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Original Article

The Healthiness of Commercial Butter in Malaysia: Evaluation of the Physicochemical and Microbial Quality

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Abstract

Food safety includes all health aspects of the food, especially microbiological quality. Butter, particularly commercial butter is one of the highly consumed foods in Malaysia. The research objective was to investigate the microbiological, physicochemical characteristics and structure of fatty acids of a commercial cows' butter (CCB) sold in Malaysia. Chemical, microbial quality and fatty acid compositions of 7 randomly purchased butter samples from the market were analyzed. The results show the existence of substantial variances in physicochemical parameters between butter samples. The range of pH was between 3.32 to 4.90. The moisture content of all commercial cows' butter samples except sample 5 and 6 was in international standard range. Peroxide and iodine values of all samples were in range of international standard limit. The determination of fatty acids composition by gas chromatography showed the prevalence of the saturated fatty acids dominated by palmitic acid, with a low rate of unsaturated fatty acids, dominated by oleic acid. The results also show the presence minimum of total aerobic mesophilic bacteria (TBC) as well as psychrotrophic bacteria, while coliforms bacteria were not detected. Moulds and yeasts were detected in all samples as minimum counts except samples 2 and 4 which were free from them. Therefore, it was found that the microbiological quality of commercial butter samples is generally good and thus health safety.

Keywords: *Butter; Physicochemical; Microbiological; Fatty acids; Colifom; Moulds and Yeasts*.

Introduction

Consumption of milk and dairy products (yogurt, butter, ice cream, and cheese) was associated with health benefits including low risk of hypertension, improved bone health and reduced risk of diabetes due to dietary compounds (El-Aidie *et al.* , 2017; El-Garhi *et al.*, 2018). Butter as the one of dairy products has a significant role in human nutrition (Tvrzicka *et al.*, 2011).

Butter is a dairy product that plays an important role in the human diet, not only because of its nutritional components (including energy, fat-soluble vitamins, and essential fatty acids) but also because of its effect on mouth-feel and hence its ability to make our food much palatable (Ghasemloy Incheh *et al.*, 2017). The main idea of Butter manufacturing is the separation of fat from milk. Fat is the main composition of butter (80%), in addition to 20% of water, proteins and milk sugar. Butter has a pale yellow/cream colour, and a smooth consistency so that they spread easily and melt in the mouth. It is used in cooking, or as bakery ingredients (Vanderghem *et al.*, 2010).

Historically, butter has been an expensive commodity and is still high in price despite widespread production (Varnam & Sutherland, 2001). One of the most important features of butter, which increases the sale is its flavor, which the main reason for the increase in the price of sale compared to other fats (Jebson, 1994). Many fatty products compete with butter, such as vegetable oil-based margarine, blended spreads and liquid vegetable oils. However, none of them can match the desired flavor and the mouth-feel of butter imparts to food. Thus, butter has the highest value in the market depending on the flavor and identity of natural milk (Rady & Badr, 2003).

Butter is a suitable environment for microbial growth because of the 20% butter's components of water, proteins and milk sugar (Catsberg & Kempen-van Dommelen, 2013). Chemical rancidity is the main source of butter spoilage, in addition, many forms of microbial problems produced by hydrolysis such as putrid odors, rancid flavor, and cheesy (Rady & Badr, 2003). Bacterial diversity was studied in many research for butter samples resulting from lipolysis and spoilage at below 5^{o}C temperatures such as *Lactococci, Lactobacilli, Enterococci, Molds, and Yeasts* (Benkerroum & Tamime, 2004; Samet-Bali, Ayadi, & Attia, 2009). In addition, *Staphylococcus aureus and Listeria monocytogenes* appear as one of the types of pathogenic bacteria in the butter stored at low temperatures (Adam MR, 1995). Bad conditions of storage and transportation are the most important factors that decrease quality and nutritional value of butter (Tofangsazan, *et al.* 2009; Koca, Kocaoglu-Vurma *et al.* 2010). There were no clear studies on the quality of commercial butter sold in the Malaysian market, so the current study objective is to evaluate the microbiological, physical, and fatty acid structure of commercial butter in Malaysia.

