



*Antioxidant and Some Quality Characteristics of Cheeses Manufactured Using Soy Drink*

*Soya İçeceği Kullanılarak Üretilen Peynirlerin Antioksidan ve Bazı Kalite Özellikleri*

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**Abstract**

Plant-based milk substitutes have increasingly consumed around the world owing to its a good deal of human health positive effects. Cow's milk allergy, lactose-intolerance, calorie anxiety, and the prevalence of hypercholesterolemia, vegan diets play an essential role in preferring consumers towards these products. Products with plant-based substitutes, nutritionally deficient but rich in bioactive ingredients, can be great options for improving health. We investigated the changes in some chemical properties of cheeses producing from cow's milk containing 0%, 15%, and 25% soy drink in this study. FRAP values of cheeses containing 0%, 15%, and 25% soy drink were measured as 2390.76±44.37, 3367.69±32.63, 3993.84±13.05 µmol Trolox/g cheese, respectively. Due to an increased concentration of soy drink substitutes, their antioxidant activities were increased, but the dry matters of cheeses gradually were decreased. Cheese, which contains 25% soy drink, was found to be approximately two times the antioxidant activity of cheese without soy drink. This work is a preliminary study for soy drink substitution for cheese manufacture.

**Keywords:** Cheese, Soy drink, Antioxidant activity

**Abbreviations:** TA, titratable acidity; DM, dry matter

**Özet**

Bitki bazlı süt ikameleri, insan sağlığına olumlu etkilerinden dolayı dünya çapında giderek daha fazla tüketilmektedir. İnek sütü alerjisi, laktoz intoleransı, kalori kaygısı ve hiperkolesterolemi yaygınlığı, vegan diyetler tüketicilerin bu ürünlere yönelmesinde önemli rol oynuyor. Bitki bazlı ikame maddelere sahip, besin açısından yetersiz ancak biyoaktif maddeler açısından zengin ürünler, sağlığı iyileştirmek için harika seçenekler olabilir. Bu çalışmada %0, %15 ve %25 soya içeceği içeren inek sütünden üretilen peynirlerin bazı kimyasal özelliklerindeki değişimleri araştırdık. %0, %15 ve %25 soya içeceği içeren peynirlerin FRAP değerleri sırasıyla 2390.76 ± 44.37, 3367.69 ± 32.63, 3993.84 ± 13.05 µmol Trolox/g peynir olarak ölçüldü. Soya içeceği ikame maddelerinin artan konsantrasyonu nedeniyle, antioksidan aktiviteleri arttı, ancak peynirlerin kuru maddeleri kademeli olarak azaldı. %25 soya içeceği içeren peynirin, soya içeceği bulunmayan peynirin antioksidan aktivitesinin yaklaşık iki katı olduğu tespit edildi. Bu çalışma, peynir üretimi için soya içeceği ikamesi için bir ön çalışmadır.

**Anahtar kelimeler:** Peynir, Soya içeceği, Antioksidan aktivite

## 1. INTRODUCTION

Recently, plant-based milk and its products have an increasing trend as they are a cheap alternative to the poor economic groups and consumers in places where cow's milk supply is insufficient (Sethi, Tyagi, & Anurag, 2016). The water-soluble extracts of legumes, oilseeds, cereals, or pseudo cereals that resemble cow's milk in appearance are called as plant-based milk substitutes, or plant extracts (Mäkinen, Uniacke-Lowe, O'Mahony, & Arendt, 2015). They are prepared by breaking down and reducing the size of plant sources, extracted in water, and then filtrated and homogenized, being an alternative to cow's milk (Silva, Silva, & Ribeiro, 2020). Various plant-based milk contains functional components, phenolic compounds, unsaturated fatty acids, antioxidant activity, and bioactive compounds such as phytosterols and isoflavones with health-promoting benefits. However, they are lack nutritional value compared to cow's milk (Aydar, Tutuncu, & Ozcelik, 2020). They have plentiful antioxidant activity and fatty acid, reducing the risk of cardiovascular diseases, cancer, atherosclerosis, and diabetes (Zujko & Witkowska, 2014). They are used to substitute for cow's milk in the diet for demanding individuals due to health issues concerning milk and dairy products consumption, mentioned functional properties, and individual consumption patterns (Mäkinen, Uniacke-Lowe, O'Mahony, & Arendt, 2015). Cow's milk allergy, lactose-intolerance, calorie anxiety, and the prevalence of hypercholesterolemia, vegan diets play an important role in tending consumers

