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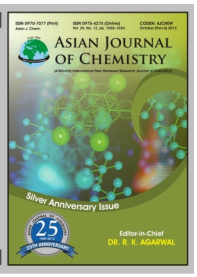

Evaluation of Chemical and Microbiological Characteristics and Fatty Acid Profiles of Butter Samples Collected from the Black Sea Region of Turkey

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The physico-chemical and microbiological characteristics and fatty acid composition of butter samples produced in the Black sea region of Turkey were analyzed. The mean percentages of total solids, non-fat solids and fat were 85.36, 2.71 and 82.64 %, respectively; the pH and titratable acidity, 4.64 and 0.55 %, respectively; the salt and ash contents, 0.51 and 0.81 %, respectively and the peroxide content, 1.19 meq O_2/kg fat. The average colour values of L, a and b for the butter samples were 78.49, -1.3 and 24.74, respectively. The mean counts of aerobic mesophilic bacteria, enterococci, lactic acid bacteria, yeast and mould, coliform and *Escherichia coli* organisms in the butter samples were 6.01, 5.81, 4.22, 5.14, 2.76 and 2.29 log CFU/g, respectively. *Staphylococcus aureus* was not detected in any of the samples. The predominant fatty acids in the butter samples were palmitic acid (33.72 %), oleic acid (20.79 %), myristic acid (11.17 %) and stearic acid (10.47 %).

Key Words: Butter, Composition, Fatty acid, Microbiology.

INTRODUCTION

Butter is produced from cream or yoghurt in dairy factories and on farms in Turkey; the traditional butter made from yoghurt is called 'Yayik butter' and has been produced for centuries[1]. Factory-produced butter is generally made from cream, packaged in aluminium foil, plastic or paper and then stored at between 4 and 18 ºC [2]. According to the Turkish Butter Standards, butter is described as "a dairy product obtained from cream or yoghurt" and as "having a characteristic flavour, odour and consistency"[3]. The components of butter are legislated in a number of countries and the product usually includes 80-84 % milk fat, 15.3-15.9 % water, 1 % non-fat dry matter and 0.03-1.8 % salt[4]. To produce butter from cream, the cream is first pasteurized at 90-95 ºC for 15-20 min, followed by cooling to 15-20 ºC. The cream is then churned for *ca.* 60-70 min, at which time the fat globules coalesce. The buttermilk is removed and the butter is washed once or twice before the slabs are wrapped[2].

In the Black sea region, milk products are mainly produced in small factories that generally use simple methods and with little regard to the raw material source, quality and hygiene history of milk used. For these reasons, the composition and hygiene qualities of dairy products are not guaranteed[5].

Many studies have investigated the microbial content and chemical qualities of milk and milk products produced in Turkey. However, there is no published information about the chemical and microbiological quality of butters produced in the Black sea region of Turkey. In the present study, we therefore aimed to determine the chemical and microbiological characteristics and fatty acid content of butter samples collected from that region during two production periods and three sub-regions and to discuss the legal stipulations in Turkey.

EXPERIMENTAL

In 2009 and 2010, a total of 88 samples of butter manufactured by 44 different firms were obtained randomly from markets in the East, Middle and West sub-regions of the Black sea region of Turkey. The butter was produced in October-November 2009 (first period) or May-June 2010 (second period). The samples (1 kg) were transported to the laboratory in an insulated container at 4 ºC and analyzed upon arrival.

Chemical analysis: The total solids content of the butter samples was determined by heating at 103 ± 1 ºC to constant weight[6] and the percentage of non-fat solids was calculated with the method of Van Gulik[7]. Titratable acidity and salt and ash content were determined according to the methods described by Bradley *et al.*[8]. The pH was measured using a

pH meter (model pH 510, Eutech Instruments, Singapore) equipped with a probe for solids (Sensorex, Garden Grove, CA, USA) and according to the methods of the Association of Official Analytical Chemists[9].

Colour measurement: Colour analyses on the butter samples were conducted with a colourimeter (Chroma Meter CR-300; Minolta, Osaka, Japan) to measure the Hunter L, a and b values. Measurements were performed on subsamples taken from 5 separate areas of the butter sample.

