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Araştırma Makalesi (Research Article)

Comparison of the Compositions of Butter and Butter Oil Produced in the Province of Hakkari**

Evin SEVMİŞ[1], Seval ANDİÇ[2*], Şehriban OĞUZ[3]

[1]Van Yüzüncü Yıl Üniversitesi, Fen Bilimleri Enstitüsü, Van, Türkiye
[2,3]Van Yüzüncü Yıl Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, Van, Türkiye
[1]https://orcid.org/0000-0001-7812-8870 [2]https://orcid.org/0000-0002-8306-0222 [3]https://orcid.org/0000-0001-6889-9487
*Corresponding author e-mail: sevalandic@yyu.edu.tr

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Abstract: The aim of this study was to determine and compare certain properties of various butter and butter oils obtained from the province of Hakkari in Turkey. Butter and butter oil samples were produced by local producers using traditional methods from the same raw materials. Fat percentage and total dry matter were determined using the gravimetric method, peroxide values (PV), thiobarbituric acid (TBA), and cholesterol levels were measured using spectrophotometry, and acidity was determined by titration. The water activities (a_w) of the samples were measured using a water activity meter. In addition, coliform bacteria, yeast and mould, and lipolytic microorganism counts were determined. Total dry matter and fat values of the butter oils were significantly higher than those of the butter samples. The fat-free dry matter, a_w, ADV, cholesterol, and TBA values of the butter samples were generally higher than those of the butter oil samples. The counts of yeast and mould, lipolytic microorganism and coliform bacteria for the butter samples also exceeded those of the butter oil samples. Conversely, peroxide values were higher overall for the butter oil than the butter samples.

Hakkari Bölgesinden Temin Edilen Tereyağı ve Sadeyağların Bileşimlerinin Karşılaştırılması

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Anahtar kelimeler

Tereyağı,
Sadeyağ,
Kolesterol,
Su aktivitesi,
Peroksit değeri,
TBA.

Öz: Yapılan bu çalışmada, Hakkari bölgesinden elde edilen tereyağı ve sadeyağların bazı özelliklerinin belirlenmesi ve karşılaştırılması amaçlanmıştır. Tereyağı ve sadeyağ örnekleri, yerel üreticiler tarafından aynı hammaddeden geleneksel yöntemlerle üretilmiştir. Tereyağı ve sadeyağ örneklerinde yağ ve toplam kurumadde tayinleri gravimetrik yöntemle, peroksit değeri, TBA ve kolesterol tayinleri spektrofotometrik yöntemlerle, asitlik tayini ise titrimetrik yöntemle yapılmıştır. Örneklerin su aktiviteleri (a_w) su aktivite tayin cihazı ile ölçülmüştür. Bununla birlikte örneklerdeki koliform grubu, maya-küf ve lipolitik mikroorganizma sayıları da belirlenmiştir. Çalışmadan elde edilen analiz bulgularına göre genelde sadeyağların toplam kurumadde ve yağ değerlerinin tereyağlarına göre istatistiki olarak önemli düzeyde yüksek olduğu bulunmuştur. Tereyağı örneklerinin yağsız kurumadde, a_w, asitlik, ADV, kolesterol, TBA değerlerinin genelde sadeyağ örneklerinden yüksek olduğu bulunmuştur. Ayrıca tereyağı örneklerine ait koliform bakteri, maya-küf ve lipolitik mikroorganizma sayılarının da sadeyağ örneklerinden daha yüksek olduğu

tespit edilmiştir. Buna karşın, peroksit değerlerinin genellikle sadeyağlarda daha yüksek olduğu tespit edilmiştir.

** Bu çalışma, Evin SEVMİŞ'in "Hakkari Bölgesinden Temin Edilen Tereyağı ve Sadeyağların Bileşimlerinin Karşılaştırılması" isimli yüksek lisans tezinden üretilmiştir.

1. Introduction

Milk is produced by mammals after they give birth and constitutes a vital source of nourishment for their offspring. It is of high nutritional value and can be converted into more stable products to increase its longevity as well as into products with distinct aromatic and structural properties (Mehta, 2015). Milk-based products were discovered by chance in the distant past and have been used by humans ever since. Until the development of industrial techniques for food production, dairy products had always been produced in small batches using traditional techniques. As with other food products, however, they are now frequently manufactured on an industrial scale using standardized methods, reflecting modern technological developments in food processing and serving the needs of growing populations with their concomitant increase in demand for food. Heat processing during production, fermentation, and the concentration of certain nutrients are some of the techniques used alone or in combination with industrial food production. Butter, the subject of the present paper, is one dairy product frequently subjected to industrial methods of food production (Fox et al., 2017).

