

# Determination of antioxidant activities in milks obtained from simmental breed of cattle

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## Abstract

In recent years there has been an increasing demand for foods containing bioactive compounds that have positive effects on health. Milk is among these foodstuffs. Milk also contains antioxidant compounds that reduce or prevent the risk of various diseases. Many bioactive compounds such as proteins, vitamins (E and C), retinol, beta carotene, glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) form antioxidant activity in milk. In this study, it was aimed to investigate the GSH level, GSH-Px, SOD and CAT activities in milk which was obtained from Simmental breed of cattle and raised in Tunceli province. For this purpose, milk samples were obtained from the enterprises producing milk of simmental breed of cattle at the central district of Tunceli province. The data on the milk parameters we obtained were found to be consistent with the levels reported for dairy cattle.

**Keywords:** Milk, antioxidant, glutathione, glutathione peroxidase, catalase.

## Simmental ırkı sığırlardan elde edilen sütlerde antioksidan aktivitelerin belirlenmesi

### Özet

Son yıllarda sağlık üzerine olumlu etkileri olan biyoaktif bileşenleri içeren gıdalara olan talep giderek artmaktadır. Süt bu gıda maddeleri arasındadır. Süt, hastalık risklerini azaltıcı ya da önleyici nitelikte antioksidan bileşenlerini de yapısında bulundurmaktadır. Proteinler, vitaminler (E ve C), retinol, beta karoten, glutatyon (GSH), katalaz (CAT), süperoksit dismutaz (SOD) ve glutatyon peroksidaz (GSH-Px) gibi birçok biyoaktif bileşen sütün antioksidan aktivitesini oluşturmaktadır. Bu çalışmada Tunceli ilinde satılan sütlerin GSH düzeyi ile GSH-Px, SOD ve CAT

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*aktivitelerinin araştırılması amaçlanmıştır. Araştırma örnekleri Tunceli ili merkez ilçesinde satılan sütlerden elde edilmiştir. Elde edilen süt parametrelerine ait veriler, süt sığırları için rapor edilen seviyeler ile tutarlı bulunmuştur.*

**Anahtar kelimeler:** Süt, antioksidan, glutatyon, glutatyon peroksidaz, katalaz.

## 1. Introduction

Optimum nutrition is the approach to intake of nutrients and bioactive compounds in sufficient quantities required to fulfill the functions of the body and to mitigate the disease risks that may occur in the future [1]. Nutritionists make suggestions to people about the consumption of nutritious foods containing compounds with important functions in the mechanisms that regulate metabolism and health. These non-nutritional compounds can be summarized under the title of bioactive compounds. Studies have shown that bioactive compounds have positive effects on health through various mechanisms such as substrate in biochemical reactions, cofactor or inhibitor in enzymatic reactions, absorbent in eliminating undesirable compounds in the intestines, fermentation substrate for useful bacteria, inhibitor preventing growth of harmful bacteria, and catching agent for reactive and toxic chemicals [2].

Antioxidant substances are one of the bioactive food compounds researched mostly in recent years, which form a protective shield against the effects of oxygen, causing cells to deteriorate and other harmful substances entering the body. [2, 3]. Antioxidant in the composition of foods are defined as “free oxygen radicals formed in physiological conditions in humans, or substances capable of reducing the adverse effects of one or both of the free nitrogen radicals”. Antioxidant compounds also play a role in reducing the risk of antibacterial, anticarcinogenic and cardiovascular diseases in the body by preventing the oxidation of compounds which can be oxidized [4]. While free radicals and antioxidant mechanisms are in balance in physiological conditions, the shift of this balance toward oxidants causes “oxidative stress” called as tissue damage. Free radicals cause peroxidation of membrane lipids leading to increased permeability of the membrane and disruption of the ionic balance of the cell. Measurement of thiobarbituric acid reagents such as malondialdehyde, conjugated dienes and lipid hydroperoxides are the lipid peroxide determinants that indicate oxidative stress of tissues. Under normal conditions, antioxidant defense mechanisms have been developed in the body as enzymatic [such as SOD, CAT and GSH-Px] and nonenzymatic [reduced glutathione and vitamins (A, C, E)] to prevent the formation of free radicals and damage caused by them.

In order for metabolism to be protected from oxidative damage, consumption of antioxidant-containing foodstuffs has a positive effect on the individual [4, 5]. Milk is a complex nutrient that naturally contains many components that act as antioxidants along with basic nutrients which can enable the newborn to survive. Milk is a complex nutrient that naturally contains. Many dairy compounds such as casein, whey proteins, peptides and amino acids formed by their decomposition, vitamins E, A and C, carotenoids, enzymes and lactic acid bacteria show antioxidant properties beneficial to health due to their various properties [5-9]. Some enzymes in the milk can block radical

formation or remove radicals and other peroxides with  $H_2O_2$ . Enzymes with antioxidant activity in milk are SOD, CAT and GSH-Px [6, 10, 11].

