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Characteristics of Iranian traditional butter produced in Sarab in comparison with European and national standards

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Summary. *Background/Objective:* Due to the widespread use of traditional butters (TB) and the lack of reliable data and regarding insufficient supervision on their production process, this study aims to compare microbiological, physicochemical and organoleptic characteristics of Sarab's TB with European standards (ES) and national standards (NS). *Methods:* In this descriptive-analytical study, fifty samples of TB were collected from the farmers of different locations of Sarab, Iran. Microbiological, physicochemical and organoleptic characteristics of butter samples were compared with ES and NS. The SPSS statistical software package version 16 and One Sample t- Test were used for comparing the mean value of parameters with standard values. *Results:* Our results showed that the salt content, iodine index and acidity of all samples were consistent with ES. The peroxide value, moisture and solids-non-fat content of some butter samples were higher and the fat content of 74% of them was lower than the ES values. The saponification index of 26% of samples was out of the ES range. Coliform bacteria and psychrotrophic bacteria count were higher than NS in 8% and 4% of samples, respectively. In contrast to NS, E. coli and coagulase positive staphylococcus were detected in 10% of samples. Molds and yeasts count in all samples were lower than the NS value. Score of organoleptic properties in all samples were more than 8 points. *Conclusion:* Despite high quality and low microbial contamination of butter samples in present study, more supervision on the production process of traditional products is recommended.

Key words: butter, microbiological, physicochemical, organoleptic, Iran

Introduction

Various dairy products are manufactured from raw milk. Among them, butter is a popular traditional food which is consumed as a fat source, directly or as a component of processed foods (1, 2). Due to the high amount of fats, fat- soluble vitamins and some minerals in butter it has a considerable nutritional value (2). Butter is a water-in-oil emulsion. Sweet cream and/or fermented cream are used for butter making. It has 2% of salt (If the produced butter is salted), 15.6-17.5% of water, 4% of protein, and the remaining part is fat (3).

The consumption of milk, cheese and butter within some countries in 2014 were as follow: Canada: 73.3, 12.4, and 2.8; United States: 71.6, 15.5, and 2.5; European Union: 62.4, 17.9, and 3.7; and Iran: 27.8, 4.8, and 0.3 kilograms (kg) per capita, respectively (4-6). The consumption of butter within Asia was 0.1-3.9 (kg) per capita in 2014 (4).

Sensory evaluation methods which are used by several industries are critical in promotion, production and the quality of foodstuffs. Sensory evaluation is a scientific field which is related with all methods of stimulating, measuring, analyzing and explaining human responses to the physical and sensory properties of foods and materials. These properties are perceived by senses such as taste, smell, touch, sight and hearing. The utilization of sensory methods can lead to the development of healthier foodstuffs (7).

Bacteria, yeasts, molds and viruses are the most common microorganism in dairy products. Some of the bacteria which are found in milk are useful (e.g., lactic acid bacteria) for fermenting the milk naturally, but pathogenic bacteria such as Salmonella spp., Staphylococcus aureus, Escherichia coli, Mycobacterium tuberculosis, Listeria spp. and Brucella spp. are also found in milk (8).

The surface discoloration and off-flavor are caused by yeasts and molds. Lactic acid bacteria are responsible for too strong acidity. The oxidization of fat content and then the rancidity of butter are the results of lipolytic bacteria activities, while the degradation of casein and so the cheesy taste of butter are related to proteolytic bacteria activities. Coliform bacteria, enterobacteria and other bacteria cause coloring and undesirable tastes in the butter (9, 10).

The per capita consumption of butter in Iran is increasing (11). The traditional dairy products are common foods in some cities of Iran such as Sarab (12). There is a risk of microbial contamination for all traditional dairy products which are transported to market without any pasteurization process (13). There are some studies on microbiological and physicochemical characteristics of traditional dairy products that had been conducted in some cities of Iran (14-18), such as Roofegari Nejad et al.'s study that some of the physicochemical characteristics and also fatty acid composition of traditional butters (TB) in East Azarbaijan were evaluated in it (14). The microbiological quality of TB was also assessed by Farrokh Eslamloo et al. (15), Mousavi et al. (16), and Mirzaei et al. (17) in Urmia, Garmsar, and Tabriz, respectively. Mirzaei et al. also assessed the prevalence of methicillin-resistant Staphylococcus aureus in raw milk and dairy products in Sarab (18). Due to the lack of reliable data about microbiological and physicochemical characteristics of TB and regarding insufficient supervision on the production process of them, this study aims to assess the microbiological and physicochemical characteristics of TB in Sarab.

Materials and Methods

Samples

Fifty samples of TB were collected from the farmers of different locations of Sarab, Iran. Butter samples were taken in 100 mL sterilized plastic dishes and transported to the Nobel laboratory in Tabriz, Iran and Azargol dairy products company's laboratory in Sarab, Iran.