While some dairy products are produced directly from milk, without changing the proportions of any of its components, others are made by increasing the concentration(s) of one or more of its components. Butter contains higher percentages of milk fats than other dairy products and is generally produced by churning and kneading the cream derived from milk. The qualities of a given butter vary according to the properties of the raw materials, such as ripened or non-ripened cream or yogurt (Muehlhoff et al., 2013).

Butter is a dairy product that is 80-90% milkfat by weight, 0-2% fat-free dry milk substances, and maximum 16% water by weight as defined by the Turkish Food Codex (Notification No. 2005/19) (Turkish Food Codex, 2005). To produce butter using modern industrial techniques, milk cream is pasteurized and pre-fermentation is carried out using a starter culture. Salt may be added to butter for taste. The end product should be packed using proper materials in such a way as to prevent contamination. The shelf life of butter, which is limited in even the best of circumstances (such as during refrigeration) and particularly brief under suboptimal conditions, depends on the correct application of the aforementioned methods; water activity is also a factor. When butter is produced using traditional domestic methods, the cream is usually processed in its raw form and is prone to contamination by numerous microorganisms present in the immediate environment. For this reason, butter, a valuable dairy product, is converted to butter oil, especially in hot climates and/or in locations with inadequate cooling facilities.

Butter oil is called as a dairy product produced from milk and/or dairy products, from which almost the entirety of its fat-free dry matter and water has been removed, and thus contains 99-100% milk fat by weight (Turkish Food Codex, 2005). Certain dairy products similar to butter oil are also produced in Africa, Asia, and the Middle East. In India, butter oil is called "ghee", in the Middle East it is known as "maslee" or "samn", and "roghan" in Iran. The types of milk used to produce these different butter oils also vary (Atasoy and Türkoğlu, 2010).

According to recent data, the quantity of butter produced in Turkey is 57,609 tons/year (TÜİK, 2016). This figure excludes butter produced by individual households and small- and medium-sized enterprises, which are also the primary producers of butter oil; hence, no statistical data regarding production quantities of the latter exists. Butter is a valued product due to its organoleptic properties and nutritional value. It is especially valued for its distinct and preferred flavor, although its essential fatty acids (linoleic, linolenic, and arachidonic acids), fat-soluble fatty acids (A, D, E, and K), and conjugated acid contents all pose potential health implications (Palmquist, 2006). Butter is also controversial due to its cholesterol content; excessive consumption has been linked to an increased risk of cardiovascular diseases and cancer (Nestel et al., 2005). Moreover, butter is highly susceptible to microbial contamination as a result of its high water activity.

Numerous studies have been published on butter and butter oil produced outside of Turkey. Although many studies have analyzed butter from Turkey, few studies have been conducted on Turkish butter oil. The number of articles investigating the differences between butter and butter oil, both of which are derived from the same raw materials, is limited as well. No study examining the differences between butter and butter oil samples which produced from the same batch of raw materials under normal conditions of production has been published.

In eastern and southeastern Turkey, small household enterprises that raise livestock and produce dairy products convert a portion of the butter they produce into butter oil either for immediate consumption or to store for later. The decision to convert butter into butter oil may be influenced by an inability to properly store butter and/or personal preferences; butter oil has extended longevity at room temperature, whereas butter requires either refrigeration, freezing, or salinization to preserve it for long periods. There are no standardized methods, however, for the kind of production performed in small household enterprises (Kirazcı and Javidipour, 2008; Fındık and Andiç, 2017). These producers may apply excessive heat to the product or keep it at elevated temperatures for too long. Scientific studies should be conducted to determine the changes undergone by dairy products as a result of such practices. In one study, the researchers investigated the differences between butter oils processed under laboratory conditions using standard methods and butter produced under normal production conditions and used as raw materials for the aforementioned butter oils. The authors of the study concluded that there were significant differences between the butter and butter oils in respect of their contents (Fındık and Andiç, 2017). The present study also attempts to detect dissimilarities between butter and butter oil samples which produced under normal production conditions.

The aim of this study was to determine certain microbiological and chemical properties of butter and butter oils produced by small family establishments using the same raw materials in uncontrolled conditions. Ten samples of butter produced by 10 different family enterprises located in the Hakkari region of Turkey and the butter oils made from them were analyzed. Chemical and microbiological tests were carried out on the butter and butter oil samples produced using traditional methods, and the results were then compared.

