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## Some chemical and microbiological properties of the butter and the butter oil produced from the same raw material

Osman Fındık<sup>a</sup>, Seval Andiç<sup>b,\*</sup>

<sup>a</sup> Directorate of Provincial Food Agriculture and Livestock, Şırnak, Turkey
<sup>b</sup> University of Yüzüncü Yıl, Faculty of Engineering, Department of Food Engineering, Van, Turkey

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# 1. Introduction

Butter is a dairy product produced by the churning of milk, voghurt or -as the case generally is-of cream; and it displays varying properties based on the qualities of the raw material from which it is produced. Butter also is a valuable product as it is utilized directly as a breakfast favorite, as an ingredient in the preparation of many dishes or as cooking oil for some hot dishes (Dvořák, Lužová, & Šustová, 2016; Çakmakçı, Gündoğdu, Dağdemir, & Erdoğan, 2014) In the related Turkish Food Codex (2005) (Notification No. 2005/ 19); butter is defined as a product which has minimum 80 g/100 gand maximum of 90 g/100 g milk fat in net weight. Butter also has high water content and water activity to support microbiological and enzymatic processes. Since the butter has proven difficult to preserve in years back when cooling techniques have not been developed to perfection -especially in the countries with a hot climate-the milk fat left after the precipitation of water and solid matter has been gathered and processed to yield a more durable

\* Corresponding author. E-mail address: sevalandic@yyu.edu.tr (S. Andiç).

### ABSTRACT

In this study, it was aimed to determine various characteristics of butter samples obtained from Van market and butter oil produced from these butters. Fat, acidity value, water activity (a<sub>w</sub>), peroxide value (PV), lipolysis (ADV), cholesterol, total fatty acid, free fatty acid compositions and conjugated linoleic acid determinations were made in butter and butter oil samples. Minimum and maximum values of the butter and butter oil samples respectively for free fatty acids, cholesterol, a<sub>w</sub>, peroxide and acidity values were determined as; 2415–27640 and 2026–14666 (mg/kg), 258–334 and 214–262 (mg/100 g fat), 0.96 –1.00 and 0.48–0.82, 1.2–7.4 and 4.3–10.5 (meqO<sub>2</sub>/kg fat), and 0.2–2.2 and 0.05–1.7 (mL/100 g) respectively. Conjugated linoleic acid content of butter and butter oil varied from 0.5 to 1.7 and from 0.2 to 0.9 (g/100 g in total fatty acids) respectively. In general yeast–mould, lipolytic bacteria and lactic acid bacteria counts and among total free fatty acids contents, acidity values and ADV was observed.

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product. This product is called as "butter oil", or, as "sadeyağ" in our country.

Butter oil, is manufactured in various countries in Africa, Middle East and Asia by melting in the butter at 110–140 °C. This end product is named differently in various countries in which it is manufactured, like "ghee" of India, "maslee" of Middle East and "roghan" of Iran (Atasoy & Türkoğlu, 2010; Sserunjogi, Abrahamsen, & Narvhu, 1998). In Turkey, "Sadeyağ" is produced largely in the Southeast and East Anatolian regions, by melting in the butter at 80 °C or somewhat higher temperatures, and is produced largely due to the insufficiency of the existing preservation techniques. Butter oil, which forms by removal of water and majority of the solid matter that gets separated from the milk fat during the melting process -due the differences in their specific weights-is an ingredient of importance, especially for the rural populations (Batun, Kirazcı, Küçük, Çoksöyler, & Javidipour, 2004).

Butter oil is defined in the codex in our country as a product containing at least 99 g/100 g milk fat in net weight (Turkish Food Codex, 2005), while The International Dairy Foods (IDF) defines it in the related standard as the product which has at least 96 g/100 g of milk fat and at most 0.3 g/100 g water (IDF, 1997).







There is, as the current situation holds, very little data present regarding the general properties of the butter oil manufactured in our country, especially for those produced in the rural regions. This study attempts to determine the amount of some of physicochemically and microbiologically significant component within butter, chiefly the amount of important components for health as cholesterol and conjugated linoleic acid (CLA), and their states after being processed into butter oil. Reviewing the previous literature revealed several studies regarding the ingredients of butter, yet there is hardly any data concerning butter oil. This is a bit unfortunate; as it is a commonly known fact that the amount of components such as CLA and cholesterol in the dairy products may change as a result of various factors (Temperature holds a great deal of importance amongst those). It is crucial to have knowledge on the amount of change these ingredients undergo in the heating process, since butter oil is consumed in high amounts in the rural regions. Other parameters that may get altered in the process include the water activity, the microbial load and the free acidity.

In this study; the aim was to demonstrate some physicochemical and microbiological characteristics of butter and butter oil, as both have a high degree of importance in nutrition and economics. A further emphasis was given on the characteristics of butter oil -which has a very few number of studies in its regard- and to determine its differences with its raw material, butter.

#### 2. Materials and method

#### 2.1. Chemicals

Ether, heptane, hexane, fatty acids methyl ester mixtures, Conjugated Linoleic acid standart, Cholesterol standard, internal standard (C13:0) and methanol were from Sigma Chemical Co., (St. Louis, MO, USA) mix FAME standard was from Supelco (Supelco Park, Bellefonte, PA, USA) and free fatty acids (FFA) standards used were from Fluka, (Buchs, Switzerland). Violet Red Bile Agar (VRBA), Potato Dextrose Agar (PDA), Tributyrin Agar (TBA), DE MAN, ROGOSA and SHARPE Agar (MRS), potassium hydroxide (KOH), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), chloroform and acetic acid were purchased from Merck (Darmstadt, Germany). M17 agar was obtained Oxoid (Drongen, Belgium).