towards different milk and milk product alternatives (Vanga & Raghavan, 2018). Many vegan food products such as plant-based yogurt, cheese, kefir, butter, ice cream, etc. are produced by using plant-based milk substitutes as an essential ingredient (Aydar, Tutuncu, & Ozcelik, 2020). Therefore, developing new alternative food products from a mixture of plant and animal-based milk has become inevitable to benefit consumers from plants' bioactive properties. To their declared nutritious and healthful effects, the consumption of foods obtained from soy and the incorporation of soy milk (aqueous extract of soybean) and its by-products in human diets are increasing (Rinaldoni, Campderrós, & Pérez Padilla, 2012). Singh, Vij, Hati, Singh, & Bhushan (2016) stated that soy drink obtained from conventional process averagely comprise of 3.4% protein, 1.8% of lipids, 1.5% of carbohydrate (sucrose, raffinose, and stachyose), and 0.5% of ash and it is the only vegetarian food containing all essential amino acids and economically more applicable than cow milk. Based on researches related to plant-based milk substitutes, we designed cheese by substituting soy drink because the common cheese production process is not available in soy cheese production. Our study aimed to evaluate changes of some quality characteristics and antioxidant properties of cheeses produced by replacing cow's milk with soy drink in different concentrations.

## 2. MATERIAL AND METHODS

### 2.1. Material for Cheese Production

Cow milk and soy required for the production of cheese were obtained from local businesses. White cheese culture containing *Lactococcus lactis* (mesophilic), *Lactococcus cremoris* (mesophilic) strains were used as a starter culture (Mystarter culture, CT 211 in 10 units/500 l lyophilized powder). Cheese production was carried out using rennet (Yayla, Maysa Food) whose power is 1/16.000 MCU/ml as a coagulating enzyme.  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  (Merck) was used for soy drink coagulation in the trials.

### 2.2. Preparation of Soy Drink

Soy drink was obtained by making some modifications in the method applied by Li, Xia, Zhou, & Xie (2013) on condition that the dry matter of soy drink is of the equivalent of the dry matter of cow's milk. Soybeans were kept overnight in 0.1% (w / v) sodium bicarbonate solution (solid: liquid = 1: 3, w/v). Then, soybeans were washed with clean water, and their peel was removed. Soybeans separated from their skins were blended at 95 °C with a water-soybean mixture (soy: water = 1: 4.5, w/v). The mix was shredded with a blender (Waring 8011EB) at 18000 rpm for 2 minutes. The obtained slurry was heat-treated at 85-90 °C for 10 minutes. Then it was filtered through cheesecloth, thereby not having soy residue. Soy drink containing 9-11% water-soluble dry matter, and 12-12.5% total dry matter was prepared.

### 2.3. Coagulating of Soy Drink

$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  (Merck) was utilized to coagulate soy drink because soy proteins are not capable of coagulating with starter cultures. As Ting, Kuo, Lien, & Sheng (2009) was studied, the coagulating concentration for soy drink was 0.5% (w/v)  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ .

