Abstract

In this research, Sanliurfa butterfat, collected from local small-sized dairy plants located in Karacadağ Region of Sanliurfa, were stored at two different temperatures (4 and 20 °C) for 12 months. The butterfat samples have been analysed to determine some biochemical parameters (free fatty acids, acid value, peroxide value, induction time, and pH) and fatty acid composition on the 1st, 30th, 90th, 120th, 180th, 270th and 360th days of storage. The mean value of free fatty acids, acid value and peroxide value of the butterfat stored in refrigerator conditions (4 °C) were lower, and the induction time was higher than the butterfat stored at room temperature (20 °C) (P<0.05). Furthermore, the acid value, free fatty acids value, C16:1, C17:1, C18:2n6c and MUFA of the butterfat increased significantly during the storage period (P<0.05). The ratio of saturated fatty acid of the butterfat decreased during the storage (P<0.05). According to the results of free fatty acids, traditional Sanliurfa butterfat at room temperature (20 °C) could be stored safely for a maximum of 3 months, while this period could be up to 6 months for the butterfat stored at refrigerated temperature (4 °C).

Key words: Sanliurfa butterfat; storage; biochemical parameters; storage stability; fatty acid composition
Introduction

Sanliurfa butterfat (traditional anhydrous butterfat), which is a traditional dairy product of Turkey, is produced commonly in the Karacadağ Region located in the borders of Sanliurfa, Diyarbakir and Mardin. The similar traditional anhydrous butterfat is also produced in some countries in Asia, Africa and the Middle East. However, they are known by different names. For example, it is known Ghee in India, Maslee or Samn in Arab countries and Rohhan in Iran. These traditional products differ from each other in terms of milk type used in the production and specific parts of the production stages (Sserunjogi et al., 1998; Haenlein and Wendorff, 2006; Olfa et al., 2009). Sanliurfa butterfat has been protected by geographic indication since 06 July 2018 in Turkey, and it has been registered officially as Sanliurfa sadeyağı (Urfa yağı) (Turkish Patent Institute, 2018). In the geographic indication, Sanliurfa butterfat is defined as ‘A product that is produced from Awassi sheep milk, of which water and the non-fat dry matter is removed, and contains at least 99 % milk fat by weight’. Sanliurfa butterfat is generally produced after the melting step of churned butter that is produced from yoghurt. After the melting step, water and residue part of butter are removed to obtain anhydrous butterfat. In the region, the anhydrous butterfat is preferred because of taste-aroma properties and is widely used in the production of various desserts prepared by a frying process such as Baklava.

In the literature, quality characteristics, storage stability and fatty acid profile of Samn and Ghee which are produced from different milk types (cow, sheep and goat milk) with traditional and industrial scale, have been investigated (Sawaya et al., 1984; Al-Kalifah and Al-Kahtani, 1993; Mariod et al., 2010; Jing et al., 2016). Studies about the anhydrous butterfat of Turkey were mainly related with quality characteristics, storage stability and fatty acid composition of the butterfat produced traditionally in different provinces (Batun et al., 2004; Kirazci and Javidi-pour, 2008; Findik and Andiç, 2017; Sevmis, 2019). Furthermore, Kaya (2000) investigated the properties and stability of anhydrous butterfat produced from milk and yoghurt in a laboratory condition, while the other investigated the storage stability and fatty acid composition of anhydrous butterfat that was produced from raw and pasteurized sheep milk (Ozkani and Kaya, 2007). On the other hand, a limited number of studies have been conducted on the composition and properties of traditional Sanliurfa butterfat (Atasoy and Turkoğlu, 2010; Altun et al., 2017; Yokuş, 2019). However, the storage stability of Sanliurfa butterfat has not been investigated in these studies.

