its maximum for goat milk, the effect of production conditions should meet the purpose of products during production processing.
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No potential conflict of interest was reported by the authors.

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References
Albera E, Kankofer M. 2010. Antioxidants in colostrum and milk of sows and cows. Reprod Domest Anim. 44:606–611.
Arockiaraj J, Palanisamy R, Bhatt P, Kumaresan V, Gnanam AJ, Pasupuleti M, Kasi M. 2014. A novel murrel Channa striatus mitochondrial manganese superoxide dismutase: gene silencing, SOD activity, superoxide anion production and expression. Fish Physiol Biochem. 40:1937–1955.
Calamari L, Gobbi L, Russo F, Cappelli FP. 2015. Pattern of γ-glutamyl transferase activity in cow milk throughout lactation and relationships with metabolic conditions and milk composition. J Anim Sci. 93:3891–3900.
Castillo C, Hernandez J, Bravo A, Lopez-Alonso M, Pereira V, Benedito JL. 2005. Oxidative status during late pregnancy and early lactation in dairy cows. Vet J. 169:286–292.
Chung IM, Kim JJ, Lim JD, Yu CY, Kim SH, Sang JH. 2006. Comparison of resveratrol, SOD activity, phenolic compounds and free amino acids in Rehmannia glutinosa at different stages of growth under temperature and water stress. Environ Exp Bot. 56:44–53.
Díaz-Castro J, Pérez-Sánchez LJ, Ramírez L-FM, López-Aliaga I, Nestares T, Alférez MJ, Ojeda ML, Campos MS. 2012. Influence of cow or goat milk consumption on antioxidant defence and lipid peroxidation during chronic iron repletion. Br J Nutr. 108:1–8.
Filipovic D, Kasapovic J, Pejić S, Niciforovic A, Pajović SB, Radijočić MJ. 2003. Superoxide dismutase activity in intact, extracted, or calcium depleted raw cow milk. Cent Eur J Occup Environ Med Hung. 9:284–289.
Filipović D, Kasapović J, Pejić S, Ničiforović A, Pajović SB, Radijočić MB. 2005. Superoxide dismutase activity in various fractions of full bovine milk. Acta Aliment. 34:219–226.
França EL, Nicomedes TR. 2010. Time-dependent alterations of soluble and cellular components in human milk. Biol Rhythm Res. 41:333–347.
Gašl T, Ribiczeň-Szabó P, Stadler K, Jakus J, Reiczigel J, Kóvér P, Mézes M, Sümeghy L. 2006. Free radicals, lipid peroxidation and the antioxidant system in the blood of cows and newborn calves around calving. Comp Biochem Physiol B Biochem Mol Biol. 143:391–396.
Gao DX, Jia J. 2010. Study on the garlic’s SOD extraction and the application of SOD-yogurt. Food Sci Technol. 35:220–222.
Gao R, Yuan Z, Zhao Z, Gao X. 1998. Mechanism of pyrogallol autoxidation and determination of superoxide dismutase enzyme activity. Bioelectrochem Bioenerget. 45:41–45.
Granelli K, Bjorck L, Appelqvist LA. 2010. The variation of superoxide dismutase (SOD) and xanthine oxidase (XO) activities in milk using an improved method to quantitate SOD activity. J Sci Food Agric. 67:85–91.
Gutiérrezrezipiso C, Velasco I, Garciaescobar E, Garciaerrano S, Rodríguezpacheco F, Linares F, Ms RDA, Rubiomartín E, Garridosanz L, Cobosbrazo JF. 2014. Does dietary iodine regulate oxidative stress and adiponectin levels in human breast milk? Antioxid Redox Signal. 20:847.
Hamed H, Feki AE, Gargouri A. 2008. Total and differential bulk cow milk somatic cell counts and their relationship with antioxidant factors. Comptes Rendus Biologies. 331:144–151.
Hassan AA, Ashry GME, Soliman SM. 2011. Effect of supplementation of chelated zinc on milk production in ewes. Food Nutr Sci. 2:706–713.
Hicks CL, Bucy J, Stofer W. 1979. Heat inactivation of superoxide dismutase in bovine milk. J Dairy Sci. 62:529.
Homayouni-Tabrizi M, Shabestarini H, Asoodeh A, Soltani M. 2016. Identification of two novel antioxidant peptides from camel milk using digestive proteases: impact on expression gene of superoxide dismutase (SOD) in hepatocellular carcinoma cell line. Int J Pept Res Ther. 22:1–9.
Juma A, Shen X. 2017. Lipopolysaccharide derived from the digestive tract provokes oxidative stress in the liver of dairy cows fed a high-grain diet. Lipopolysaccharide derived from the digestive tract provokes oxidative stress in the liver of dairy cows fed a high-grain diet. J Dairy Sci. 100:666–678.
Kamal M, Foukani M, Karoui R. 2017. Effects of heating and calcium and phosphate mineral supplementation on the physical properties of rennet-induced coagulation of camel and cow milk gels. J Dairy Res. 84:220.
Kiyosawa I, Matuyama J, Nyui S, Yoshida K. 2014. Cu,Zn- and Mn-superoxide dismutase concentration in human colostrum and mature milk. Biosci Biotechnol Biochem. 57:676–677.
Kljanian MG, Preciat MT. 2017. Effect of pH on temperature controlled degradation of reactive oxygen species, heat shock protein expression, and mucosal immunity in the sea cucumberStichopus adianotus. PLoS One. 12:e0175812.
Kozaczynski L, Hadziosmanović M, Cvrtila Z, Majić T, Karadjojle I. 2004. Effect of lactation and number of somatic cells on the composition and quality of goat milk. Tierarztliche Umschau. 59:453–463.
Lan Li, Zhang MX, Yuan JH. 2008. Influence of different methods of cell-wall broken on SOD activity produced by bacteria. Sci Technol Food Ind. 29:108–111.
Li ZL, Mo L, Le G, Shi Y. 2014. Oxidized casein impairs antioxidant defense system and induces hepatic and renal injury in mice. Food Chem Toxicol. 64:86.
Lifeng Y, Jinjing L, Shengmin W, Shenghu Z, Guixiang J, Aihua G. 2014. Seminal superoxide dismutase activity and its relationship with semen quality and SOD gene polymorphism. J Assist Reprod Genet. 31:549–554.
Liochev SI, Fridovich I. 2010. Mechanism of the peroxidase activity of Cu, Zn superoxide dismutase. Free Radic Biol Med. 48:1565–1569.