Microbiological analysis: Ten gram butter samples were dispersed in 90 mL of sterile 0.85 % (w/v) NaCl solution at 45 ºC in a Stomacher laboratory blender (Smasher; AES Chemunex, Bruz, France). Ten-fold dilutions ranging from 10^{-1}-10^{-8} were prepared and incubated under the following conditions to determine the microbial contents of the samples: for total aerobic mesophilic bacterial growth, plate count agar at 30 ºC for 72 h[10]; for lactic acid bacteria (LAB), MRS agar (adjusted to pH 5.4 with acetic acid) at 30 ºC for 72 h[11]; for coliform bacteria, violet-red bile agar at 37 ºC for 24 h[12]; for *Escherichia coli*, chromocult TBX agar at 44ºC for 24 h[13]; for *Staphylococcus aureus*, Baird-Parker agar (incorporating 5 % egg yolk tellurite emulsion) at 37 ºC for 24 h[14]; for yeasts and moulds, yeast glucose chloramphenicol (YGC) agar at 25 ºC for 5 days[15] and for enterococci, kanamycin esculin agar at 37 ºC for 48 h[16].

Fatty acid analysis: The butter samples were warmed to 37 ºC immediately prior to analysis, then vortexed vigorously to achieve sample uniformity. The method described by Zeppa *et al.*[17] was used for the analysis of fatty acid methyl esters (FAMEs). For transesterification of the lipids in 0.4 mL of butter, 4 mL of isooctane was added to the sample and shaken slowly for 0.5 min. The samples were kept in the dark for 6 min; 2 mL of 2 N potassium hydroxide in methanol was then added and neutralized with 1 N HCl. The upper layer was removed and analyzed with a Shimadzu gas chromatography unit with a flame ionization detector (Model GC-2010, Shimadzu Corporation, Kyoto, Japan). The fatty acids were separated in a capillary column DB 23 (60 m × 0.25 mm i.d., 0.25 µm) (J & W Scientific, Folson, CA, USA) with an injected volume of 1 µL. The operating conditions and procedures were as follows; injector port and detector temperatures were held at 270 and 280 ºC, respectively. The carrier gas was helium at a pressure of 150 kPa; the split ratio was 10^{-2}; the column temperature was maintained at 130 ºC for 2 min, then ramped at 6.5 ºC/min to 170 ºC and at 2.75 ºC/min to 215 ºC, held at 215 ºC for 6 min, raised again to 240 ºC at 40 ºC/min and finally held at 240 ºC for 10 min. Fatty acid methyl esters were identified by comparison of their retention times with those of standard references (Supelco 37 Component FAME Mixture, Bellefonte, PA, USA) and results were expressed as w/w (%) total fatty acid.

Statistical analysis: Statistical analyses were performed using SPSS version 17.0 software (SPSS Inc. Chicago, Illinois); the effects of geographic region and sampling period were analysed with ANOVA and Duncan's test for multiple comparisons ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Chemical characteristics: The results of the chemical analysis of the 88 butter samples are provided in Table-1. The mean values of total solids, non-fat solids, fat, titratable acidity, pH, salt, ash and peroxide were 85.36, 2.71, 82.64, 0.55, 4.64, 0.51, 0.81 % and 1.19 meq O_2/kg of fat, respectively. According to the Turkish Food Codex and TS 1331 Butter Standards, butter must contain minimums of 80 % milk fat and 84 % total solids and maximums of 2 % non-fat solids and 5 meq O_2/kg fat[3,18]. In this study, 27 of 88 samples (30.68 %) did not comply with the TS 1331 Butter Standards for total solids content, 17 of 88 (19.32 %) did not comply for fat content and 59 of 88 (67.05 %) did not comply for non-fat solids content. However, all 88 samples satisfied the criteria for peroxide consumption. Altun *et al.*[4] found that 80, 90 and 70 % of butter samples were non-compliant with the aforementioned butter standards for moisture content, non-fat solids and fat content, respectively. The mean titratable acidity values in the present study were higher than these of Sagdic *et al.*[1]. However, the mean pH values were similar to those reported in earlier studies[1,10]. The mean salt content was similar to the value reported by Sagdic *et al.*[1] but lower than these of Mourad and Nour-Eddine[19]. In addition, the mean ash percentage in present study was lower than that reported by Samet-Bali *et al.*[10].