Sanliurfa butterfat is produced intensely in the spring months in the Karacadağ region of the province of Sanliurfa. The region has a wide range of pasture areas where sheep breeding is widely livelihood for the villagers. The traditional butterfat is stored in the cellar part of the house at room temperature in the region. The major risk during the storage of the traditional butterfat is lipid oxidation. The oxidation causes mainly the deterioration of quality parameters such as colour, flavour, aroma and nutritive value. The secondary oxidation products such as aldehyde, ketone, organic acids, which occurs after the degradation of primary oxidation products, are responsible for rancid flavour and aroma (Choe and Min, 2006; Rahila et al., 2018). Furthermore, it is stated that the primary and secondary oxidation products are toxic to consumers (Rahila et al., 2018). Especially unsaturated fatty acids in edible oils and fats cause severe technological problems because of their susceptibility to oxidation during processing and storage. In this context, storage temperature has a significant role in the oxidative stability of the product. In this study, Sanliurfa butterfat samples produced ultimately from sheep yoghurt in Karacadağ Region have been analysed to determine some biochemical parameters and fatty acid composition. For this purpose, fatty acid composition, pH, free fatty acids, acid value, peroxide value and induction period of the butterfat, stored at 4 and 20 °C, were examined during the storage period.

Materials and methods

Material

Sanliurfa butterfat samples were collected from 17 small-sized dairy plants in the Karacadağ region of Sanliurfa province. For the research, freshly produced butterfat was taken from each plant, and the butterfat was filled into glass jars. Then half of the jars were stored at 4 °C, while the remaining jars were stored at 20 °C in dark for 12 months.

Methods

pH (Cemeroğlu, 1992), free fatty acids (AOAC, 1977), acid value (AOAC, 1977), peroxide value (AOAC, 2000), induction time (Laubli and Bruttel, 1986) and fatty acid composition (TS EN ISO 12966-2, 2017) were determined on the 1st, 30th, 90th, 120th, 180th, 270th and 360th days of storage period.

In order to determine the induction time, approximately 3.5 g butterfat sample was analysed with the Rancimat 743 (Metrohm, Herisau, Switzerland) instrument at 120 °C. The airflow rate was set to 20 L/h.

The samples were esterified to determine the fatty acid composition according to TS EN ISO 12966-2 (TS EN ISO 12966-2, 2017). Operating conditions for GC (Thermo Quest Trace GC 2000 Series; Oshawa, Canada) were as follows; Detector: FID (Agilent Tech. Inc., CA, USA) at 280 °C, Column: Hewlett Packard capillary column HP-88, 100 m×0.25 mm×0.20 µm film (Agilent Technologies Ltd., Santa Clara, CA, USA), Injection mode/volume: Split (1/50) 5 µL at 250 °C, Flow rates: H₂: Air: N₂ = 33:370:30 mL min⁻¹. Oven temperature: 50 °C for 2 min, increased to 180 °C at a rate of 20 °C min⁻¹, then increased to 230 °C at a rate of 5 °C min⁻¹, and held for 8.5 min. Injection volume was 1 µL.
Statistical analysis

Two-way ANOVA and the Tukey multiple comparison test were performed using Minitab software (Minitab, State College, Pa) (Yildiz and Bircan, 1994). Results are expressed as means and standard deviation. All the analysis was carried out three replicates.

Result and discussion

Some biochemical parameters of Sanliurfa butterfat

Average values and statistical groups of some biochemical parameters of Sanliurfa butterfat in terms of storage temperature and duration were presented in Table 1. The mean pH value of the butterfat, stored at refrigerated conditions, was higher than the butterfat stored at room temperature during the storage. The mean pH value of the butterfat samples, stored at refrigerated condition, increased during the period. On the other hand, the mean pH value of the butterfat stored at room temperature increased until the 90th day of the storage period and then decreased from the 90th day to 360th day of the period. This situation may be related to the fact that low molecular weight fatty acids, formed as a result of hydrolysis, remove faster from the fat matrix, especially in the butterfat stored at room temperature.