2. Materials and Methods

In this study, the butter and butter oil samples to be analyzed were supplied by local producers from the province of Hakkari in southeastern Turkey. Butter samples (1 kg) produced by local producer in uncontrolled conditions and the butter oil samples (1 kg) derived from them were placed into sterile containers and transferred to the Dairy Technology Lab of the Food Engineering Department, Engineering Faculty, Van Yüzüncü Yıl University, maintaining an unbroken cold chain.

2.1. Physicochemical analysis of butter and butter oil

Fat percentage and fat-free dry matter analyses of the samples were performed using the relevant ISO (International Organization of Standardization) reference methods (ISO, 2003). The acidity (lactic acid) analysis was also analyzed according to the ISO standard method (ISO, 2012). Water activity values of the samples were determined by using AquaLab LITE brand device (Decagon Devices, Inc., Washington, USA), while the amount of free fatty acids was analyzed using the BDI (Bureau of Dairy Industries) method (IDF, 1991).

2.2. Determination of peroxide

The determination of the peroxide values was determined according to the AOAC International standard method. First, 0.3 g of the butter or butter oil samples was placed into a 20 mL glass tube. Chloroform and a methanol mixture (9.6 mL) with a volume ratio of 70:30 (v/v) were then added to the glass tube. These were mixed over a vortex to obtain a solution. A 0.05 mL quantity of ammonium thiocyanate solution (30% volume ratio) and 0.05 mL of ferrous chloride solution (35% volume ratio) containing 2% 10 M HCl were added to the previous mixture. The blanks were prepared by adding all the aforementioned solutions excluding the samples. The absorbance values of all the resulting solutions were measured using a spectrometer set at 500 nm.

To determine the concentrations, a series of ferric chloride solutions with concentrations varying between 0.25-10 mg/L were prepared, and the absorbance values obtained from this series were used. The peroxide values of the set of solutions were specified as meq O_2/kg fat (Egan et al., 1981).

2.3. Determination of cholesterol

The spectrophotometric method was used to determine the cholesterol levels of the samples. Four mL of lipid extract was dehydrated in a water bath at 55-60 °C under nitrogen. Fifteen mL of 15% KOH solution which in 90% ethanol was used to saponify the lipid remnants at 75 °C during 20 minutes. Following this, 5 mL distillate water was added to the sample tubes and cooled to room temperature. The cholesterol in the samples was extracted two times using 10 mL hexane and 4 mL of this extract was transferred to a different tube. The contents of the tube containing extract were again evaporated under nitrogen. 3 mL of 0.25% FeSO4 which in acetic acid and 1 mL of concentrated H_2SO4 were added to the tube and then the contents of the tube were mixed and cooled. After 10 minutes, measurements were taken using a spectrophotometer set at 490 nm wavelength. The standart curve was derived from the absorbance values of the serial of cholesterol standards prepared by 30-150 mg/mL concentrations (Sigma Chemical Co., St. Louis, MO, USA) and this curve was used to determine the final results (Searcy and Bergquist, 1960).

2.4. Thiobarbituric acid test (TBA test)

10 g sample with 50 mL of water was subjected to maceration for 2 minutes. Following maceration, the sample was washed with 47.5 mL water and transferred to a distillation flask. Two mL of 4 M HCl was introduced in order to reduce the pH value to below 1.5. A few glass beads were placed into the distillation flask to prevent the formation of foams, and distillation was regulated so that 50 mL of distillate was obtained every 10 minutes. Five mL of distillate was transferred to a closed tube and 5 mL of TBA reagent (0.2883 g TBA/100 mL of 90% glacial acetic acid) was added. The tubes were shaken and placed in a water bath for 35 minutes. Afterward, the tubes were cooled in cold water during 10 minutes. The absorbance values (D) were obtained by comparison with the blank prepared by using 5 mL water and 5 mL reagent (1 cm tube diameter).

TBA values (mg malondialdehyde/kg of the sample) were determined to equal D multiplied by a factor of 7.8. Since 450 nm was recommended for green pigments and 532 nm to 538 nm for red pigments, these values were used in the analyses (Kristensen et al., 1999).