GSH is a tripeptide consisting of glutamic acid, cysteine and glycine, with a higher intracellular concentration. Glutathione, an important reductase agent and antioxidant, maintains the oxidoreduction balance of the cell and protects the cells from harmful effects of endogenous and exogenous oxidants [12, 13]. In addition to protecting -SH groups in proteins and acting as coenzymes in certain reactions, it also plays an important role in the transportation of amino acids and protein and DNA synthesis [14].

SOD is a metalloenzyme that catalyzes the conversion of the superoxide anion, the first step in the antioxidants defense, to  $H_2O_2$  by decomposition. The superoxide radical is formed by the addition of an electron to oxygen or the reduction of the molecular oxygen in aerobic cells by taking an electron. Since the superoxide radical contains an unpaired electron, it leads to cell death as a consequence of discharge of cellular energy deposits and the accumulation of toxic metabolites despite it is neither too reactive nor a strong oxidant [15]. The most important function of this radical is that it is a  $H_2O_2$  source and its conversion into a highly active hydroxyl radical by Fenton and Haber Weiss reaction in the presence of transition metals. In this case, the activity of CAT and GSH-Px enzymes is increased and the level of  $H_2O_2$  formation is under control [15, 16].

CAT protects cells from  $H_2O_2$ , which is formed as a result of oxidoreduction events and demonstrates a toxic effect. By the effect of this enzyme,  $H_2O_2$  is decomposed and becomes harmless and the decomposition product, oxygen, is in the free state [17]. In addition, CAT is reported to be required to optimize the antioxidative properties of superoxide dismutase [18]. CAT has an important role in the case of oxidative stress, even if it is not essential for some cell types under normal conditions.

GSH-Px is the most effective antioxidant enzyme responsible for the destruction of intracellular hydroperoxides. It is known that GSH-Px has two different types, one with and without selenium. It is used for the reduction of organic  $H_2O_2$  in every enzyme. The GSH-Px enzyme, which contains selenium as a prosthetic group in erythrocytes and other tissues, catalyzes the decomposition of  $H_2O_2$  and lipid peroxides by reduced glutathione; thus, it protects membrane lipids and hemoglobin against oxidation by peroxides [19, 20].

In the literature, there is no study reported investigating the determination of antioxidant activities in milks obtained from simmental breed of cattle with regard to the parameters specified in this study. Therefore, this study is thought to make a contribution to the related literature. For this purpose, it was aimed to determine GSH level as well as GSH-Px, SOD and CAT activities of milk obtained from Simmental breed of cattle raised in Tunceli.

## **2. Material and method**

The material of the study is the milk samples, simmental breed cattle milk, obtained from 10 separate enterprises in the central district of Tunceli province. Ethical committee approval was not required because milk samples were offered for sale. . In

the study, the milk was preferred milked in a single milking time (morning) in order to ensure homogeneity in each sample.

Milk samples were centrifuged at 3000 g for 10 minutes, and the fat deposited on the top was removed. GSH and SOD, GSH-Px and CAT levels were determined in the remaining milk.

GSH concentration was determined according to the method specified by Beutler et al [21]. The GSH level is based on the fact that SH groups with 5,5'-dithiobis- (2-nitrobenzoic acid) (Ellmann's solution) form yellow color and this color is measured at 412 nm. GSH level was given as  $\mu\text{mol/g}$  protein.

GSH-Px activity was measured according to the method of Beutler [22]. GSH-Px activity is calculated spectrophotometrically as the decrease in optical density of the system at 340 nm following NADPH oxidation. GSH-Px activity was calculated as U/g protein.

SOD activity is based on the principle that the nitroblue tetrazolium (NBT) reduction in the medium of the superoxide groups resulting from an enzymatic reaction in the reaction medium are inhibited by the SOD present in the sample. In the method, xanthine-oxidase, which is the product of superoxide groups, enters the reaction, resulting in reduction of the substance, formazan is formed that gives its highest absorbance at 560 nm. As the enzyme added to the medium undergoes dismutation of the produced groups, the NBT reduction reaction slows down and, as a result, the absorbance readings in the spectrophotometer decrease. Thus, SOD activity is indirectly determined by determining the suppression of formazan formation [23].

CAT activity was determined according to the method reported by Aebi [24]. It is based on the measurement of the absorbance reduction at 240 nm when the  $\text{H}_2\text{O}_2$  in the activity measurement medium is converted to water by CAT. For the  $\text{H}_2\text{O}_2$  amount, the results of CAT activity were given in k/g protein.