TABLE-1
PHYSICOCHEMICAL CHARACTERISTICS AND THE L-, a-, b-VALUES OF BUTTER SAMPLES COLLECTED FROM THE EAST, MIDDLE AND WEST BLACK SEA REGIONS OF TURKEY IN OCTOBER-NOVEMBER 2009 AND MAY-JUNE 2010

Variable	Region*			Period**		Average
	East	Middle	West	First	Second	
Total solids (%)	84.13 ± 4.39^{bc}	86.78 ± 3.18^{a}	85.86 ± 3.38^{ab}	84.85 ± 3.48^{A}	85.86 ± 4.21^{A}	85.36 ± 3.87
Non-fat solids (%)	2.74 ± 1.2^{a}	3.01 ± 1.32^{a}	2.57 ± 1.09^{a}	2.46 ± 1.12^{B}	2.97 ± 1.18^{A}	2.71 ± 1.17
Fat (%)	81.39 ± 3.92^{b}	83.77 ± 2.46^{a}	83.29 ± 2.95^{a}	82.39 ± 3.23^{A}	82.89 ± 3.59^{A}	82.64 ± 3.4
TA (%)	0.57 ± 0.33^{a}	0.39 ± 0.24^{b}	0.6 ± 0.28^{a}	0.66 ± 0.36^{A}	0.44 ± 0.19^{B}	0.55 ± 0.3
pH	4.57 ± 0.32^{a}	4.8 ± 4.78^{a}	4.62 ± 0.47^{a}	4.41 ± 0.35^{A}	4.86 ± 0.36^{A}	4.64 ± 0.42
Salt (%)	0.67 ± 0.31^{a}	0.45 ± 0.32^{b}	0.4 ± 0.23^{b}	0.54 ± 0.29^{A}	0.48 ± 0.32^{A}	0.51 ± 0.31
Ash (%)	0.95 ± 0.43^{ab}	0.68 ± 0.39^{bc}	0.74 ± 0.47^{b}	0.88 ± 0.47^{A}	0.75 ± 0.42^{A}	0.81 ± 0.45
Peroxide (meq O_2/kg fat)	1.32 ± 0.99^{a}	1.26 ± 0.91^{a}	1.05 ± 0.56^{a}	1.16 ± 0.69^{A}	1.24 ± 0.93^{A}	1.19 ± 0.82
L-	77.62 ± 1.6^{b}	79.33 ± 2.06^{a}	78.92 ± 2.01^{a}	78.44 ± 1.92^{A}	78.54 ± 2.07^{A}	78.49 ± 1.98
a-	-0.79 ± 0.96^{a}	-1.81 ± 0.53^{b}	-1.53 ± 0.68^{b}	-1.3 ± 0.88^{A}	-1.29 ± 0.88^{A}	-1.3 ± 0.88
b-	26.53 ± 5.45^{b}	23.61 ± 3.54^{a}	23.62 ± 4.49^{a}	24.76 ± 4.91^{A}	24.73 ± 4.95^{A}	24.74 ± 4.91

Values are expressed as mean ± standard deviation. *a-c and ** A-B: means with different letters in a row (ie within the same category) are significantly different ($p < 0.05$).

The butter production period had a significant effect only on the titratable acidity and pH values ($p < 0.05$). The total solids and fat content of the butter samples produced in the Middle Black sea region were significantly higher than samples produced in the East and West regions ($p < 0.05$). However, the salt and ash contents and titratable acidity were highest in the West Black Sea region ($p < 0.05$).

Colour properties: On the Hunter colour scale, L, a and b values represent light to dark, red (+a) to green (-a) and yellow (+b) to blue (-b), respectively. The average values for the butter samples analyzed were 78.49 for L, -1.3 for a and 24.74 for b (Table-1). The colour properties of the butter samples were not affected by the production period ($p > 0.05$), but did vary with the production region. Samples from the East Black sea region were darker, redder and yellower than samples from the other regions ($p < 0.05$). One possible explanation for this finding is that the animal feeds in that region may contain higher levels of β-carotene. In most cases, when appearance factors (especially colour) cause selection or rejection impulses for butters, their potential interrelationships with other basic properties (*e.g.* flavour) cannot be ignored. It is well documented that certain colourants infer a relationship between the product and a most probable source of origin, irrespective of the actual flavour[20].

Microbiological properties: The results of the microbiological analysis of the butter samples are shown in Table-2. The mean combined yeast and mould count for the samples was 5.14 log CFU/g. Seventy eight of 88 samples were above the critical limit of 4 log CFU/g for yeast and mould content set by the Turkish Food Codex Microbiological Criteria Notification[21]. By comparison, Karagozlu and Ergonul [22] investigated 45 butter samples collected from Manisa in Turkey and found that only 3 of the samples were under the critical limit for yeast and mould content.

