



## Lipid Peroxidation Risk in White and Kashar Cheese

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**Abstract:** Lipid oxidation is responsible for the development of many diseases with the loss of characteristics of foods and therefore by leading the loss of quality and formation of toxic compounds. Dairy products are also very susceptible to oxidative degradation because they contain polyunsaturated fatty acids. In this study, the oxidative deterioration levels of 50 Kashar cheese and 50 White cheese marketed in Bilecik city were evaluated using Thiobarbituric Acid (TBA), Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) tests. While TBA, TAS and TOS values of the kashar cheese samples were found as 0.057-1.552 µgMA/g (0.526±0.299 µgMA/g), 0.020-1.522 µmol Trolox equiv/L (0.853±0.222 µmol Trolox equiv/L) and between 1.322-4.140 µmol H<sub>2</sub>O<sub>2</sub> equiv/L (2.691±0.626 µmol H<sub>2</sub>O<sub>2</sub> equiv/L), respectively, TBA, TAS and TOS values of the white cheese samples were found as 0.047-1.427 µgMA/g (0.475±0.352 µgMA/g), 0.029-0.311 µmol Trolox equiv/L (0.094±0.062 µmol Trolox equiv/L) and between 2.028-3.937 µmol H<sub>2</sub>O<sub>2</sub> equiv/L (3.180±0.503 µmol H<sub>2</sub>O<sub>2</sub> equiv/L), respectively.

**Keywords:** Kashar cheese, Lipid peroxidation, White cheese.

## Beyaz ve Kaşar Peyniri'nde Lipid Peroksidasyon Riski

**Öz:** Lipid oksidasyonu, gıdaların has özelliklerini kaybederek kalite kaybına ve toksik bileşiklerin oluşumuna yol açarak birçok hastalığın gelişmesinden sorumludur. Süt ürünleri de çoklu doymamış yağ asitlerini fazla miktarda içermesinden dolayı oksidatif bozulmaya çok duyarlıdır. Bu araştırmada, Bilecik'te tüketime sunulan 50 adet kaşar peyniri ve 50 adet beyaz peynirin oksidatif bozulma düzeyleri Tiyoarbiturik Asit (TBA), Toplam Antioksidan Seviyesi (TAS) ve Toplam Oksidan Seviyesi (TOS) testi kullanılarak değerlendirildi. Kaşar peyniri örneklerinin TBA, TAS ve TOS değerleri sırasıyla 0.057-1.552 µgMA/g (0.526±0.299 µgMA/g), 0.020-1.522 µmol Trolox equiv/L (0.853±0.222 µmol Trolox equiv/L) ve 1.322-4.140 µmol H<sub>2</sub>O<sub>2</sub> equiv/L (2.691±0.626 µmol H<sub>2</sub>O<sub>2</sub> equiv/L) arasında tespit edilirken, beyaz peynir örneklerinin TBA, TAS ve TOS değerleri sırasıyla 0.047-1.427 µgMA/g (0.475±0.352 µgMA/g), 0.029-0.311 µmol Trolox equiv/L (0.094±0.062 µmol Trolox equiv/L) ve 2.028-3.937 µmol H<sub>2</sub>O<sub>2</sub> equiv/L (3.180±0.503 µmol H<sub>2</sub>O<sub>2</sub> equiv/L) arasında tespit edildi.