The mean free fatty acids value of the butterfat stored at room temperature was higher than the value of the samples stored at the refrigerated condition during the storage period. The free fatty acids values of butterfat are very close to each other on the first day of the period, after that time the free fatty acids value of the butterfat increased during the storage period, however, the increasing rate of the free fatty acids value of the samples at room temperature was faster than the samples at refrigerated temperature. This difference may be due to the positive effect of the temperature on the hydrolysis reactions. It was stated that the maximum value of the free fatty acids of butterfat is defined as 0.36 % LA in Turkish Standard (Anonymous, 1995) and this value is defined as 0.3 % LA in IDF Standard (IDF, 1997). In terms of the limit value of free fatty acids (Anonymous, 1995), the maximum storage period could be six months for the samples stored at refrigerated temperature, while it could be only three months for the samples stored at room temperature. Similar to the results, it was reported that though the ratio of saturated fatty acids of ghee was greater than 65 %, its recommended shelf life was 3-6 months at room temperature (Rahila et al., 2018).

The free fatty acids value of traditionally produced Sarm was reported as 1.22-1.21 % (oleic acid) (Mariod et al., 2010). The free fatty acids value of traditional butterfat produced in Hakkari/Turkey and Van/Turkey, 0.02-0.14 % (lactic acid) (Sevmiş, 2019), 0.14-4.30 % (oleic acid) (Kirazcı and Javidipour, 2008), respectively. Free fatty acids of butterfat produced from pasteurized and raw milk was reported as 0.28 and 0.15 % (oleic acid) (Ozkani and Kaya, 2007). That value of fresh Sanliurfa butterfat was reported as 0.02-0.05 % (lactic acid) (Yokus, 2019). In this context, the free fatty acids value of Sanliurfa butterfat stored for 12 months at two different temperatures is compatible with the literature.

The acid value of the samples stored at room temperature was higher than the samples stored at refrigerated conditions (P<0.05). In addition, the acid values of the butterfat stored at two different temperatures increased during the storage period. Nevertheless, the acid value of

Table 1. Average values and groups of some biochemical parameters of Sanliurfa butterfat in terms of storage temperature and duration

| Temperature | Time (Day) | pH  | Free fatty acids (% LA) | Acid value (mg KOH/g) | Peroxide value (meq O₂/kg) | Induction time (h) |
|-------------|------------|-----|-------------------------|-----------------------|-----------------------------|-------------------|
| 4 °C        | 1          | 4.68±0.11d | 0.06±0.03e | 0.52±0.21d | 0.47±0.23d | 6.15±0.19d |
|             | 30         | 5.50±0.07ab | 0.20±0.03c | 0.94±0.21ab | 0.61±0.23bc | 5.91±0.19ab |
|             | 90         | 5.41±0.07ab | 0.28±0.03abc | 1.76±0.21abc | 1.07±0.23abd | 5.75±0.19abc |
|             | 180        | 5.60±0.07bc | 0.31±0.03bcd | 1.96±0.21bcd | 1.33±0.23cde | 5.20±0.19cde |
|             | 270        | 5.69±0.07bc | 0.33±0.03bcd | 2.07±0.21cde | 1.86±0.24de | 3.84±0.19de |
|             | 360        | 5.50±0.07ab | 0.37±0.03cde | 2.29±0.21e | 2.10±0.23ab | 3.16±0.19ab |
| 20 °C       | 1          | 4.82±0.07cd | 0.05±0.03e | 0.54±0.21c | 0.48±0.23d | 6.09±0.19a |
|             | 30         | 5.77±0.07d | 0.26±0.03cde | 1.36±0.21cd | 0.73±0.23bc | 5.95±0.19a |
|             | 90         | 5.61±0.07bc | 0.32±0.03cdef | 2.01±0.21cdef | 1.68±0.23bc | 5.62±0.19bc |
|             | 180        | 5.49±0.07de | 0.49±0.03def | 3.05±0.21def | 1.83±0.23cdef | 4.50±0.19cdef |
|             | 270        | 5.24±0.07de | 0.51±0.03de | 3.19±0.21def | 2.02±0.23cdef | 3.66±0.19cdef |
|             | 360        | 4.91±0.07de | 0.65±0.03e | 4.05±0.21c | 2.17±0.23ab | 2.92±0.19ab |