The presence of coliform bacteria in food products is a result of insufficient heat treatment or recontamination after heat treatment[23]. In our study, 44 of 88 samples (50 %) were contaminated with coliform bacteria. The TS 1331 Butter Standard[3] set the acceptable limit for coliform bacteria at 1 log CFU/g for pasteurized butter; in our study, only 4.5 % of samples were below the limit. Gun[24] reported coliform bacteria counts of between 1.3 and 2.62 log CFU/g in Karin butter samples, which is similar result to that of our study. However, Sagdic *et al*.[25] did not detect coliform bacteria in their study of Yayik butter.

The *E. coli* content of food products is considered a reliable indicator of faecal contamination and indicates the possible presence of enteropathogenic and/or toxigenic microorganisms[26]. The Turkish Food Codex states that butter should contain no *E. coli*[21]. We found that 23 of the 88 butter samples in our study were contaminated with *E. coli.* By comparison, Karagozlu and Ergonul[22] reported that all their butter samples were contaminated with *E. coli*.

The Turkish Food Codex and TS 1331 Butter Standard set no restrictions for the total aerobic mesophilic bacteria counts in butter. However, butter is classified according to its total aerobic mesophilic bacteria content as very good quality (< 6 log CFU/g), good quality (6-6.3 log CFU/g) and poor quality (> 6.3 log CFU/g)[23]. According to that classification, 51.14, 18.18 and 30.63 % of the butter samples in our study were categorised as very good, good and poor quality, respectively.

Staphylococcus aureus is an important cause of mastitis and is often found in milk products that are insufficiently heat treated[23]. According to the Turkish Food Codex, the *S. aureus* count must not exceed 2 log CFU/g[21]. However, we did not detect it in our samples. Similarly, Karagozlu and Ergonul[22] did not detect *S. aureus* in any of their butter samples from Manisa city.

Enterococci play a major role in the butter ripening process and development of the characteristic butter aromas[19]. However, Giraffa[27] stated that the presence of enterococci in milk products may be due to inadequate sanitary conditions during production. We detected enterococci in all butter samples, with a mean level of 4.22 log CFU/g.

Lactic acid bacteria (LAB) plays an important role in the development of the flavour characteristics of dairy products. The production of diacetyl and acetaldehyde and conversion of lactose to lactic acid by the LAB are their main contributions to the flavour of cultured milk and milk products[28]. The current study reports a mean LAB count in the butter samples of 5.81 log CFU/g, which is higher than that reported by Sagdic *et al*.[25].

We noted regional variations in the microbial content of the samples; the total aerobic mesophilic bacteria counts were highest in the West Black sea region but coliform and *E. coli* counts were highest in the Middle Black sea region ($p < 0.05$). In this study, there was a negative correlation between these microorganisms and titratable acidity. Many authors have indicated that survival and growth of these microorganisms

TABLE-2
SOME MICROBIOLOGICAL CHARACTERISTICS OF BUTTER SAMPLES COLLECTED FROM THE EAST, MIDDLE AND WEST BLACK SEA REGIONS OF TURKEY IN OCTOBER-NOVEMBER 2009 AND MAY-JUNE 2010 PRESENTED AS log CFU/g

Microorganisms	Region*			Period**		Average
	East	Middle	West	First	Second	
Yeast-Mould	5.02 ± 0.91^{a}	4.94 ± 0.66^{a}	5.34 ± 0.86^{a}	5.34 ± 0.89^{A}	4.95 ± 0.77^{A}	5.14 ± 0.86
Enterecocci	4.18 ± 1.03^{a}	3.88 ± 0.94^{a}	4.22 ± 0.98^{a}	3.82 ± 0.92^{B}	4.62 ± 0.88^{A}	4.22 ± 0.98
AMB	5.76 ± 0.57^{bc}	6.03 ± 0.62^{b}	6.22 ± 0.7^{ba}	5.82 ± 0.55^{B}	6.19 ± 0.71^{A}	6.01 ± 0.66
LAB	5.67 ± 0.56^{a}	5.66 ± 0.89^{a}	6.01 ± 0.61^{a}	5.68 ± 0.64^{A}	5.95 ± 0.67^{A}	5.81 ± 0.67
Coliform	2.36 ± 0.88^{b}	3.41 ± 0.46^{a}	2.74 ± 0.98^{b}	2.74 ± 1.11^{A}	2.78 ± 0.81^{A}	2.76 ± 0.93
E. coli	1.68 ± 0.66^{bc}	2.62 ± 0.93^{ab}	2.44 ± 0.7^{b}	2.17 ± 1.03^{A}	2.35 ± 0.74^{A}	2.29 ± 0.82

S. aureus was not detected in any samples. Values are expressed as mean ± standard deviation. Means were calculated for samples where coliform bacteria and *E. coli* were detected. * a-c and ** A-B: means with different letters in a row (*i.e.* within the same category) are significantly different ($p < 0.05$).

may be inhibited by lactic acid production[29,30]. Additionally, in present study, the yeast and mould counts were highest in samples from the first production period, whereas enterococci counts were the highest in the second period ($p < 0.05$).