Materials and Methods

Collection of samples

A total of 6 random samples of refrigerated commercial cows butter were aseptically collected from different market in different areas, Malaysia during November-December period (2016). All samples were taken in sterilized container and transported under refrigerated condition to the laboratory. Analyses were started without any delay.

Physicochemical analyses

Chemical quality of butter samples was investigated by measurement of peroxide value, iodine value and also determination of saponification value according to the methods described by (Idoui *et al.*, 2010). Digital pH meter (pH Model 510, Dutch instruments Pltd., Malaysia) was used to evaluate the samples' pH, and based on (Marth, 1978) methods, the samples moisture content was measured. To determine the peroxide value, 1g of butter was dissolved in acetic acid/chloroform (3v/2v). 0.5 ml of saturated KI was added, and the combination titrated by a solution of sodium thiosulfate in the existence of starch as indicator. To determine iodine value, a weight of butter dissolved in hexane was added to 0.1M iodine monochloride in acetic acid. After 10 min, a solution of 0.1M of KI was added and then, the liberated iodine in the mixture was titrated with a solution of 0.1M sodium thiosulfate in the presence of starch as indicator. To determine saponification value, 2 g of butter was dissolved in excess alcoholic KOH and heated for 30 min at 100°C. The titration of the mixture was carried out with chlorhydric acid (0.5 N) using phenolphthalein as indicator.

Fatty Acid Composition

According to (Christie, 1989) methods, the samples' structure of fatty acid was analyzed. For butter-fat samples, the preparation of fatty acid methyl esters were conducted by implementing 2M methanolic potassium hydroxide solution and extracted with 15ml of N-hexane and dried over anhydrous CaC12 salt. The analysis of the aliquot of the supernatant for free fatty acids was processed by gas chromatography (GC1000, DANI instruments SPA, Pavia, Italy) equipped with flame ionization detector and a polar capillary column: ECTM-5 (30m ×0.32mm×0.25µm film thickness) with a mobile phase of 5% phenyl and 95% methylpolysiloxane. The length of the column was 30m. The operative circumstances temperatures were: (injector 210^{o}C, detector 260^{o}C, and column 50^{o}C for 2min, and then upgraded by 4^{o}C/min and

during 15min the temperature reached 250^{o}C). Flow rate nitrogen gas used at 1mL/min.

Microbiological analyses

Microbial quality of commercial butter was investigated according the American Public Health Association (Marth, 1978). The total aerobic mesophilic bacterial counts (TBC) were estimated using plate count agar medium and plates were incubated at 32°C for 48-72hours. Total coliforms were determined on violet red bile agar medium and the plates were incubated at 37°C for 24h. Moulds and yeasts were determined on Oxytetracycline glucose agar medium and the plates were incubated at 25°C for 3-7 days. Plate count agar incubated at 6°C for 7 to 10 days for psychotropic bacteria.

Results

Physicochemical properties

The analysis of physiochemical properties of the commercial butter samples are illustrated in table 1. The finding of the analysis revealed that there were a difference between samples in chemical scores. Samples' pH ranged between 3.32 to 4.90, Similar results are found by Berhe, Seifu, & Kurtu, (2013) and Mourad & Nour-Eddine, (2006). The moisture content of the study samples varied between 14.6 % and 18.6%. The moisture content of samples 5 and 6 was out of international standard range. According to obtained results, The upper legal limit for moisture in butter is 16%, according to Rashidi & Shabani, (2017). The high level of moisture in butter may have an effect on its microbiological and physicochemical quality furthermore high moisture can activate lipases, stimulate the growth of microorganisms and hydrolysis of the triglycerides (Idoui *et al.* 2013). The peroxide value ranged between 0.27 and 1.48meq/ kg of butter samples. The all samples peroxide value was in the range of international standard limit (1.7 mEq/ kg). This value is measured as an indicator of fat oxidation. Oxygen, metal ions and light as oxidant factors can affect on this value (Ghasemloy *et al.*, 2017). Therefore, the early phase of lipid oxidation can be detected by estimating the peroxide value as an oxidative indicator (Samet-Bali et al., 2009). Iodine value of butter samples ranged from 26.22 to36.32 mg I/100g. According to Iranian national standard, Iodine value for butter samples is 26-40 (Ghasemloy *et al.*, 2017).