**Anahtar Kelimeler:** Beyaz peynir, Kaşar peyniri, Lipid peroksidasyonu.

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## INTRODUCTION

Lipid oxidation is a free radical complex chain reaction in which the formation of free radicals (initiation), the formation of hydroperoxides (diffusion), and the formation of non-radical degradation products (termination) (1,2). The most important free oxygen radicals are superoxide radical, hydrogen peroxide, hydroxyl radical and singlet oxygen, Hydroperoxyl radical is lipid radical (alkyl radical), lipid peroxy radicals (active peroxide), lipid alkoxy radicals (active oxide) (3). The most important effects of free radicals are lipid peroxidation. Hydroperoxides which occur in the chain phase of lipid oxidation and are not stable compounds cause the oxidation of pigments and vitamins and therefore dark organic polymers occur by polymerization. They turn into various metabolic forms, such as aldehydes, ketones, alkanes, alkenes, alcohols, carboxylic acids and polymerization products by facing structural degradation in chain openings (3-5). These aldehydes, which are the result of the oxidation of fatty acids, are considered as the main cause of undesirable odour and taste formation in food. Texture change, damage of vitamin and essential fatty acids lead to decrease in nutritional value and shelf life of the products and toxic compounds occur in advanced oxidation formation (3,6,7). Initial oxidation products in lipid-containing foods usually emerge at later stages of storage. As oxidation progresses, deterioration accelerates and oxidation becomes unstoppable (8,9). Products such as malondialdehyde, which predominantly result from the degradation of three or more double-linked fatty acids as a result of this reaction, are substances presenting mutagenic and carcinogenic effects determining the severity of oxidation (3,10-12). Malonaldehyde (MDA) and free radicals are toxic biological substances that cause lipid, protein, carbohydrate oxidation and DNA (deoxyribonucleic acid) damage in living organisms (2,13). As a result; pathologies such as diabetes mellitus, necrotizing enterocolitis (NEC), patent ductus arteriosus (PDA),

hypoxic ischemic encephalopathy (HIE), premature retinopathy (ROP), bronchopulmonary dysplasia (BPD), periventricular leukomalacia (PVL), intraventricular haemorrhage (IVH) and many other diseases develop (2,3,14). For this reason, many analytical methods have been developed to determine lipid oxidation. These analytical methods have been characterized based on the principle of measuring secondary products such as aldehydes and polymers and the measurement of oxidant or primary peroxide products (15). Currently, the most widely accepted methods for measuring free radicals are the use of thiobarbituric acid reactive substance malondialdehyde (MDA) or ABTS (2,2-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid) markers (3,16). In most studies the Thiobarbituric Acid (TBA) reaction is generally used because determination of MDA level is the most preferred way used to determine the oxidation in foods (11,17-20). Procedures involving thiobarbituric acid (TBA) which is an analytical reagent are widely used to determine the progress of oxidation in dairy products since lipid oxidation is one of the important quality parameters for dairy products (21-23). Liang (9) notes that the TBA test has shown positive results only in oils containing oleic and linoleic acid to a certain extent. For this reason, lipid oxidation level can be determined by measuring Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) besides TBA test in oils containing polyunsaturated fatty acids. Kashar cheese and white cheese are more affected by chemical, microbiological and physical changes in terms of their structural properties. In particular, lipid oxidation can be easily observed due to the presence of polyunsaturated fatty acids in their structure at high level and therefore reaction products that can cause human health can occur. Nevertheless, there was no study on literature in which lipid oxidation level in food was determined by determining TAS and TOS. For this purpose, in this study lipid oxidation levels of white cheese and

kashar cheese marketed in Bilecik city were determined by TBA, TAS and TOS tests.

## MATERIALS and METHODS

### Materials

In this study, 50 kashar cheese and 50 white cheese samples which are marketed at the existing stores in Bilecik city, were provided according to random sampling method without threatening to the routine sales procedure and packing material brought to the laboratory under the cold chain and kept at +4°C until the analyses were completed.

### Methods

#### Thiobarbituric Acid (TBA) Measurement

10 g of kashar cheese and white cheese sample were subjected to maceration for 2 minutes with 50 mL distilled water and then transferred to a distillation flask by being washed with 47.5 mL of water. 2.5 mL of 4 M HCl was added to the distillation apparatus and distilled in a way that 50 mL of distillate was obtained within 10 minutes. 5 mL is taken from distillation and transferred to a tube and a 5 mL of TBA solution (in 90% glacial acetic acid) was added and it was waited in boiling water bath for 35 minutes. At the end of this duration, the tubes were cooled and the absorbance values were read against the standard solution in the spectrophotometer at 538 nm wavelength and the results were given as µgMA/g (24, 25).