Fatty acid composition: Chromatograms of fatty acids obtained from standards and butter samples are shown in Fig. 1 and the fatty acid compositions of butter samples are shown in Table-3. The mean saturated fatty acid (SFA) percentage of the butter samples was 73.63 % and the predominant ones were palmitic acid (C16:0) (32.15 %), myristic acid (C14:0) (12.71 %), stearic acid (C18:0) (12.11 %) and lauric acid (C12:0) (4.53 %). Sagdic *et al.*[25] reported a lower saturated fatty acid content of butter from cows' milk (67.06 %) than present study. Their palmitic acid, myristic acid, stearic acid and lauric acid contents were 33.72, 11.17, 10.47 and 2.4 %, respectively. Palmitic acid, myristic acid and lauric acid elevate the serum cholesterol level while stearic acid does not influence it[31]. In present study, the amounts of some other important saturated fatty acids, namely butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0) and capric acid (C10:0), were 2.16, 2.04, 1.4 and 3.14 %, respectively. These results approximate those reported by Ledoux *et al.*[32]. In contrast, the butyric, caproic, caprylic and capric acid contents of Turkish butter obtained from cows' milk were 1.83, 1.18, 0.81 and 1.97 %, respectively[25], all of which were considerably lower than in our study. Butyric acid is a potent inhibitor of cancer cell proliferation, whereas it is also responsible for the rancidity of butter resulting from lipolysis[2,33]. Gun and Simsek[2] further stated that caproic acid, caprylic acid and capric acid are responsible for problems such as sharp pepper and soapy tastes.

From the current study, the mean monounsaturated and polyunsaturated fatty acid contents of butter samples were 23.12 and 1.62 %, respectively and the most abundant monounsaturated fatty acid was oleic acid (C18:1 *cis*) (20.79 %). A similar result was reported for yayik butter by Sagdic *et al.*[25]. Gun and Simsek[2] reported an oleic acid value of 22.68 % for Karinyagi (traditional butter), which was higher than our figure. Oleic acid is a convenient baseline fatty acid for comparison purposes and assuming it to be neutral puts the effects of other fatty acids into perspective[31].

Humans do not have the enzymatic capacity to synthesize polyunsaturated fatty acids, so they must be consumed in the diet[2]. Linoleic acid (C18:2 *cis*) and linolenic acid (C18:3 *cis*), which are essential fatty acids, were 0.35 and 0.43 %, respectively. Sagdic *et al.*[25] stated that the linoleic acid and linolenic acid proportions in their butter samples were 0.16 and 1.18 %, respectively, which vary markedly from our results. Furthermore, the mean *trans*-unsaturated fatty acid (TUFA) (%) contents of butter samples were minimal; the amount of elaidic acid (C18:1 *trans*) was 0.45 % and linolelaidic acid (C18:2 *trans*) was 0.39 %. *trans*-Unsaturated fatty acids are important because they increase the low-density-lipoprotein (LDL) cholesterol (harmful cholesterol) level and decrease the high-density-lipoprotein (HDL) cholesterol (beneficial cholesterol) level[34].

Short chain fatty acid (SCFA) [except butyric acid (C4:0) and capric acid (C10:0)] and medium chain fatty acid (MCFA) [including tridecanoic acid (C13:0), myristoleic acid (C14:1) and pentadecanoic acid (C15:0)] levels were higher overall in butter produced in the West Black sea region than the other regions ($p < 0.05$). In that region, cows are fed silage and cereal-rich rations indoors, whereas in the other regions, the cows are fed fodder during the winter season and graze during the summer season. Especially in the East Black sea region, dairy cows have access to grassy meadows for long periods of the year.