Lipko-Przybylska J, Kankofer M. 2012. Antioxidant defence of colostrum and milk in consecutive lactations in sows. Ir Vet J. 65:171–175.

Lugonja N, Spasić SD, Laugier O, Nikolić-Kokić A, Spasojević I, Orešćanin-Dušić Z, Vrvić MM. 2013. Differences in direct pharmacologic effects and antioxidative properties of mature breast milk and infant formulas. Nutrition. 29:431–435.

Ma S. 2006. Study on SOD & CAT and their heat stability in milk. J Dairy Sci Technol. 7–8.

Ma S. 2007. Change of activity on SOD, CAT, GSH-Px, GST in fermented milk. J Dairy Sci Technol. 67–68.

Marenjak TS, Pirsilj J, Poljičakmilas N, Milinkovićtur S, Beerlubi B, Benić M. 2008. Milk components, SOD activity and oxidative stability of milk in two breeds of dairy goats. 5th International Symposium on the challenge to sheep and goats milk sectors; 17–21 April 2007; Alghero, Italy; International Dairy Federation, p. 120–122.

Marenjak TS, Poljičakmilas N, Pirsilj J, Ljubic BB, Tur SM. 2009. Oxidative stability and quality of raw Saanen and Alpine goats milk. Archiv Für Tierzucht. 52:637–646.

Maruyama K, Iso H, Ito Y, Watanabe Y, Inaba Y, Tajima K, Nakachi K, Tamakoshi A. 2009. Associations of food and nutrient intakes with serum IGF-I, IGF-II, IGFBP-3, TGF-β1, total SOD activity and sFas levels among middle-aged Japanese: the Japan Collaborative Cohort study. Asian Pac J Cancer Prev. 10:7.

Nagasawa T, Kiyosawa I, Kuwahara K, Fukuwatari Y, Suzuki T. 1972. Changes of disc gel electrophoretic patterns of human milk protein with duration and temperature of storage of the milk. J Dairy Sci. 55:289.