Physicochemical analysis

International Dairy Federation (IDF) Standard 80 (2001) was used for determination of moisture, solids-non-fat and fat contents of the TB. The procedure for determination of moisture content of butter was as follow: A known mass of butter was dried in the presence of pumice stone at 102 °C ± 2 °C. The remaining dry mass was weighed to determine the loss in mass. For determination of the solids-non-fat content, water from a known mass of butter was evaporated. The fat was extracted with pentane (with a boiling point of 36 °C) and the mass of remaining substances was determined. The fat content of butter was calculated by subtracting the mass fraction of water content and solids-non-fat content from the total mass fraction of substances (equal to 100%).

IDF Standard 12 (2004) was used for determination of salt content of the TB. The salt content was determined through melting a test portion of butter by adding boiling water and then titration of the dissolved chlorides in the mixture with a solution of silver nitrate, using potassium chromate as indicator.

The acidity of samples was determined by an approximately similar method described by Idoui et al, 2013 (10). For the acidity, 20 g of butter were dissolved in ethanol. The mixture was titrated with sodium hydroxide in the presence of phenolphthalein as indicator.

We determined peroxide index, iodine index and saponification index according to the methods described by Idoui et al, 2013 (10). For peroxide index, 5 g of butter were dissolved in acetic acid/chloroform mixture with the 3:2 volume ratios. Then we added 0.5 ml of saturated KI solution. The mixture was titrated with a solution of sodium thiosulfate until the appearance of the light yellow color. After that the starch was added as indicator and the titration was continued until the blue color was disappeared. For the determination of the iodine index, 0.5 g of butter was dissolved in chloroform and Hanus solution mixture. Then, the mixture was titrated with a sodium thiosulfate solution

in the presence of starch as indicator. For determination of the saponification index, 2 g of butter was dissolved in alcoholic potassium hydroxide and heated for 30 minutes at 100 °C. Then the mixture was titrated with hydrochloric acid (0.5 N) in the presence of phenolphthalein as indicator.

Microbiological analysis

We prepared the samples and performed serial dilutions according to IDF (19). The microbial counting was performed on the following media and conditions: Plate count agar incubated at 6.5 °C for 10 days for psychrotrophic bacteria (20, 21); Baird-Parker agar base at 37 °C for 24h and 48h for Coagulase Positive Staphylococcus (20, 21); pour-plate method, plate count agar media incubated at 25 °C for 5 days for molds and yeasts; lauryl sulfate tryptose broth medium at 37 °C for 24-48h for Escherichia coli. We determined coliform bacteria count by the most probable number technique (MPN) on MacConkey broth medium for probable coliform test (22) at 30 °C for 24h. All tubes with positive results (production of gas) were used for confirmatory tests; so, they were incubated at 30 °C for 24-48h. Then we used the MPN table for determination of the coliform number.

Sensory evaluation

The butter samples were evaluated by a trained 12-member sensory panel aged from 25 to 35 years. All of them were familiar with the characteristics of the Sarab's TB. A ten-point hedonic scale was used for the assessment of the texture, color, odor, flavor and overall acceptability of the samples, where 10 indicated "extremely desirable" and 1 indicated "undesirable". Each panelist scored the characteristics of samples in an appropriate location with enough privacy.

For quality evaluation of our butter samples, we compared the values of physicochemical content and microbial count of samples with National standards (NS) and European standards (ES) (23-25).

Statistical analysis

We used the SPSS statistical software package version 16 for all statistical analysis. The mean ± standard error (±S.E) was represented for amounts of different parameters. One Sample t- Test was used for comparing the mean value of parameters with standard values.

Results

Fifty samples of TB were examined and table 1 shows the physicochemical characteristics of them. The peroxide value, moisture and solids-non-fat content of 88%, 62% and 78% of butter samples were higher than the ES, respectively. According to the results of our study the fat content of 74% of butter samples were lower than the standard values. The salt content, iodine index and the acidity of all samples were consistent with the standards. The saponification index of 26% of samples was out of the standard range.

Microbiological characteristics of the samples

Table 1. Physicochemical characteristics of the Sarab's traditional butter (n=50)

Physicochemical Characteristics	Minimum	Maximum	Mean (SE)	ES	*P**
Moisture content (%)	0.78	33.50	17.35 (0.886)	Up to 16% m/m	0.134
Solids-non-fat content (g/100g)	0.44	21.70	5.30 (0.683)	Up to 2% m/m	< 0.001
Fat content (%)	68.00	90.00	79.14 (0.741)	Min. 82% m/m	0.252
Salt content (%)	0.10	0.99	0.62 (0.032)	Up to 2%	< 0.001
Acidity (%)	0.10	0.99	0.53 (_0.039)	1.2 mmol/100 g of fat	< 0.001
Peroxide value ($mEqO_2/kg$)	0.10	1.20	0.65 (0.037)	Up to 0.3	< 0.001
Iodine index (mg I_2/100 g)	26.00	40.00	32.06 (0.733)	26-40	0.206
Saponification Index (mg KOH/g)	222.20	235.90	231.10 (0.537)	225-235	0.045

SE, Standard Error of means; ES, European Standards
One Sample t- Test
* $p < 0.05$

are shown in table 2. Thirty six percent and 32% of samples were contaminated by coliforms and psychrotrophic bacteria, respectively. The coliform bacteria count in 8% and the psychrotrophic bacteria count in 4% of samples were higher than the NS. The E. coli and coagulase positive staphylococcus were detected in 10% of samples while according to the NS contamination of butter samples by these two organisms is not acceptable. Fifty percent of samples were contaminated by molds and yeasts but these organisms count in all samples was lower than the standard value.