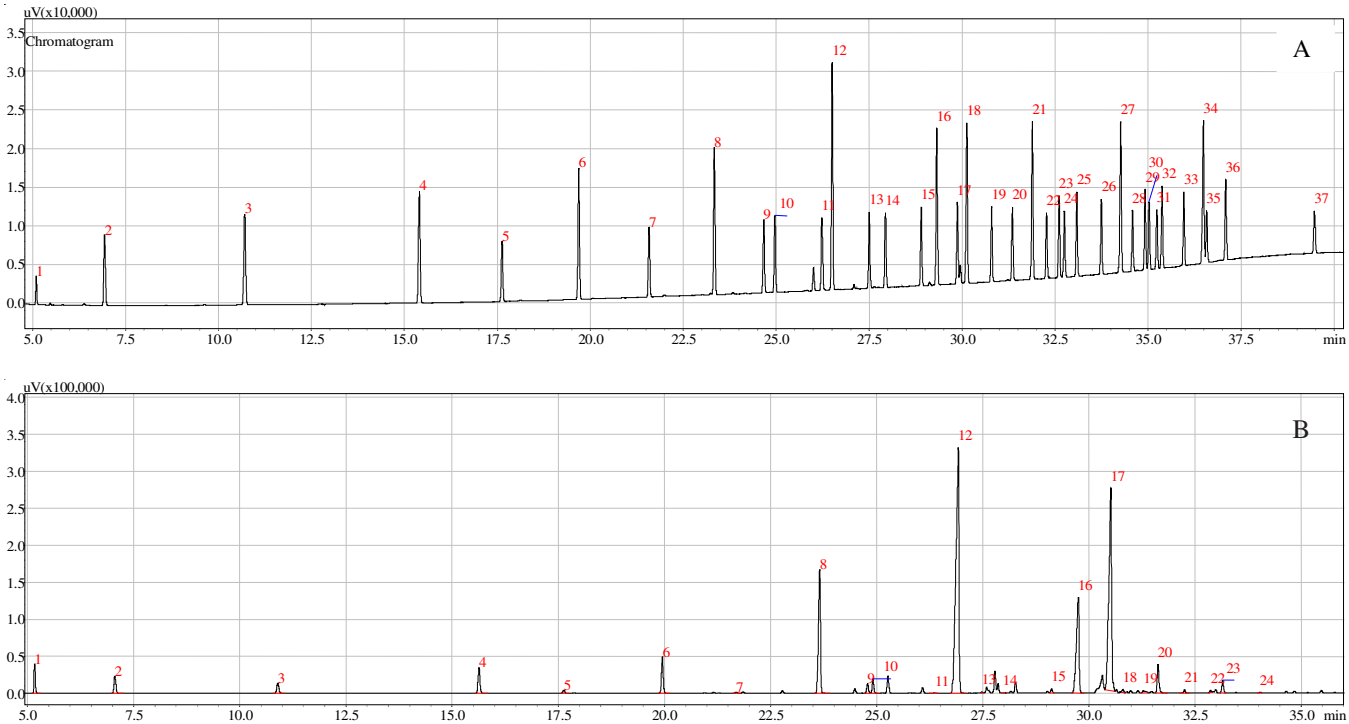

Fig. 1. GC chromatograms of fatty acids obtained from (A) standards and (B) butter samples ([1]C4:0; [2]C6:0; [3]C8:0; [4]C10:0; [5]C11:0; [6]C12:0; [7]C13:0; [8]C14:0; [9]C14:1; [10]C15:0; [11]C15:1; [12]C16:0; [13]C16:1; [14]C17:0; [15]C17:1; [16]C18:0; [17]C18:1 *trans*; [18]C18:1 *cis*; [19]C18:2 *trans*; [20]C18:2 *cis*; [21]C20:0; [22]C18:3 (γ); [23]C20:1; [24]C18:3; [25]C21:0; [26]C20:2; [27]C22:0; [28]C20:3 (n-6) *cis*; [29]C22:1; [30]C20:3 (n-3) *cis*; [31]C20:4; [32]C23:0; [33]C22:2 *cis*; [34]C24:0; [35]C20:5 *cis*; [36]C24:1 and [37]C22:6 *cis*)

TABLE-3
FATTY ACID COMPOSITION OF BUTTER SAMPLES COLLECTED FROM THE EAST, MIDDLE AND WEST BLACK SEA REGIONS OF TURKEY IN OCTOBER-NOVEMBER 2009 AND MAY-JUNE 2010