2.5. Microbiological analysis

A 10 g sample of each butter and butter oil was homogenized in sterile physiological saline solution (0.85% NaCl – 90 mL) at 40 °C, for the microbiological analyses. This solution was used to prepare serial dilutions. The coliform bacteria counts were carried out using MacConkey Agar (Merck, Darmstadt, Germany) and the samples were incubated at 35 °C for 48 hours. Potato Dextrose Agar (PDA) (Merck, Darmstadt, Germany) was used to determined the yeast and mould counts and its pH was adjust to 3.5 by tartaric acid solution. The Petri dishes were incubated at 21 °C for 7 days. Tributyrin Agar (TBA) (Merck, Darmstadt, Germany) was used to performed the lipolytic microorganisms counts, then samples were incubated at 30 °C for 3 days. The counts of microorganism were transformed by using a logarithmic (log10) operation.

2.6. Statistical analysis

For the present study, the Kolmogorov-Smirnov test was used to test the normality of grouped data; all 10 groups exhibited normal distribution for all analyses. Because of this, parametric tests and the independent t-test were employed for comparison of the data. The results obtained were analyzed using SPSS software (SPSS, 1999). All samples were analyzed in triplicate and the results of analysis were shown in “mean and standard deviation” format.

3. Results and Discussion

3.1. Chemical analysis results

The fat ratios of the butter samples ranged from 67.34% ± 0.48 to 87.10% ± 0.14, while those of the butter oils were between 92.90% ± 0.07 and 99.44% ± 0.22 (Table 1). The dry matter ratios of varied from 68.50% ± 1.07 - 90.19% ± 0.06 and 99.22% ± 0.07 - 99.78% ± 0.14 for the different butters and butter oils, respectively (Table 1). From a statistical perspective, the differences between the various butters and the butter oils produced from them with respect to fat ratios and dry matter ratios were significant ($p<0.01$, $p<0.05$, respectively). However, the fat ratios of some of the butter and butter oil samples fell below the lower limits set by the Turkish Food Codex (2005) (80% and 99%, respectively). The fat values of the samples were, however, generally consistent with values obtained in similar studies and with related standards (Kirazcı and Javidipour, 2008; Fındık and Andiç, 2017). In terms of fat-free dry matter, there was no statistically significant difference between the butter oils and butter samples used as raw materials for the former ($p>0.05$) (Table 1).

Our analyses revealed that the degree of acidity of the butter oils was lower than that of the butter samples, a statistically significant difference ($p<0.05$) (Table 1). Acidity in butter increases in the serum phase; since the serum is removed during the conversion of butter to butter oil, the degree of acidity decreases (Tavlaşlar-Hocalar, 2011). The higher acidity in butter poses a risk because it accelerates hydrolysis reactions and lowers the oxidation stability (Deeth and Fitz-Geralds, 2006). Therefore, the reduced acidity of butter oils constitutes an advantage for product stability. However, given that in uncontrolled conditions each batch of butter produced has a different acidity value and that a different amount of serum is extracted from each butter during the process of butter oil production, the degree of acidity in butter oils differs.

The free fatty acid or acid degree value (ADV) is a measurement of the degree of lipolysis, an important biochemical reaction involving factors such as the water content of fatty foods, enzymes of various origins, and acidity (Egan et al., 1981). In the present study, ADV results for the butter and butter oil samples were 0.45 ± 0.02 - 14.17 ± 0.73 meq/100 g fat and 0.32 ± 0.00 - 3.05 ± 0.63 meq/100 g fat, respectively (Table 1).

An increase in ADV indicates that triglycerides have undergone hydrolysis, the speed and degree of which depend on reaction conditions. The characteristics of the output are affected by the amount and type of diglycerides, monoglycerides, and free fatty acids present. While short-chain fatty acids synthesized by hydrolysis have a major effect on flavor and aroma, unsaturated long-chain fatty acids lead to a decrease in oxidation stability (Nawar, 1996). Atamer and Sezgin (1984) found that aroma defects become clearly noticeable when the ADV reaches 1.8 mg KOH/g fat. Downey (1980) reported that butters with free fatty acid levels below 20 mg NaOH/100 g milk fat were of good quality, while those with free fatty acid levels above 40 mg NaOH/100 g milk fat generated increased lipolytic aroma. The results of our study indicated that a lipolytic aroma was apparent in most butter samples. Water solubility increases as chain length decreases. The reason that free fatty acid values of the butter oils were lower than those of the butter samples might be due to the fact that fatty acids are removed from the latter in conjunction with the serum (Nawar, 1996). Our ADV results were consistent with the findings of Kirazcı and Javidipour (2008) and Fındık and Andiç (2017).