### 2.4. Cheese Manufacture Protocol

The method described by Sahingil, Hayaloglu, Kirmaci, Özer, & Simsek (2014) with some modifications was used in cheese manufacturing. Cow's milk was pasteurized at 68°C for 10 min, then soy drink was added at rates 0%, 15%, and 25% and cooled to 32-33°C (Table 1). Commercial cheese starter cultures mix (*Lactococcus lactis* and *Lactococcus cremoris*) were added at the level of 3% (w/v) to decrease the pH of milk mixtures around 6.0 and then,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (0.02%w/w),  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  (0.5%w/w), and rennet (66.6 µL/L cow's milk) were added, respectively. The mixtures were left undisturbed for 45 min. After 45 min, the curds (pH 5.0) were cut into small cubes (2.5x2.5x2 cm) by a sterile stainless-steel knife and then, heated and incubated to 40 °C for syneresis rapidly for one hour and followed to add sodium chloride (1.5-2% w/w) to them. Then, the curds were filtrated with cheesecloth and pressed by 2 kg weight overnight. Cheeses, packed in airtight bags, were kept at 4°C for analyses.

**Table 1.** Composition of milk mixtures manufactured cheeses.

Cheese samples	Milk composition of cheeses
A	100% cow's milk+0%soy drink
B	85% cow's milk+15%soy drink
C	75% cow's milk+25%soy drink

## 2.5. Physicochemical Analyses of Cheese Materials

The total dry matter, titratable acidity, and pH measurements of cow's milk and soy drink were determined according to standard methods of the Association of Official Analytical Chemists (AOAC, 1990). Their soluble solids (°Brix) were measured with a hand Hand-Refractometer with ATC (Automatic Temperature Compensation).

## 2.6. Physicochemical Analyses of Cheese

The total dry matter, titratable acidity and pH measurements of manufactured cheeses were performed according to standard methods of the Association of Official Analytical Chemists (AOAC, 2005).

## 2.7. Antioxidant Activity Assay [Iron (III) Ion Reducing Antioxidant Power Method (FRAP)]

The iron (III) ion reducing antioxidant power (FRAP) experiment was carried out with some modifications in the method described by Benzie & Szeto (1999). FRAP reagent (10: 1: 1) was prepared by mixing 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride solution, and 10 mM TPTZ solution in 40 mM hydrochloric acid (HCl). Then, 100 µL of the sample was mixed

with 3 mL of FRAP reagent and incubated for 4 min. Incubated samples were measured at absorbance 593 nm. Reading was done by adding Frap reagent using water without sample for blank. Trolox was used as a positive control. FRAP values of samples were calculated by comparing Trolox with µmol Trolox / g cheese.

## 2.8. Statistical Assay

Results obtained from analyses of samples were statistically analyzed using SPSS software (version 20.0). Duncan's multiple ranges tests were carried out for mean separation between treatments ( $p < 0.05$ ). All analyses were performed in triplicate. All data were presented as mean±standart deviation.

## 3. RESULTS AND DISCUSSION

Before cheese processing, some chemical characteristics of cow's milk and soy drink required for cheese production are given in Table 2. As can be seen from Table 2, cow's milk and soy drink approximately possess the same total dry matter (%) were utilized for cheeses to be manufactured with a soy drink. Some chemical properties of raw materials used cheese manufacture by Elsamani, Habbani, Babiker, & Mohamed Ahmed (2014) are similar to the measurements in this study. The initial pH of the combined milk types for cheese production was 6.59 for cow's milk and 6.75 for soy drink (Table 2). After cheese manufacture, the pH values of cheeses were measured between 4.77-5.30.