FA (%)	Region*			Period**		Average
	East	Middle	West	First	Second	
C4:0	1.12 ± 0.57^{a}	2 ± 0.41^{a}	2.36 ± 0.37^{a}	2.23 ± 0.49^{A}	2.09 ± 0.45^{A}	2.16 ± 0.47
C6:0	1.99 ± 0.39^{ab}	1.9 ± 0.32^{bc}	2.24 ± 0.31^{a}	2.03 ± 0.44^{A}	2.06 ± 0.29^{A}	2.04 ± 0.36
C8:0	1.31 ± 0.32^{bc}	1.34 ± 0.16^{ab}	1.55 ± 0.15^{a}	1.43 ± 0.23^{A}	1.37 ± 0.27^{A}	1.4 ± 0.24
C10:0	2.94 ± 0.72^{a}	2.98 ± 0.51^{a}	3.34 ± 0.36^{a}	3.09 ± 0.52^{A}	3.19 ± 0.61^{A}	3.14 ± 0.56
C11:0	0.34 ± 0.1^{b}	0.32 ± 0.08^{b}	0.4 ± 0.06^{a}	0.37 ± 0.09^{A}	0.33 ± 0.08^{A}	0.35 ± 0.09
C12:0	4.2 ± 0.79^{bc}	4.92 ± 0.84^{a}	4.46 ± 0.43^{ab}	4.48 ± 0.81^{A}	4.58 ± 0.69^{A}	4.53 ± 0.75
C13:0	0.1 ± 0.03^{b}	0.1 ± 0.02^{b}	0.13 ± 0.02^{a}	0.12 ± 0.03^{A}	0.1 ± 0.02^{A}	0.11 ± 0.3
C14:0	12.7 ± 0.86^{a}	12.79 ± 1.29^{a}	12.63 ± 0.92^{a}	12.64 ± 0.82^{A}	12.77 ± 1.19^{A}	12.71 ± 1.01
C14:1	0.87 ± 0.35^{b}	0.84 ± 0.23^{b}	1.11 ± 0.16^{a}	0.98 ± 0.24^{A}	0.89 ± 0.32^{A}	0.94 ± 0.28
C15:0	1.18 ± 0.36^{b}	1.21 ± 0.45^{b}	1.64 ± 0.29^{a}	1.46 ± 0.34^{A}	1.21 ± 0.44^{A}	1.34 ± 0.42
C15:1 *cis*	0.04 ± 0.02^{b}	0.35 ± 0.13^{a}	0.28 ± 0.08^{a}	0.23 ± 0.17^{A}	0.21 ± 0.16^{A}	0.22 ± 0.16
C16:0	32.24 ± 1.91^{a}	32.83 ± 1.55^{a}	31.39 ± 1.97^{a}	31.77 ± 1.91^{A}	32.53 ± 1.78^{A}	32.15 ± 1.85
C16:1	0.35 ± 0.25^{a}	0.39 ± 0.2^{a}	0.48 ± 0.3^{a}	0.21 ± 0.13^{B}	0.6 ± 0.19^{A}	0.4 ± 0.25
C17:0	0.07 ± 0.05^{a}	0.05 ± 0.02^{a}	0.06 ± 0.01^{a}	0.05 ± 0.01^{A}	0.07 ± 0.04^{A}	0.06 ± 0.03
C17:1	0.22 ± 0.06^{a}	0.23 ± 0.09^{a}	0.27 ± 0.04^{a}	0.24 ± 0.05^{A}	0.25 ± 0.08^{A}	0.24 ± 0.06
C18:0	12.41 ± 2.05^{a}	11.88 ± 1.26^{a}	12.04 ± 1.79^{a}	12.91 ± 1.44^{A}	11.31 ± 1.56^{B}	12.11 ± 1.68
C18:1 *trans*	0.46 ± 0.11^{a}	0.4 ± 0.1^{a}	0.49 ± 0.17^{a}	0.49 ± 0.1^{A}	0.41 ± 0.15^{A}	0.45 ± 0.13
C18:1 *cis*	21.36 ± 2.09^{a}	20.63 ± 1.85^{a}	20.4 ± 1.26^{a}	20.25 ± 1.22^{B}	21.34 ± 2.06^{A}	20.79 ± 1.75
C18:2 *trans*	0.38 ± 0.1^{a}	0.35 ± 0.13^{a}	0.42 ± 0.11^{a}	0.39 ± 0.12^{A}	0.38 ± 0.11^{A}	0.39 ± 0.12
C18:2 *cis*	0.36 ± 0.18^{a}	0.33 ± 0.15^{a}	0.36 ± 0.18^{a}	0.39 ± 0.16^{A}	0.31 ± 0.16^{B}	0.35 ± 0.16
C20:0	0.28 ± 0.12^{a}	0.33 ± 0.2^{a}	0.29 ± 0.11^{a}	0.3 ± 0.17^{A}	0.31 ± 0.16^{A}	0.3 ± 0.14
C20:1 *cis*	0.06 ± 0.02^{a}	0.05 ± 0.01^{a}	0.06 ± 0.02^{a}	0.06 ± 0.02^{A}	0.05 ± 0.02^{A}	0.05 ± 0.02
C18:3	0.44 ± 0.09^{a}	0.44 ± 0.01^{a}	0.39 ± 0.03^{a}	0.45 ± 0.09^{A}	0.39 ± 0.06^{A}	0.43 ± 0.08
C21:0	1.19 ± 0.41^{a}	1.16 ± 0.44^{a}	1.47 ± 0.47^{a}	1.29 ± 0.4^{A}	1.15 ± 0.47^{A}	1.27 ± 0.45
Others	1.91 ± 0.44^{a}	1.69 ± 0.64^{ab}	1.31 ± 0.46^{bc}	1.59 ± 0.49^{A}	1.68 ± 0.64^{A}	1.64 ± 0.56
SCFA	12.9 ± 2.43^{bc}	13.47 ± 1.05^{ab}	14.36 ± 0.75^{a}	13.63 ± 1.83^{A}	13.52 ± 1.51^{A}	13.57 ± 1.65
MCFA	47.48 ± 1.71^{a}	48.51 ± 1.83^{a}	47.65 ± 2.19^{a}	47.42 ± 1.52^{A}	48.35 ± 2.19^{A}	47.88 ± 1.91
LCFA	39.61 ± 2.46^{a}	38.01 ± 2.26^{a}	37.99 ± 2.08^{a}	38.96 ± 1.65^{A}	38.13 ± 2.85^{A}	38.54 ± 2.33
SFA	73.06 ± 1.66^{a}	73.82 ± 2.13^{a}	74.01 ± 1.32^{a}	74.27 ± 1.35^{A}	72.99 ± 1.85^{B}	73.63 ± 1.72
MUFA	23.37 ± 1.77^{a}	22.88 ± 1.63^{a}	23.08 ± 1.38^{a}	22.46 ± 1.17^{B}	23.77 ± 1.65^{A}	23.12 ± 1.56
PUFA	1.66 ± 0.31^{a}	1.59 ± 0.32^{a}	1.6 ± 0.28^{a}	1.67 ± 0.33^{A}	1.56 ± 0.25^{A}	1.62 ± 0.29