In the present study, cholesterol values for butter and butter oil varied between 240.64 ± 0.54 - 420.64 ± 0.53 mg/100 g fat and 121.37 ± 0.80 - 376.09 ± 0.24 mg/100 g fat, respectively (Table 1). Cholesterol is an important compound associated with cardiovascular disorders. Experiments have shown that the cholesterol ratios of butter oil are usually substantially lower than those of butter. Our results were within the ranges of those obtained by Fındık and Andiç (2017) for commercially made butters (262.62-334.47 mg/100g fat) and the butter oils produced from those butters (214.58-258.59 mg/100 g fat). In the aforementioned study, the cholesterol ratios of all butter oil samples were lower than those of the respective butter samples. However, it should be stressed that the butter oils in their study were manufactured according to standardized processing methods under laboratory conditions, whereas in our study the butter oils were produced under uncontrolled conditions.

Table 1. Results of chemical analyses of butter and butter oil samples produced by using the same raw materials (mean ± standard deviation, n = 3)

Sample No	Butter	Butter oil	Butter	Butter oil	Butter	Butter oil
	Fat (%)		Dry matter (%)		Fat-free dry matter (%)	
1	80.62 ± 0.88^{a}	$99.24 \pm 0.05^{b*}$	84.00 ± 0.38^{a}	$99.78 \pm 0.14^{b*}$	3.38 ± 1.27^{a}	0.54 ± 0.08^{a}
2	80.60 ± 0.84^{a}	$99.05 \pm 0.07^{b*}$	82.14 ± 1.26^{a}	$99.22 \pm 0.07^{b*}$	1.54 ± 2.11^{a}	0.17 ± 0.14^{a}
3	84.50 ± 0.70^{a}	$99.44 \pm 0.22^{b*}$	84.93 ± 0.49^{a}	$99.71 \pm 0.02^{b*}$	0.43 ± 0.20^{a}	0.27 ± 0.25^{a}
4	87.10 ± 0.14^{a}	$99.17 \pm 0.04^{b**}$	89.74 ± 0.83^{a}	$99.51 \pm 0.05^{b*}$	2.64 ± 0.68^{a}	0.34 ± 0.10^{a}
5	79.50 ± 0.70^{a}	$92.90 \pm 0.07^{b*}$	90.19 ± 0.06^{a}	97.45 ± 1.44^{a}	10.69 ± 0.77^{a}	$4.55 \pm 1.37^{b*}$
6	79.62 ± 0.88^{a}	$99.34 \pm 0.08^{b*}$	81.63 ± 0.79^{a}	$99.53 \pm 0.04^{b*}$	2.01 ± 0.08^{a}	0.19 ± 0.12^{a}
7	83.87 ± 0.17^{a}	$99.33 \pm 0.07^{b**}$	84.95 ± 0.77^{a}	$99.39 \pm 0.11^{b*}$	1.07 ± 0.95^{a}	0.06 ± 0.03^{a}
8	83.88 ± 0.17^{a}	$99.28 \pm 0.19^{b**}$	88.46 ± 0.82^{a}	$99.76 \pm 0.005^{b*}$	4.59 ± 0.99^{a}	$0.48 \pm 0.19^{b*}$
9	82.60 ± 0.84^{a}	$99.36 \pm 0.19^{b*}$	83.79 ± 0.55^{a}	$99.64 \pm 0.13^{b*}$	1.19 ± 0.29^{a}	0.27 ± 0.05^{a}
10	67.34 ± 0.48^{a}	$99.12 \pm 0.17^{b**}$	68.50 ± 1.07^{a}	$99.54 \pm 0.01^{b*}$	1.16 ± 1.56^{a}	0.41 ± 0.19^{a}
	Acidity (%)		ADV (meq/100 g fat)		Cholesterol (mg/100 g fat)	
1	0.06 ± 0.00^{a}	$0.04 \pm 0.00^{b*}$	0.45 ± 0.02^{a}	0.36 ± 0.03^{a}	389.99 ± 0.56^{a}	$274.42 \pm 0.68^{b**}$
2	0.11 ± 0.00^{a}	$0.06 \pm 0.00^{b*}$	1.02 ± 0.06^{a}	$0.32 \pm 0.00^{b*}$	381.03 ± 0.80^{a}	$287.48 \pm 0.64^{b**}$
3	0.08 ± 0.00^{a}	0.07 ± 0.00^{a}	1.04 ± 0.27^{a}	0.45 ± 0.18^{a}	340.65 ± 0.54^{a}	$296.93 \pm 0.94^{b*}$
4	0.16 ± 0.00^{a}	$0.05 \pm 0.01^{b*}$	1.88 ± 0.00^{a}	$0.64 \pm 0.22^{b*}$	420.64 ± 0.53^{a}	$376.09 \pm 0.24^{b*}$
5	0.07 ± 0.00^{a}	$0.03 \pm 0.00^{b*}$	0.99 ± 0.00^{a}	0.58 ± 0.13^{a}	391.92 ± 0.43^{a}	$283.09 \pm 0.20^{b**}$
6	0.43 ± 0.06^{a}	$0.16 \pm 0.00^{b*}$	14.17 ± 0.73^{a}	$3.05 \pm 0.63^{b*}$	300.65 ± 0.62^{a}	$121.37 \pm 0.80^{b**}$
7	0.06 ± 0.01^{a}	$0.02 \pm 0.00^{b*}$	0.71 ± 0.02^{a}	0.41 ± 0.13^{a}	335.93 ± 0.45^{a}	$350.95 \pm 0.01^{b*}$
8	0.06 ± 0.00^{a}	0.08 ± 0.00^{a}	4.35 ± 0.09^{a}	$0.78 \pm 0.10^{b**}$	373.70 ± 0.77^{a}	$343.95 \pm 0.96^{b*}$
9	0.33 ± 0.01^{a}	$0.13 \pm 0.00^{b*}$	10.40 ± 0.37^{a}	$0.67 \pm 0.28^{b**}$	280.61 ± 0.63^{a}	$335.93 \pm 0.47^{b**}$
10	0.17 ± 0.03^{a}	0.14 ± 0.01^{a}	1.04 ± 0.02^{a}	0.80 ± 0.16^{a}	240.64 ± 0.54^{a}	$370.83 \pm 0.32^{b**}$