The difference between the means indicated by different letters is statistically significant at the level of P<0.05; LA: Lactic acid
the samples stored at room temperature increased faster than those samples stored at refrigerated conditions. This situation was due to the high rate of hydrolysis reactions in triglyceride molecules with the effects of storage temperature and the water (Pop and Boltea, 2014). The limit of acid value of butterfat was not defined in the Turkish Food Codex Regulation (Anonymous, 2005) and Turkish Standard (Anonymous, 1995). However, it was reported that the quantity of free fatty acids and oxidation-by-products of ghee decreased as the acid value decreased (Jing et al., 2016). The acid values of Samn, Tibetan Ghee and ghee produced from Nuami and Najdi sheep milk were reported as 1.21–2.58 mg KOH·g⁻¹ (Mariod et al., 2010), 0.02–0.27 mg g⁻¹ (Jing et al., 2016) and 1.82–1.91 mg KOH·g⁻¹ (Sawaya et al., 1984), respectively. The acid value of traditional butterfat produced in Van, Hakkari and Sanliurfa were reported as 0.05–1.79 mg KOH·g⁻¹ (Fındık and Andić, 2017), 0.32–3.05 mg KOH·g⁻¹ (Sevmiş, 2019) and 0.520–8.208 mg KOH·g⁻¹ (Yokuş, 2019), respectively. In this context, the acid value of Sanliurfa butterfat stored at two different temperatures for 12 months was parallel to aforementioned literature.

On the first day of the storage period, the peroxide value of butterfat was determined 0.47–0.48 meq O₂·kg⁻¹. Then the peroxide value increased during the storage period. In this context, the peroxide value of the samples stored at room temperature was found significantly higher than that samples stored at refrigerated conditions on the 12th months of the storage (P<0.05). This situation showed that the storage temperature is an important factor for oxidative stability of the butterfat. The Turkish Food Codex Regulation (Anonymous, 2005) and the Turkish Standard (Anonymous, 1995) do not contain any criteria regarding the peroxide value of butterfat. The maximum peroxide value of Ghee is limited as 0.6 meq O₂·kg⁻¹ by the Codex Alimentarius Standard 280–1973 (Codex Alimentarius Commission, 1997), while it was limited that the peroxide value of anhydrous milkfat should be at most 0.1 meq O₂·kg⁻¹ by International Dairy Federation Standard (IDF, 1997).

Similar to the results obtained in this study, it was reported that peroxide values of butterfat produced by different methods and stored in dark condition at 60, 70 and 80 °C ranged between 0.54–1.87 meq O₂·kg⁻¹ and peroxide values of the samples increased by increasing storage temperature (Özbayram, 2000). The peroxide value of Samn and Ghee were reported as 1.5–2.0 meq O₂·kg⁻¹ (Mariod et al., 2010) and 0.07–5.93 meq O₂·kg⁻¹ (Jing et al., 2016), respectively. The peroxide values of butterfat that was produced in Van and Hakkari were reported as 0.87–12.84 meq O₂·kg⁻¹ (Batan et al., 2004; Kirazci and Javidipour, 2008; Fındık and Andić, 2017) and 1.19–5.79 meq O₂·kg⁻¹ (Sevmiş, 2019). The average peroxide value of butterfat produced from yoghurt in a laboratory was reported as 0.21 meq O₂·kg⁻¹ (Kaya, 2000), while the peroxide values of butterfat samples made from pasteurized and raw milk were reported as 1.21 and 0.98 meq O₂·kg⁻¹ in another laboratory-scale production (Özkanli and Kaya, 2007). The mean peroxide value of the fresh Sanliurfa butterfat was reported as 0.12–0.34 meq O₂·kg⁻¹ (Yokuş, 2019). The obtained peroxide value of fresh Sanliurfa butterfat in the study was in accordance with the literature and the relevant legislation. As a result of storage for more than 3 months at both storage temperatures, the peroxide value of butterfat was found to be higher than the limits, reported in the relevant legislation. The registration certificate of butterfat has not contained any limitation regarding the use of antioxidant. Therefore, the use of natural antioxidants may be studied for extending the shelf life of the butterfat. Besides, inadequate packaging of Şanlıurfa butterfat is one of the reasons for short shelf life. The air in the packages can be removed by vacuum packaging, or replaced by inert gas to prevent lipid oxidation.