FA: Fatty acids; SCFA: Short chain fatty acids; MCFA: Medium chain fatty acids; LCFA: Long chain fatty acids; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids. Values are expressed as mean ± standard deviation. *a-c and **A-B: means with different letters in a row (ie within the same category) are significantly different ($p < 0.05$).

Palmitoleic acid, stearic acid and oleic acid levels in our butter samples were influenced by seasonal variations ($p < 0.05$). Palmitoleic acid and oleic acid levels were higher in May-June (second period) than in October-November. Generally, cows graze in pastures (herbaceous plants, including grass) during spring and summer and these plants are rich in unsaturated fatty acids[35]. The significantly higher levels ($p < 0.05$) of stearic acid in the first period (12.91 %) compared to the second period (11.31 %) are surprising because the presence of stearic acid in milk fats mainly results from the movement of unsaturated C_{18} fatty acids in feedstuffs (C18-rich grass) to the udder[35]. Overall, butter from summer milk contained more unsaturated fatty acids and long chain fatty acids and less short and medium chain saturated fatty acids, than butters from winter milk. These observations are confirmed by Ledoux *et al.*[32] and Wolf *et al.*[36].

Conclusion

At present, there are no recognized standards for the chemical contents of butter in Turkey. As a result, it is not possible to state that the samples analyzed in the current study were fit for human consumption. However, most of the samples in present study did not comply with the Turkish Butter Standards[3] and Turkish Food Codex[18] criteria for acceptable content of total solids, fats and non-fat dry matter. In the broader picture, the season influenced the saturated and monounsaturated fatty acid composition of the various butters. Fatty acid profiling revealed that palmitic acid was the predominant saturated fatty acid and that oleic acid was the major monounsaturated fatty acid. Myristic acid and stearic acid were also found in high quantities.

Overall, the results of our microbiological analyses indicate that poor hygiene standards exist during manufacturing, packaging and marketing of butter in the Black Sea Region of Turkey. The presence of coliform bacteria and *E. coli*, together with the high total microorganism counts, suggests that there is inadequate heat treatment of milk and cream and/or low hygiene standards in processing and packaging. To improve the microbiological profiles of butter and hence make it a safer food product, the milk or cream must be adequately pasteurized and the latest techniques, equipment and practices employed throughout the production process.

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