**Table 2.** Some physicochemical properties of raw materials used in cheese manufacture.

	Cow's milk	Soy drink
<b>pH</b>	6.59±0.03	6.75±0.02
<b>TA (%)</b>	0.10±0.00	0.49±0.03
<b>DM (%)</b>	12.80±0.41	12.02±0.01
<b>°Brix</b>	9.90±0.14	12.03±0.25

TA (%): Titratable acidity %, DM (%): Dry matter %

When their titratable acidity values are viewed, the titratable acidity values of cheeses containing 0% and 25% soy drink substitutes were detected close to each other (Table 3). Hussein, Suleiman, Ilesanmi, & Sanusi (2016) detected the titratable acidity value of 25% soy drink added cheese was 1.38% while we measured the titratable acidity of C cheese with similar milk content as 0.72%. This situation results from the distinctness of cheese manufacture protocol. Our milk mixtures were coagulated with the addition of calcium sulfate, starter cultures, and rennet, while their protocol is required to acid coagulation with lime juice. Hence, the pH measurement of their cheese (4.61) was lower and closer than ours (4.77). The pH value of their control cheese (manufactured from 100% cow's milk) was 5.10, while that of A cheese produced with the same milk composition was 5.30. The pH values of soy cheeses produced by Li, Xia, Zhou, & Xie (2013) with several production protocols are also compatible with our study. Balogun, Oyeyinka, Kolawole, Joseph & Olajobi (2019) produced soy-tigernut cheese prepared with various milk mixtures. When we consider cheeses that are the same as the proportions of soy drink in our study, the pH values of cheeses were increased as the quantity

of soy were increased in the milk mixture for cheese production. And, the titratable acidity values of soy-tiger nut cheeses varied from 0.21 to 0.32.

For cheese, total dry matter is a critical parameter in terms of nutritional value and shelf life of cheese. The highest total dry matter value ( $50.62 \pm 1.34$ ) belonged only to the cheese manufacture from cow's milk. Depending on the quantity of soy drink substitutes in cheese manufacture, the total dry matter (%) was diminished. The incorporation of cow's milk-soy drink significantly ( $p < 0.05$ ) influenced the dry matter content of manufactured cheeses. It was observed that the total dry matter is decreased by 15-16%, with the addition of about 15-25% soy drink to cheese milk. Soy-based beverage substitution at a rate of 15-25% in cheese milk resulted in a 15-16% reduction in total dry matter values. This indicates that the nutritional value of cheeses manufactured with soy drink substitution will be lower owing to the decrease (%) of the total dry matter. It is difficult to syneresis of the cheese with soy drink substitution due to different coagulation mechanisms of soy drink (calcium sulfate) and cow's milk (starter cultures and rennet). Hussein, Suleiman, Ilesanmi, & Sanusi (2016) reported the total dry matter (%) of cheese whose milk proportion is 75% cow milk and 25% soy drink as 51.88% when cheese with similar milk proportion in this work was 34.63%. However, their control cheese (100% cow's milk) had 4% more total dry matter by comparison with our A cheese. While cheese produced from only cow's milk has the highest total dry matter, the

difference of total dry matter between cheeses has gradually increased with soy drink substitutes. The total dry matter values of B and C cheeses added soy drink in the present study were additionally similar to those (32.75 and 33.31g/100 g) described by Matias, Bedani, Castro, & Saad (2014). Balogun, Oyeyinka, Kolawole, Joseph & Olajobi (2019) measured the dry matter of soy-tiger-nut cheeses containing 75% and 85% soy drink as 35.79% and 37.28%, respectively. Increasing tiger-nut drink in milk mixture led to a decrease in dry matter. Besides, Arise, Opaleke, Salami, Awolola & Akinboro (2020) investigated the possibility of producing a cheese-like product by substituting soy drink with a variable proportion of almond drink. The pH, titratable acidity, and dry matter measurements of cheeses were respectively reported as 6.45-6.90; 0.23-0.33; 73.55-75.51. These cheeses had less acidity and higher pH than the cheeses in this work. Depending on the production method and milk types, the dry matters of cheeses produced by Arise, Opaleke, Salami, Awolola & Akinboro (2020) were higher than ours. Giri, Tripathi, & Kotwaliwale (2018) examined the physicochemical properties of soy and dairy milk cheese spreads. The values of pH, acidity, and total dry matter for their soy and dairy milk cheeses were 6.25; 6.30, 0.39; 0.30, and 50.38; 45.52, respectively. There are differences between our values and Giri, Tripathi, & Kotwaliwale (2018) values due to some differences in our raw materials and cheese production conditions.