Induction time is known as an expression of resistance to oxidation reactions for oil or fat-containing foods. In the storage period, the products of oxidation reactions occurring in oil or fat-containing foods cause various quality losses such as taste-aroma, odour, texture and unwanted changes in the colour and shorten the shelf life of foodstuffs or raw material. In addition, it was also reported that the products, which occur in the oxidation reaction, can cause serious health risks (Gorji et al., 2016). It was found that the induction times (hours, h) of the Sanliurfa butterfat samples decreased significantly during the storage period at two different storage temperatures (P<0.05). The induction time of butterfat stored at room temperature was 6.09±0.19 h on the 1st day of the period. The value decreased rapidly to 2.92±0.19 h on 360th days of the storage period. A similar decreasing trend was observed for the induction time of the butterfat stored in refrigerator conditions. Besides, the induction time of butterfat stored at two different temperatures had decreased rapidly in the last 6 months of the period (Table 1). The mean induction time of Ghee produced from Buffalo milk was reported as 8.2 h (Fatouh et al., 2005). Similar to the results obtained in this study, it was reported that the induction time of butterfat was 3.2–27 h (Özbayram, 2000). In another research, the induction time of Sanliurfa butterfat was reported between 0.23–13.64 h (Yokuş, 2019). This difference may have been due to the milk type and milk composition used in the production of butterfat, production parameters, storage temperature and duration.

**The fatty acid composition of Sanliurfa butterfat**

The mean values of the saturated fatty acids composition of Sanliurfa butterfat samples in terms of storage temperature and duration are presented in Table 2. The ratios of total saturated fatty acids (SFA), and C4:0, C6:0 vs C8:0 of the fatty acid composition of Sanliurfa butterfat stored at two different temperatures decreased during the storage period. This may be particularly due to the oxidation of short-chain fatty acids. As a result of this situation, the free fatty acids, acid value and peroxide value of butterfat increased during the period. On the other hand, SFA ratio of the butterfat varies between 66.25–69.01 % during the storage period. The fatty acid composition of traditional butterfat may have been due to the milk type and milk composition used in the production of butterfat, production parameters, storage temperature and duration.
are the major fatty acids. Similar to the results obtained in this study, total SFA ratio of traditionally produced Samn was reported as 68.79 % (Mariod et al., 2010) and that of value of traditional butterfat produced in Van/Turkey was reported as 69.18 % (Findik and Andič, 2017). The ratios of palmitic, stearic and myristic acids of Samn were reported as 35.13 %, 15.51 % and 13.24 %, respectively (Mariod et al., 2010). Similar to the results obtained in this study, the ratios of SFA, palmitic, stearic and myristic acid of butter sold in markets of Rio de Janeiro/Brazil were reported as 63.2 %, 31.4 %, 11.7 % and 11.5 %, respectively (Nunes et al., 2017). Compared to the results obtained in this study, the total SFA, myristic and palmitic acid of traditional Tibetan ghee was reported as 40.99 g/100 g, 3.92-9.85 g/100 g and 22.19-28.04 g/100 g, respectively (Jing et al., 2019). The species of mammals, the climate of the regions and grass pasture qualities can affect the fatty acid composition results.

In the composition of saturated fatty acids, the ratio of butyric (C4:0), caproic (C6:0), caprylic (C8:0), capric (C10:0) and lauric (C12:0) acids of Sanliurfa butterfat stored at 4 °C for 360 days were calculated as 1.28-1.95 %, 1.07-1.14 %, 1.05-1.32 %, 3.70-4.21 % and 2.71-2.85 %, respectively. Similar ranges were observed for the butterfat stored at room temperature during the storage period. On the other hand, the ratio of pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) of the samples were as 1.60-1.79 % and 0.87-1.05 %. The total ratios of trace-saturated fatty acids of the butterfat were calculated averagely as 1.24%. The difference between the mean values of some saturated fatty acids (C4:0, C6:0, C14:0, C17:0 and C22:0) and total SFA during the storage period were found statistically significant (P<0.05).