The sensorial characteristics of the samples are shown in Table 3. The scores of sensory evaluation ranged from 8 to 10 for all butter samples. According to the sum of scores, most of the samples were extremely desirable with respect to color, texture, odor, flavor and overall acceptability.

Discussion

This study was conducted to assess the physicochemical, microbiological, and sensory characteristics of TB in Sarab. In comparison with the study that conducted by Honfo et al. (26), our results showed higher values for moisture and fat content. In other similar studies, the moisture content, peroxide, and iodine index were higher and saponification index was lower than our results (1, 10, 26). Different sources for preparation of butter, shea kernels in Honfo et al.'s study and goat milk in Idoui et al.'s study, is probable reason for the differences between between our results and theirs. Since the activation of lipases, stimulation of the growth of microorganisms and hydrolysis of triglycerides are related to the existence of water in butter, physicochemical and microbiological attributes of TB can be affected by the high level of moisture in it (27).

In Hermida et al.'s study the solids-non-fat content of samples ranged from 1.14 to 3.12 g/100 g of butter and fat content was between 81.09 and 85.52% (28). Different methods that were used for determination of these parameters may explain the differences between our results and the results of Hermida et al.'s study. The average salt content of samples in our study was higher than the salt content that was reported in Dadgostar et al's study (29). Acidity of samples in O. Samet-Bali et al.'s (30) and Ayegnon et al.'s (31) studies was higher and in G. Simões et al.'s study (32) was lower than our results. Various sources (the milk of different animals or plants) for preparation of butter, different methods for determination of physicochemical characteristics, the precision degree of examinations,

Table 2. Microbial characteristics of the Sarab's traditional butter (n=50)

Organisms	Minimum	Maximum	Mean (SE)	NS	*P**
Total coliforms (cfu/g)	0.00	120.00	8.06 (3.231)	< 20	0.001
E. coli (cfu/g)	0.00	1.00	0.10 (0.042)	0	0.024
Psychrotrophic count (cfu/g)	0.00	1×10^{6}	2.14×10^{4} (2×10^{4})	$< 10^{4}$	0.570
Coagulase Positive Staphylococcus (cfu/g)	0.00	2.00	0.12 (0.054)	0	0.032
Molds and Yeasts (cfu/g)	0.00	40.00	6.32 (1.373)	$< 10^{2}$	< 0.001

SE: Standard Error of means; CFU: Colony forming unit; NS, National standards
One Sample t- Test
* $p < 0.05$

Table 3. Sensory evaluation of the Sarab's traditional butter (n=50)

Sensorial characteristics	Fairly desirable (%)	Desirable (%)	Extremely desirable (%)	Mean (SE)
Color	6	4	90	9.84 (0.072)
Texture	2	8	90	9.88 (0.054)
Odor	4	28	68	9.64 (0.079)
Flavor	10	28	62	9.52 (0.095)
Overall acceptability	0	28	72	9.72 (0.064)

and the farmers' skill in preparation of traditional butter may explain the differences between the results of several studies.

In comparison with our results, higher levels of total coliforms, E.coli, molds and yeasts, and psychrotrophic bacteria counts were recorded by other studies (9, 31). In Mirzaei et al.'s study the contamination of butter samples by coagulase positive staphylococcus aureus was higher than our samples (17, 18). Idoui et al. (2010) revealed that the samples had no contamination by staphylococcus but the values of molds and yeasts were higher than our results (1). The contamination by molds can occur during the preparation process and also during the storage of food products (33). Diverse primitive methods of processing, different levels of hygiene during the production process of butter, the precision degree of examinations, various environmental conditions during the storage of TB, and the farmers' skill in preparation of it may explain the different microbial quality of samples in several studies.

Score of organoleptic properties in all samples of present study were more than 8 points. Other studies reported lower scores than our study (26, 31, 34). It seems that higher score of organoleptic properties in our samples were because of higher quality of TB and panelists' long time familiarity with Sarab's TB.

Lack of identical production process for TB and non-random sampling are the limitations of our study. The concurrent assessment of physicochemical characteristics and microbiological quality of samples and comparison of them with ES and NS were main strengths of present study. Although there are 170 units for preparation of this product in Sarab, we assessed just 50 units of them but due to the sample size of our study was obtained through Krejcie and Morgan table (35) and sampling was performed in valid and well-known units with high experience in butter production, it seems that the results of our study are generalizable.

Conclusion

This study suggests that the quality of physicochemical and organoleptic characteristics and microbial load of traditional butter were acceptable, but because of the lack of sufficient data more studies and supervision on the production process of traditional products is recommended. Through studies on the traditional products and evaluation of their quality in comparison with commercial dairy products, the preparation units can get license number for their products. Using the traditional dairy products which have license number is in accord with food safety standards and these products are more acceptable for populations.

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