Table: 1. Physicochemical properties of commercial purchased cow butter samples

	Treatments						
Microbial groups	1	2	3	4	5	6	7
Moisture (%)	16	14.6	15.8	15.7	18	18.6	16
pH	3.90	3.32	4.12	3.73	4.34	4.90	3.98
Peroxide value (meq/Kg)	0.45	0.33	0.27	0.42	0.66	1.48	0.29
Iodine value (mg I/100g)	29.84	32.98	29.45	26.46	36.32	34.53	26.22
Saponification value (mg KOH/g)	225.19	232.82	196.77	214.42	225.74	247.62	228.60

This value shows unsaturated fatty acids content and their resistance to oxidation which confirmed by showed results in Table 2. The results obtained also indicated that saponification value of butter samples ranged from 196.77 to 247.62 mg KOH/g. Saponification value of samples 3, 4 and 6 was out of standard butter range. According to Iranian national standard, the saponification value of butter sample should be in the range 225-235 (Ghasemloy *et al.*, 2017). This index shows presence of long-chain fatty acids which confirmed by showed results in Table 2.

Fatty acid composition

The fatty acid composition of the 7 selected samples of commercial cows' butter (CCB) is given in the Table 2. Eleven (11) fatty acids are identified and quantified. As shown, all CCB samples controlled a high proportion of saturated fatty acids (SFA) and it ranged between 54.9 - 62.59%. Samples 4 and 7 had higher levels of saturated fatty acids than other

samples (62.59- 60.745% respectively). It was observed that samples 2 and 3 had the same saturated fatty acids level as it was 57.37-57.63% respectively.

Table 2. Fatty acid composition (Proportion, percent weight) of commercial purchased cow butter samples.

Fatty acids	Treatments						
	1	2	3	4	5	6	7
Saturated fatty acid (S)	54.9	57.37	57.63	62.59	59.53	59.08	60.74
C6:0	0.38	0.34	0.82	0.76	0.39	0.81	0.74
C8:0	0.24	0.28	0.23	0.47	0.32	0.44	0.61
C10:0	0.36	1.82	0.73	0.81	1.57	0.99	1.65
C12:0	0.88	0.68	1.37	1.34	1.35	2.31	2.23
C14:0	10.60	10.55	9.22	10.23	11.12	9.14	8.53
C16:0	32.05	33.85	35.35	36.94	35.92	37.14	37.53
C18:0	9.84	9.31	9.42	11.53	8.33	7.76	8.89
C20:0	0.55	0.54	0.49	0.51	0.53	0.49	0.56
Unsaturated (U)	25.01	27.32	28.04	27.33	26.02	28.30	29.39
Monounsaturated	21.79	23.9	24.57	22.55	22.63	24.95	24.23
C16:1	1.68	1.77	1.43	1.22	1.26	0.55	0.49
C18:1	20.11	22.13	23.14	21.33	21.37	24.4	23.74
Polyunsaturated	3.22	3.42	3.47	4.78	3.39	3.35	5.16
C18:2	3.22	3.42	3.47	4.78	3.39	3.35	5.16

Four major fatty acids were detected in CCB samples; palmitic (C16: 0), oleic (C18: 1), myristic (C14: 0), stearic (C18: 0) acids and the palmitic acid was the greatest one (32.05%-37.53%). The previous finding are coincided with Samet-Bali *et al.*, (2009) who stated that there are four major fatty acids in Tunisian butter made from cow milk and palmitic aid was similarly the main fatty acid (33.72%). Furthermore Fatouh *et al.* (2007) and Idoui *et al.*, (2013) described that palmitic acid was the main SFA (31.89%) and (20.45-50.56%) respectively. Butter samples 6 and 7 contained a higher level of palmitic aid than all samples (37.14- 37.53% respectively). Minimum myristic acid was present in sample 7 as (8.53%) and maximum was in sample 5 as (11.12%). As it can be seen in Table 2 the stearic and oleic acids were higher level in sample 4 as (11.53%) and sample 7 as (23.74%), while they were a lower level in sample 6 as (7.76%) and sample 1 as (20.11%) respectively.