#### Total Oxidant Status (TOS) Measurement

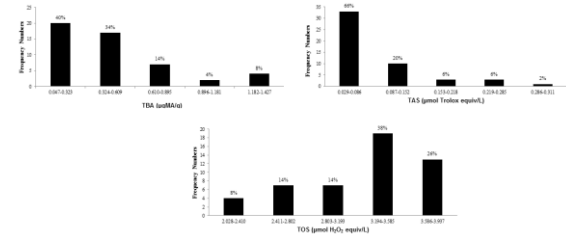
Total oxidant status measurement was determined by the method developed by Erel (26), which depends on the bases that oxidants oxidize Fe<sup>2+</sup>-o-dianisidine (ferrous ion chelator) complex to Fe<sup>+3</sup> (ferric ion) and cause an increase in absorbance by reacting with a chromogen (xyleneol ratio) in an acidic environment. The measurement was calibrated with hydrogen peroxide and the results were reported as µmol H<sub>2</sub>O<sub>2</sub> equiv/L.

#### Total Antioxidant Status (TAS) Measurement

The total antioxidant status measurement was determined by the method developed by Erel (16), which depends on the bases that the characteristic color formed by of 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical bleaches by the antioxidants in the sample added to the setting. The results are given in µmol Trolox equiv/L.

## RESULTS and DISCUSSION

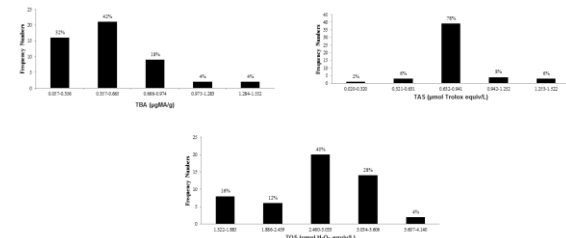
The obtained mean values of TBA, TAS and TOS amounts of the examined 50 white cheese samples are given in Table 1 and the percentage distribution and frequency numbers are given in Figure 1.



**Figure 1.** Percentage distribution and frequency numbers of TBA, TAS and TOS values determined in white cheese samples.

**Şekil 1.** Beyaz peynir örneklerinde belirlenen TBA, TAS ve TOS değerlerinin yüzde dağılımı ve frekans sayıları.

The mean values of TBA, TAS and TOS amounts of the examined 50 kashar cheese samples are given in Table 2 and the percentage distribution and frequency numbers are given in Figure 2.



**Figure 2.** Percentage distribution and frequency numbers of TBA, TAS and TOS values determined in kashar cheese samples.

**Şekil 2.** Kaşar peyniri örneklerinde belirlenen TBA, TAS ve TOS değerlerinin yüzde dağılımı ve frekans sayıları.

In the study TBA values of the white cheese samples were found as  $0.475 \pm 0.352 \mu\text{gMAg}^{-1}$  on average between 0.047-1.427  $\mu\text{gMA/g}$ , TAS values were found as  $0.094 \pm 0.062 \mu\text{mol Trolox equiv/L}$  on average between 0.029-0.311  $\mu\text{mol Trolox equiv/L}$  and TOS values were found as  $3.180 \pm 0.503 \text{H}_2\text{O}_2 \text{equiv/L}$  on average between 2.028-3.937  $\mu\text{mol H}_2\text{O}_2 \text{equiv/L}$  (Table 1). On the other hand, TBA values of the kashar cheese samples were found as  $0.526 \pm 0.299 \mu\text{gMA/g}$  on average between 0.057-1.552  $\mu\text{gMA/g}$ , TAS values were found as  $0.853 \pm 0.222 \mu\text{mol Trolox equiv/L}$  on average between 0.020-1.522  $\mu\text{mol Trolox equiv/L}$  and TOS values were found as  $2.691 \pm 0.626 \mu\text{mol H}_2\text{O}_2 \text{equiv/L}$  on average between 1.322-4.140  $\mu\text{mol H}_2\text{O}_2 \text{equiv/L}$  (Table 2).