a, b: Different letters following the mean values for butter and butter oil indicate that the difference is statistically significant.
(*: $p<0.05$; **: $p<0.01$).

Table 2. Results of chemical and microbiological analyses of butter and butter oil samples produced by using the same raw materials (mean ± standard deviation, n = 3)

Sample No	Butter	Butter oil	Butter	Butter oil	Butter	Butter oil
	Water activity (a_w)		Peroxide Values (meq O_2/kg fat)		Thiobarbituric acid (TBA mg Malondialdehyde /kg sample)	
1	0.99 ± 0.03[a]	0.56 ± 0.01[b]*	1.15 ± 0.33[a]	1.42 ± 0.29[a]	0.21 ± 0.00[a]	0.13 ± 0.00[b]*
2	0.96 ± 0.03[a]	0.51 ± 0.02[b]*	1.36 ± 0.13[a]	1.34 ± 0.31[a]	0.12 ± 0.00[a]	0.04 ± 0.00[b]*
3	0.80 ± 0.01[a]	0.50 ± 0.00[b]*	1.64 ± 0.14[a]	1.83 ± 0.10[a]	0.23 ± 0.00[a]	0.14 ± 0.00[b]*
4	0.81 ± 0.00[a]	0.43 ± 0.01[b]*	5.48 ± 0.54[a]	5.79 ± 0.11[a]	0.27 ± 0.01[a]	0.17 ± 0.01[b]*
5	0.93 ± 0.02[a]	0.78 ± 0.01[b]*	3.06 ± 0.56[a]	1.70 ± 0.15[a]	0.24 ± 0.00[a]	0.16 ± 0.01[b]*
6	0.94 ± 0.00[a]	0.50 ± 0.03[b]*	1.60 ± 0.38[a]	3.33 ± 0.34[b]*	0.26 ± 0.02[a]	0.12 ± 0.00[b]*
7	0.96 ± 0.02[a]	0.61 ± 0.02[b]*	4.87 ± 0.67[a]	1.19 ± 0.05[b]*	0.36 ± 0.00[a]	0.08 ± 0.00[b]**
8	0.99 ± 0.02[a]	0.46 ± 0.00[b]*	6.69 ± 0.04[a]	4.53 ± 0.18[b]*	0.32 ± 0.00[a]	0.07 ± 0.00[b]*
9	0.93 ± 0.01[a]	0.43 ± 0.00[b]*	1.17 ± 0.27[a]	2.40 ± 0.13[b]*	0.26 ± 0.00[a]	0.00 ± 0.00[b]*
10	0.99 ± 0.00[a]	0.42 ± 0.00[b]**	3.87 ± 0.40[a]	3.90 ± 0.31[a]	0.14 ± 0.00[a]	0.05 ± 0.00[b]*
	Yeast and mould (log cfu/g)		Lipolytic Microorganisms (log cfu/g)		Coliform Bacteria (log cfu/g)	
1	2.74 ± 0.00[a]	1.69 ± 0.00[a]	7.27 ± 0.07[a]	3.19 ± 0.70[b]*	3.37 ± 0.00	ND **
2	3.10 ± 0.09	ND *	5.80 ± 0.28[a]	5.15 ± 0.03[a]	2.17 ± 0.00	ND **
3	4.09 ± 0.27	ND *	3.84 ± 0.21	ND *	1.69 ± 0.00	ND **
4	3.51 ± 0.15	ND *	4.19 ± 0.70	ND *	1.68 ± 0.01	ND **
5	3.43 ± 0.18	ND *	4.82 ± 0.49[a]	2.73 ± 0.61[a]	3.32 ± 0.10	ND **
6	4.82 ± 0.00	ND **	7.65 ± 0.37[a]	4.17 ± 0.67[b]*	4.35 ± 0.15	ND **
7	2.77 ± 0.00[a]	2.68 ± 0.02[a]	4.50 ± 0.70[a]	3.19 ± 0.70[a]	ND	ND
8	5.24 ± 0.00	ND **	5.28 ± 0.58	ND *	2.80 ± 0.01	ND **
9	3.24 ± 1.20	ND *	6.04 ± 0.82[a]	2.19 ± 0.70[b]*	3.61 ± 0.11	ND **
10	8.70 ± 0.01	ND **	6.37 ± 0.22[a]	1.69 ± 0.00[b]*	2.65 ± 0.04	ND**