**Table 3.** Some physicochemical properties of the manufactured cheeses.

	A	B	C
<b>pH</b>	5.3±0.02 <sup>a</sup>	4.94±0.00 <sup>b</sup>	4.77±0.00 <sup>c</sup>
<b>TA (%)</b>	0.70±0.02 <sup>b</sup>	1.05±0.16 <sup>a</sup>	0.72±0.05 <sup>b</sup>
<b>DM (%)</b>	50.62±1.34 <sup>a</sup>	35.99±0.61 <sup>b</sup>	34.63±0.12 <sup>b</sup>

A: cheese from 100% cow milk; B: cheese from 85% cow milk+15% soy drink; C: cheese from 75% cow milk+25% soy drink

<sup>a-c</sup>Lowercase letters on the same line show the statistical difference ( $p < 0.05$ ).

Table 4 reveals that the antioxidant activity values of cheeses manufactured with soy drink substitutes were determined approximately 1.5-2 times that of cheese manufacture from cow's milk only. Giri, Tripathi, & Kotwaliwale (2018) showed that the bioactivity of cheese produced with soy drink was 1.6 times higher than cheese produced with dairy milk. Due to this, it can be said that soy drink substitutes improve the antioxidant activity of dairy cheese. While the antioxidant activity value of cheese manufactured with 15% soy drink was  $3367.69 \pm 32.63^b$   $\mu\text{mol Trolox/g}$  cheese, that of cheese manufactured with 25% soy drink was  $3993.84 \pm 13.05^a$   $\mu\text{mol Trolox/g}$  cheese. It is deduced that cheeses with soy drink substitution are more valuable than cow's milk cheese in terms of antioxidant activity.

Filho, Hirozawa, Prudencio, Ida, & Garcia (2014) examined the antioxidant activities of petit-suisse and quark cheeses produced from black soybeans. According to the DPPH and ABTS method, they detected that petit-suisse had higher activity than a quark. The soy milk dry matter (%) we used in our study is approximately 2.5 times the soy milk used by Filho, Hirozawa, Prudencio, Ida, & Garcia

(2014). Therefore, it can be stated that cheeses manufactured in the current study have better antioxidant activity than cheeses produced by Filho, Hirozawa, Prudencio, Ida, & Garcia (2014). For discussion, similar studies need to be carried out since there is a limited study related to the antioxidant activity of cheese added plant-drink is tested with the FRAP method.

**Table 4.** Antioxidant activity data of the manufactured cheeses according to FRAP method ( $\mu\text{mol Trolox/g cheese}$ )

Samples	$\mu\text{mol Trolox/g cheese}$
A	2390.76 $\pm$ 44.37 <sup>c</sup>
B	3367.69 $\pm$ 32.63 <sup>b</sup>
C	3993.84 $\pm$ 13.05 <sup>a</sup>

A: cheese from 100% cow milk; B: cheese from 85% cow milk+15% soy drink; C: cheese from 75% cow milk+25% soy drink

<sup>a-c</sup> Lowercase letters in the same column show statistical difference ( $p < 0.05$ ).

#### 4. CONCLUSION

Soy drink usage in cheese manufactures significantly increases the antioxidant activity of the product. Soy drink substitution is an essential operation for enhancing the functional properties of food products. Soy can be utilized in new plant-based food products for individuals with vegan diets, health issues concerning milk and dairy products consumption, calorie anxiety, and the prevalence of hypercholesterolemia. Our findings are significant for researchers desiring to develop a suitable processing system capable of providing the required functional and chemical properties of soy cheeses types from combined milk types that closely resemble dairy cheese.

Moreover, similar studies need to be increased for the literature, as there are very few studies where the antioxidant activity of plant drink added cheese is tested by the FRAP method.

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