The Lowry method [25] was used to measure the protein amount.

### **2.1. Statistical analysis**

Descriptive statistics showing the mean and standard error levels of the studied properties of the milk samples were obtained. SPSS 18.0 package was used to obtain descriptive statistical results [26].

## **3. Results and discussion**

SOD, the most important antioxidant enzyme is found in the mitochondrial matrix of hepatocytes, erythrocytes and brain cells [20]. SOD has three types including copper/zinc or iron, and milk has copper/zinc superoxide dismutase (Cu/Zn SOD) type, besides Mn-SOD activity is present in the mother's milk [6, 17]. Cu/Zn SOD enzyme that is available in the cow's milk and its each monomer consists of about 153 amino acids containing a disulfide bond,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  ions.  $\text{Cu}^{2+}$  is responsible for the transfer of electron during the enzyme activity. Although the SOD activity in milk varies depending on the animal species, it is 0-92 U/ml in Holstein cows, 1-27 U/ml in Jersey cows and 0-89 U/ml in Ayrshire cows [27, 28]. The results in this research for

the Simmental breed are similar to those of Holsteins and Ayrshires, but are above the limits reported for Jerseys. The SOD in the mother's milk is about two times higher than the cow's milk. This enzyme is resistant to the pasteurization temperature and can continue its activity in pasteurized products [20]. Since there is no research reported on SOD levels obtained for Simmental breed in the literature, this research aims to fill this gap in related literature. The findings are given in Table 1.

The combination of CAT and superoxide dismutase has been reported to be a more effective antioxidant than butylhydroxyanisole [29]. In a study by Prasad et al. (1995), it was found that the immune system was improved and the SOD activity was found to be stronger when the calves were fed with milk containing 25 ppm Cu and 100 ppm Zn [30].

Table 1. Examined Parameters in Milk

| Parameters            | Mean± Standard deviation |
|-----------------------|--------------------------|
| GSH (µmol/g protein)  | 1.08 ± 0.10              |
| GSH-Px (U/ g protein) | 3.34 ± 0.35              |
| SOD (U/ ml)           | 56.2 ± 0.24              |
| CAT (k/g protein)     | 1.26 ± 0.28              |

CAT is a hemoprotein-like enzyme found in almost all animal and vegetable tissues containing heme-bound iron in its active moiety. The CAT activity of milk is associated with membranes in the skim milk phase. The mean CAT activity determined by polarographic method in raw milk is 1-95 U/ml. Of CAT, 60% is in the cream and 40% is in the skimmed milk. CAT concentrations in raw milk samples have been found to be higher in April than in November due to seasonal changes. CAT in milk is the least resistant to heat in milk enzymes. This enzyme converts H<sub>2</sub>O<sub>2</sub> into water and molecular oxygen in a highly efficient manner [6, 31].

When liposomes containing SOD and CAT were injected into rats, survival rates of rats exposed to 100% oxygen were increased. It is also reported that increasing the susceptibility of cells to certain medicines and oxidants, and the inhibition of oxygen consumption due to both decomposition of H<sub>2</sub>O<sub>2</sub> to oxygen and direct interaction depends on the CAT characteristics of the cells [32].

The activity of glutathione peroxidase in raw milk varies between 12-32 U/ml depending on selenium concentration [6]. It decomposes hydroperoxides from lipid peroxidation of glutathione peroxidase, inhibits new radical production and oxidation, and protects erythrocytes in blood against hemoglobin oxidation. In addition, in the presence of excessive H<sub>2</sub>O<sub>2</sub>, it catalyzes the oxidation of glutathione (GSH) to oxidized glutathione (GSSG, glutathione disulfide); meanwhile H<sub>2</sub>O<sub>2</sub> is detoxified by conversion to water [33, 34].

Debski et al. (1987) reported that the bound peroxidase activity is approximately one third of the total peroxidase activity and has similar properties to that of human and cow's milk [35]. In a study conducted by Yining et al. (2011), it has been found that feeding rats with whey protein hydrolysates for 30 days increased antioxidant activity, especially demonstrated increased levels of superoxide dismutase and GSH-Px in the liver and serum [20]. In our study, antioxidant levels found in the milk of Simmental breed of cattle are compatible with levels reported for normal healthy cows [36].

It is emphasized in many studies that milk and dairy products, which have an important role in nutrition, contain protective compounds which are effective in protecting the tissues from free radical damage, and that these compounds have therapeutic properties. Studies show that regular consumption of milk and dairy products will contribute not only to protecting the individual from oxidative damage but also to improving overall health status.

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