#### 2.2. Material and sample preparation

In the study, butter samples to be used as test material were provided from retail stores in the province of Van. The samples were put in sterile containers in portions of 2 kg and transported to the Yüzüncü Yıl University Faculty of Engineering, Department of Food Engineering Laboratory to be placed in refrigerators. Each butter sample was portioned into two parts; first section to be used as the butter sample and the second to be melted on fire with a temperature of 90–95 °C and set aside for 5 min, as done in rural regions. Following this, the fat phase was separated from the remaining parts: the serum was collected at the bottom and the solid phase precipitated on the top. This fat phase samples were selected as the constituents of the butter oil samples that would be used in the tests.

#### 2.3. Physicochemical analyses of milk and cheese

The water activity  $(a_w)$  values of the butter and butter oil samples were measured using AquaLab LITE brand (Decagon Devices, Inc., Washington, USA) water activity measuring device. The fat content of the samples was determined by the ISO reference method (ISO, 2003), while the free fatty acid content was determined by the BDI method (IDF, 1991). The acidity was determined

by the percentage of lactic acid (ISO, 2012) and the peroxide content determination was conducted according to the AOAC standard method (Egan, Kirk, & Sawyer, 1981).

#### 2.4. Determination of free fatty acids

One gram of sample was mixed with 3 g of dehydrated sodium sulfate, and 0.3 mL of sulfuric acid (2.5 mol/L) and 1 mL of internal standard diluted in which solvent (C13:0, 0.5 mg/mL) was added. The mixture was extracted three times with a 3 mL ether-heptane solution of 1 mL:1 mL ratio and the solvent was transferred to a separate test tube. Before the introduction to amino propyl column, butter sample was conditioned by adding 10 mL heptane. Following this, the solvent was transferred through amino propyl column and resulting eluent was transferred to the same column for a second time, thus fixing the free fatty acids onto the column. Neutral lipids were removed from the column by passing 10 mL ether/heptane (1 mL:1 mL) through it. Free fatty acids were processed with diethyl ether containing 3 mL/100 mL formic acid and directly injected into gas chromatography device (De Jong & Badings, 1990). TB-wax MS capillary column (260  $\times$  298P L: 30 m. ID: 0.530 mm, film thickness: 0.25 µm; Thermo Scientific, Winsford, Cheshire) was used to separate the FFAs. Helium was used as the carrier gas at a flow rate of 2.6 mL/min. The injector temperature was 250 °C in split mode (1  $\mu$ L:10  $\mu$ L). The initial oven temperature (60 °C) increased to 240 °C at 10 °C/min and was maintained for 45 min. The temperature of FID was 260 °C. FFAs were identified by comparing their retention times with those of fatty acids standards (Fluka, Buchs, Switzerland) in standard samples.

#### 2.5. Determination of total fatty acids

The fat in the samples were extracted following the method of Folch (Folch, Lees, & Sloane-Stanley, 1957). Solvent phase was removed by means of a rotary evaporator (40 °C) and a 0.2 g portion of fat thusly gathered was weighed into a tube. This portion was extracted in 2 mL hexane and fatty acid methyl esters (FAME) were prepared from it by using 0.2 mL 1 mol/L methanolic KOH (AOCS, 1997). Resulting fatty acid methyl esters (FAME) were processed in Agilent 6890 N.04.13 GC-MS device (Agilent Technologies, Palo Alto, CA, USA). Helium gas was used as determinant and as capillary column carrier. One  $\mu$ L sample with a split ratio of 1  $\mu$ L:50  $\mu$ L was used for injection. Mix Supelco FAME 37 (Supelco Park, Bellefonte, PA, USA) was used as the standard, and chromatograms were evaluated using MS database (NIST). Percentages of FAMEs were quantified according to their relative area.

Total Fatty Acid determination GC-MS process conditions are listed below:

Column: Agilent J & W GC, HP–88 capillary column, 60 m x: 0.25 mm, film thickness: 0.20  $\mu$ m; Inlet temperature: 250 °C; Split ratio: 1  $\mu$ L:50  $\mu$ L; Flow rate: 0.8 mL/min helium; Oven temperature: The initial oven temperature was 60 °C and increased to 120 °C at 10 °C/min. Then oven temperature was increased to 200 °C at 14 °C/min and the final temperature maintained for 45 min.

#### 2.6. Determination of conjugated linoleic acid (CLA)

The lipids used to determine the CLA values of the samples were extracted according to Folch method (Folch et al., 1957). The methyl esters were prepared using the AOCS method (AOCS, 1997). The separation of CLA was performed on a Agilent 6890 N.04.13 GC-MS device (Agilent Technologies, Palo Alto, CA, USA) with a Agilent J & W GC, HP–88 capillary column, (L: 100 m; inner diameter: 0.25 mm, film thickness: 0.20  $\mu$ m). The injector temperature was 250 °C in split mode (1  $\mu$ L:50  $\mu$ L). Helium was used as the carrier

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