a, b: Different letters following the mean values for butter and butter oil indicate that the difference is statistically significant.
(*: p<0.05; **: p<0.01; ND: Not detected; CFU: Colony-forming unit).

The main reason for the lower cholesterol ratios of the butter oil samples compared with those of the butter samples may be that some cholesterol was removed along with sediment when butter was converted to butter oil. Cholesterol is found in milk fats as a component of the complex that comprises the fat globule membrane; in the fat-free portion of milk, it exists within a protein complex (Huppertz and Kelly, 2006).

Another reason for the lower cholesterol levels observed in butter oils could be that the cholesterol was converted into oxidation products as a result of the heat applied during the conversion process since cholesterol is oxidized at elevated temperatures (O'Connor and O'Brien, 2006). In a study by Seçkin and Metin (2005), oxidized cholesterol compounds increased substantially with elevated temperatures (180, 200, 220, and 230 °C) and increased duration of heat exposure (10, 15, and 20 minutes). The cholesterol values for the samples in the present study were generally consistent with those reported in the literature (Seçkin et al., 2005).

The present study found that the water activity of the butter samples (0.80 ± 0.01 - 0.99 ± 0.03) decreased to 0.42 ± 0.00 - 0.78 ± 0.01 for the butter oils, the differences being statistically significant ($p<0.05$ and $p<0.01$) (Table 2). Water activity, a measurement of the availability of water content that can be utilized for microbial activity and/or chemical reactions, is one of the primary factors affecting the stability of foods. Only osmophilic yeasts and certain fungus species remain active in foods with a water activity of 0.60-0.65 a_w, and all microbiological activity ceases below 0.50 a_w (Fennema, 1996). From a microbiological perspective, the data obtained in the current study indicated that butter oil is more stable than butter. Our results were consistent with the water activity levels reported by Fındık and Andiç (2017), which were 0.96-1.00 for butter and 0.48-0.82 for butter oil, by Gómez and Salguero (1992) for butter (0.904 ± 0.050), and by Samet-Bali et al., (2009) for traditional Tunisian butter (0.79). The differences between the values obtained for the samples can be attributed to differences in the raw materials and processing conditions.