**Table 1.** TAS, TOS and TBA amounts in white cheese samples.

**Tablo 1.** Beyaz peynir örneklerinde TAS, TOS ve TBA miktarları.

Properties	N	Minimum	Maximum	Mean
TBA ( $\mu\text{gMA/g}$ )		0.047	1.427	$0.475 \pm 0.352$
TAS ( $\mu\text{mol Trolox equiv/L}$ )	50	0.029	0.311	$0.094 \pm 0.062$
TOS ( $\mu\text{mol H}_2\text{O}_2 \text{equiv/L}$ )		2.028	3.937	$3.180 \pm 0.503$

**Table 2.** TAS, TOS and TBA amounts in kashar cheese samples.

**Tablo 2.** Kaşar peyniri örneklerinde TAS, TOS ve TBA miktarları.

	N	Minimum	Maximum	Mean
TBA ( $\mu\text{gMA/g}$ )		0.057	1.552	$0.526 \pm 0.299$
TAS ( $\mu\text{mol Trolox equiv/L}$ )	50	0.020	1.522	$0.853 \pm 0.222$
TOS ( $\mu\text{mol H}_2\text{O}_2 \text{equiv/L}$ )		1.322	4.140	$2.691 \pm 0.626$

According to the obtained findings, TBA values were different in kashar cheese and white cheese samples. The slightly higher TBA value in kashar cheese compared to white cheese samples may be due to the difference in the composition of fatty acids. The TBA values of kashar cheese and white cheese samples were found to be lower than TBA values of studies conducted using different types of cheese samples (19,20,24,27,28). Lipid oxidation occurs at low levels in cheese products (23,25,29,30). Hedegaard et al. (31) found that TBA values increased by the duration of storage and Chen et al. (32) reported that variations may occur in TBA values and that this is due to the unstable structure of malonaldehyde which presents in the structure of hydrogen peroxides. Meanwhile, it can be said that the different TBA values found in the study may result from the differences in the raw materials and production techniques in cheese production and the storage conditions. There is no restriction on the number of TBA ( $\mu\text{gMA/g}$ ) in the white cheese standard and the kashar cheese standard (33,34). Excessive and uncontrolled lipid oxidation causes undesirable rancid taste although the formation of the appropriate amounts of free fatty acids leads to desired flavor (35,36). Jenq et al. (37) stated that when the amount of malondialdehyde (MDA) is above  $0.055 \mu\text{g/g}$ , the oxide aroma can be realized in milk. Especially secondary oxidation products, especially aldehydes, are the most important compounds causing the change of taste in milk and dairy products (38). However lipolytic agents such as lipase and lipoprotein lipase which goes to milk from blood cause the acceleration of lipid oxidation in cheese and undesirable taste changes due to mechanical damage in milk fat globules such as homegeneration (39-41). Storage conditions such as gas permeability of the packaging material, relative humidity and temperature are also effective in this situation (31,42). Although significant changes are observed in MDA content, it has been stated that there is a correlation between TBA values and the taste of the oxides in milk and dairy products (25,43).

Nevertheless, Frankel and Neff (44) stated that the TBA reaction is not specific for MDA and many lipid oxidation products and reacts with other biological elements. Therefore, it can be stated that low TBA values in samples do not indicate the risk of oxidation because malonaldehyde reacts with other compounds, especially with proteins, and measurement of TAS and TOS values conducted in this study on dairy products is important in determining the lipid oxidation. Gutierrez (45) stated that the presence of Pro-oxidants significantly reduced the total antioxidant capacity and increased the oxidation products in the milk during storage. The obtained data in the study showing that TAS values are low and TOS values are high in cheese samples with high TBA values confirm the results obtained by Gutierrez (45) and Tripaldi et al. (23).

As a result, the formation of toxic compounds and undesirable taste development occur since foods containing polyunsaturated fatty acids at a high level such as dairy products are susceptible to oxidation. The risk of oxidative deterioration in dairy products should be considered in terms of standard quality production and public health. Lipid oxidation can be controlled by taking the necessary precautions such as the use of good technology in production, packaging and proper storage conditions. However, since there is no study similar to this study in which the lipid oxidation level was determined in cheese samples using TAS and TOS measurements, the obtained results will contribute to the field providing more data.

#### Conflict of interest

The authors declare that they have no conflict of interest.

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