The mean values of the unsaturated fatty acids composition of Sanliurfa butterfat in terms of storage temperature and time are presented in Table 3. The mean palmitoleic (C16:1) acid ratio of butterfat stored at 20 °C was found statistically (P<0.05) higher than butterfat stored at 4 °C. Also, C16:1 and C17:1 and total monounsaturated fatty acids (MUFA) ratios of the butterfat showed statistically significant changes at the level of P<0.05 during the storage period. C16:1, C17:1, C18:2n6c, MUFA of the butterfat stored at two storage temperature increased significantly during the storage period. This situation may be originated from the oxidation of the saturated fatty acids. As a result of the increasing ratios of the unsaturated fatty acids, oxidation parameters such as acid value and peroxide value of the butterfat increased during the period. On the other hand, MUFA ratios of butterfat samples stored at 4 and 20 °C for 12 months were calculated as 27.14 % and 27.43 %, respectively. The oleic (C18:1) and palmitoleic acids (C16:1) had the highest ratios of, whereas C14:1, C15:1 and C17:1 acids had the lowest the ratio (<0.5 %) among the MUFA. Similar to the results obtained in this study, total MUFA and the oleic acid ratio of Samn were reported as 27.12 % and 24.5 % (Mariod et al., 2010). However, total MUFA and oleic acid ratios of butter were reported as 30.0 % and 25.9 % (Nunes et al., 2017). In addition, total MUFA ratio of traditional butterfat made in Van-Turkey was reported as 31.08 % (Findik and Andič, 2017).

The total polyunsaturated fatty acids (PUFA), linoleic (C18:2), linolenic acid (C18:3) acid and conjugated linoleic acid ratio of the samples was found between 3.86-3.89 %, 1.89-2.29 %, 0.95-0.97 % and 0.84-0.85 %, respectively. Similar to the results obtained in this study, the total PUFA, linoleic and linolenic acid ratios of butter were reported as 2.07 %, 0.46 % and 2.63 %, respectively (Nunes et al., 2017).

It has been expressed that conjugated linoleic acid (CLA), a polyunsaturated fatty acid, has the anticancer and positive effect against many diseases such as immune system diseases, diabetes, obesity and cardiovascular diseases (Dilzer and Park, 2012). In this study, c9t11 and t10c12 isomers of CLA were detected in Sanliurfa butterfat samples. In the fatty acid composition of butter stored at both temperatures, the ratio of the c9t11-CLA isomer was found to be 0.81% and the ratio of t10c12-CLA
was 0.02-0.03 %. CLA ratio of butterfat made in Van-Turkey was reported as 0.23-0.91 % (Fındık and Andıç, 2017). In Nigeria, the mean c9t11-CLA ratio of Man Shannu, that is a kind of butterfat obtained from cow's milk, was reported as 0.23 % (Glew et al., 2006). In another research, the c9t11-CLA ratio of butter in Rio de Janeiro-Brazil was reported as 0.95% (Nunes et al., 2017).

**Conclusion**

Sanliurfa butterfat has been stored at room temperature after the production and consumed throughout the year. It is revealed that the storage for 12 months at different temperatures (4 and/or 20 °C) poses a significant risk in terms of oxidative stability of Sanliurfa butterfat with this study. In the context of the relevant codex and standards, it is concluded that the maximum storage period could be six months for the butterfat stored at refrigerated temperature, while it could be only three months for the butterfat stored at room temperature. Studies of the usage of antioxidants and packaging techniques are necessary for increasing the shelf life of the butterfat.

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**Stabilnost i sastav masnih kiselina Sanliurfa maslaca**

**Sažetak**

U ovom istraživanju prikupljeni su uzorci maslaca iz mljekara manjeg kapaciteta lociranih u regiji Sanliurfa te su skladišteni na različitim temperaturama (4 i 20 °C) tijekom 12 mjeseci. U maslacu su analizirani biokemijski parametri (slobodne masne kiseline, srednja vrijednost: 17119).

The difference presented with the different letters between the mean values is statistically significant at the level of *P*<0.05.
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