Naif A, Whittal RM, Khan SR, Siraki AG. 2015. Phenylbutazone oxidation via Cu, Zn-SOD peroxidase activity: an EPR study. Chem Res Toxicol. 28:1476–1483.

Palmquist DL, Jenkins TC. 2003. Challenges with fats and fatty acid methods. J Anim Sci. 81:3250.

Paraskevakis N. 2015. Effects of dietary dried Greek Oregano (Origanum vulgare ssp. hirtum) supplementation on blood and milk enzymatic antioxidant indices, on milk total antioxidant capacity and on productivity in goats. Anim Feed Sci Technol. 209:90–97.

Pavić V, Antunac N, Mioc B, Ivanković A, Havranek JL. 2002. Influence of stage of lactation on the chemical composition and physical properties of sheep milk. Glob Environ Econ. 47:80–84.

Ribeiro AC, Ribeiro SDA. 2010. Specialty products made from goat milk. Small Rumin Res. 89:225–233.

Sasaki H, Itoh T, Akamatsu H, Okamoto H, Horio T. 2010. Effects of calcium concentration on the SOD activity and UBV-induced cytotoxicity in cultured human keratinocytes. Photodermatol Photoimmunol Photomed. 21:9–14.

Schogor AL, Palin MF, Santos GT, Benchaar C, Lacasse P, Petit HV. 2013. Mammary gene expression and activity of antioxidant enzymes and oxidative indicators in the blood, milk, mammary tissue and ruminal fluid of dairy cows fed flax meal. Br J Nutr. 110:1743–1750.

Sharifi M, Bashtani M, Naserian AA, Farhangfar H. 2017. The Effect of increasing levels of date palm (Phoenix dactylifera L.) seed on the performance, ruminal fermentation, antioxidant status and milk fatty acid profile of Saanen dairy goats. J Anim Physiol Anim Nutr. 101:e332–e341.

Silanikove N, Leitner G, Merin U, Prosser CG. 2010. Recent advances in exploiting goat’s milk: quality, safety and production aspects. Small Rumin Res. 89:110–124.

Sumamoto J, Sulistyowati M, Soenarto 2015. Fatty acids profiles of fresh milk, yogurt and concentrated yogurt from Peranakan Etawah goat milk. Procedia Food Sci. 3:216–222.

Talukder S, Kerrisk KL, Gabai G, Fukutomi A, Celi P. 2015. Changes in milk oxidative stress biomarkers in lactating dairy cows with ovariatory and an-ovulatori oestrous cycles. Anim Reprod Sci. 158:89–96.

Tsai TY, Chu LH, Lee CL, Pan TM. 2009. Atherosclerosis-preventing activity of lactic acid bacteria-fermented milk – soymilk supplemented with Momordica charantia. J Agric Food Chem. 57:2065–2071.

Tsiplakou E, Mam A, Mavrommatis A, Chatzikontantinou M, Skliros D, Sotirakoglou K, Flemetakis E, Labrou NE, Zervas G. 2017. The effect of dietary Chlorella vulgaris inclusion on goat’s milk chemical composition, fatty acids profile and enzymes activities related to oxidation. J Anim Physiol Anim Nutr. 197:106–111.

Vyas HK, Tong PS. 2004. Impact of source and level of calcium fortification on the heat stability of reconstituted skim milk powder. J Dairy Sci. 87:1177–1180.

Wang H, Chen H, Bian J. 2000. Studies on the activity of superoxide dismutase (SOD) in cow’s milk. J Yellow Cattleen. 26:20–22.

Wawrzynkowski J, Kankofer M. 2017. Partial biochemical characterization of CuZn-superoxide dismutase extracted from eggs of hens (Gallus gallus domesticus). Food Chem. 227:390–396.

Zelko IN, Mariani TJ, Folz RJ. 2002. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radic Biol Med. 33:337–349.

Zhang JW, Cao YS, Liu XH, Bo XU. 2006. SOD activity of lactic soymilk supplemented with Momordica charantia. J Agric Food Chem. 54:11116–11120.

Zhang JW, Cao YS, Liu XH, Bo XU. 2006. SOD activity of lactic soymilk supplemented with Momordica charantia. J Agric Food Chem. 54:11116–11120.