Hydroperoxide synthesis during the first stage of oxidation was determined by peroxide analysis. Oxidation typically occurs following hydrolysis and is affected by numerous factors, including the ratio of unsaturated fatty acids, heat, light, and the presence of catalysts (Atamer and Sezgin, 1984). Peroxide values of the butter oil samples ranged from 1.19 ± 0.05 - 5.79 ± 0.11 meq O_2/kg fat while those for the butter samples varied between 1.15 ± 0.33 - 6.69 ± 0.04 meq O_2/kg fat. The reason that peroxide values for butter oil are sometimes higher than those of butter may be due to thermic oxidation caused by the high temperatures applied during production (Nawar, 1996). The Codex Alimentarius (2011) standard does not specify a maximum peroxide value for butter; however, 0.6 meq O_2/kg fat is given as a maximum peroxide value for butter oil. The peroxide values obtained for the butter and butter oil samples in our study exceeded the maximum international standards, possibly as a result of the production and/or storage conditions of the butter. Our findings were generally consistent with those that have been reported in the literature (Kirazcı and Javidipour, 2008; Fındık and Andiç, 2017; Çakmakçı and Tahmas Kahyaoğlu, 2018).

In the present study, the TBA levels of the butter samples varied from 0.12 ± 0.00 to 0.36 ± 0.00 mg malondialdehyde/kg, while those of butter oils ranged between 0.00 ± 0.00 and 0.17 ± 0.01 mg malondialdehyde/kg. The TBA values of the butter oils were significantly lower than those of the butters from which they were produced ($p<0.05$, $p<0.01$) (Table 2). Hydroperoxides formed during the initial stage of oxidation decompose into by-products for the remainder of the process. These by-products of lipid oxidation (such as malondialdehyde) are determined using the TBA test (Asha et al., 2015). Malondialdehyde and similar by-products, which contain small molecular structures, are usually removed along with the serum and/or evaporate during the heating process in the production of butter oil. Asha et al. (2015) found that storage temperature (6 ± 2 °C: T1; 32 ± 2 °C: T2; 60 ± 2 °C: T3) significantly affects the TBA values of butter oil ($p<0.01$). The TBA values of butter oil obtained in our study were consistent with those reported by Özkanlı and Kaya (2007), while the TBA values of our butter samples were also similar to the results of other studies (Çakmakçı and Tahmas Kahyaoğlu, 2018).

3.2. Microbiological analysis results

The yeast and mould counts are presented in Table 2. Our analysis determined that yeast and mould count in butter samples ranged between 2.74 ± 0.00 log cfu/g and 8.70 ± 0.01 log cfu/g, while those of most of the butter oil samples were so minute as to be undetectable. With respect to the yeast

and mould counts, the differences between the butter and butter oils were for the most part statistically significant ($p<0.05$ for 5 samples and $p<0.01$ for 3 samples). Similar results were also reported by Fındık and Andiç (2017).

The lipolytic microorganism counts of the butter oils were lower than those of the butter samples, the the differences being in most cases statistically significant ($p<0.05$ for 7 of the 10 samples) (Table 2). The lowest and highest lipolytic bacteria counts for butter were 3.84 ± 0.21 and 7.65 ± 0.37 log cfu/g, respectively. In the majority of the butter oil samples, the lipolytic bacteria count exceeded the detectable limit, being below the detectable limit in only 3 samples.

While the presence of coliform bacteria was confirmed in most of the butter samples, its presence was not detected in any butter oil samples. The differences were statistically significant ($p< 0.01$) for all but one sample in which the presence of coliform bacteria could not be detected (Table 2). Microorganisms could not be identified in butter oil as their counts were below the detectable limit due to the application of heat during production. Certain groups of microorganisms were able to remain alive in butter oils, however, because the lipid component formed a protective barrier for microorganisms (Ray, 2004). Fındık and Andiç (2017) also reported microorganism results consistent with those obtained in our study for butter and butter oils.

4. Conclusion

Upon review of our findings, the higher fat and dry matter ratio of butter oil along with its lower cholesterol levels, coliform bacteria, yeast and mould, and lipolytic bacteria counts all demonstrate its advantages, chemically and microbiologically, compared to butter. Moreover, butter oil is more resistant to chemical reactions and microbiological activity compared to butter due to its considerably lower water activity. Although lipolytic microorganisms and yeast and mould were still detected in some of the butter oil samples, the water activity levels of these samples were below the levels necessary for microbiological and/or fungal activity. Future studies should investigate whether cholesterol oxidation products occur as a result of high temperatures during the production of butter oil and if so should determine what temperature levels are safe for production, sharing the results with producers. Furthermore, peroxide, TBA, and ADV levels obtained in this study indicate that high-quality butter oil cannot be produced from low-quality butter. Therefore, if butter oils are to be produced from butter, both products should be produced under controlled conditions using standardized methods to ensure product quality and safety.

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