In the current study the content of long-chain fatty acids (C14-C18) was higher than the short-chain fatty acid (see table 2), 64.45% and 4.19%, respectively. The previous results indicated that CCB contain a higher contents of high-melting point (superior with 44°C) triacylglycerols which contains fatty acids with long-chains (Laadhar *et al.*, 2006). These results are coincided with the results of (Laadhar *et al*., 2006 and Idoui *et al*., 2010). The properties of butter can be identified by fatty acid types. Fats containing high levels of higher-melting fatty acids are hard; while fats, with a lower proportion of low-melting point fatty acids make the butter smooth (Berhe *et al.,* 2013). Among the unsaturated fatty acids, the identified fatty acids were oleic acid followed by linoleic acid and palmitoleic acid. The amount of these fatty acids were ranged between 20.11-24.4%; 3.22- 5.16% and 0.49–1.77% respectively.

In agreement with (Samet-Bali *et al.,* 2009), and as nutritional advantageous, the current study revealed that, CCB samples contain a high proportion (27.47%) of monounsaturated fatty acids (C16:1, C18:1). Polyunsaturated fatty acids depend essentially on the food while the mono unsaturated fatty acids like the oleic acid result in part from their mammary synthesis and in part from the activity of the mammary delta-9 desaturase which converts

the saturated acid on monounsaturated acid (Idoui *et al.*, 2013). Polyunsaturated fatty acids content which is more sensitive to oxidation, and the presence of natural antioxidants are the main reasons for considering CCB as resistant to oxidation according to the current study results.

CCB Microbiological characteristics

Table 3 illustrated the count of total aerobic mesophilic bacteria (TBC), total coliforms, moulds and yeasts and psychotropic bacteria presents in commercial cow butter samples. Results are given in Table (3) revealed that the TBC of CCB ranged between 0.38×10^4 and 1.15×10^4cfu/g. High counts of total aerobic mesophilic bacteria were recorded in CCB samples 4, 5 and 7. The high-quality butter, according to Gökçe *et al.* (2010) does not exceed 5.0×103cfu/g of the counts of total mesophilic aerobic bacteria. The total bacteria's high count could be because of the pasteurization defect and to the absence of salt. According to Idoui *et al*., (2010) the main reason for the increased count of total bacteria is the effect of both churning and separation processes on the breaking up of bacterial clumps. Furthermore, Gökçe *et al.,* (2010) attributed that, the total aerobic mesophilic bacterial counts were affected because of the low-quality standards of the cream used in the preparation of the butter, and the defects in the processing circumstances.

Table 3. Microbial counts populations (cfu/g) in commercial purchased cow butter samples

Parameters	Treatments						
	1	2	3	4	5	6	7
Total bacterial counts (TBC)	0.38×10^4	0.52×10^4	0.47×10^4	0.89×10^4	0.57×10^4	0.35×10^4	1.15×10^4
Total coliforms	ND	ND	ND	ND	ND	ND	ND
Moulds and yeasts	1.09×10^2	ND	1.02×10^2	ND	1.67×10^2	2.30×10^2	1.17×10^2
Total psychrotrophic bacteria	0.97×10^3	ND	3.1×10^3	1.77×10^3	1.5×10^3	2.7×10^3	2.1×10^3

ND: Not detected

Total coliforms bacteria were not found in the CCB samples. In fact, coliforms are indicators of cleanliness of handling, premises, and equipment. These results suggest that CCB is produced under the good hygienic condition. The spoilage in the food which have low water activity, refer to the faster growing of mould and yeasts than bacteria. In addition, the existence of mycotoxin risk, and the high amount of moulds and yeast considered an indicator of incorrect processing and packaging. Except sample 2 and 4, mould and yeast were found in the CCB samples and ranged between 1.02×10^2 - 2.30×10^2cfu/g. Maximum permissible level of molds in the standard is 100cfu/g (Ghasemloy *et al*., 2017). The Table also shows that the numbers of mould and yeast were higher in sample 6 compared with other samples (2.30×10^2cfu/g). Psychrotrophic bacteria were also present and the important count was detected in all tested samples (0.97×10^3 - 3.1×10^3cfu/g) except sample 2. The obtained results are similar when compared to other studies (Mourad & Nour-Eddine, 2006 and Idoui *et al.*, 2010).

Conclusion

In the current study, we evaluated physicochemical and microbiological quality as well as the composition of fatty acids of 7 commercial cows' butter samples. The results obtained from the physicochemical composition shows the moisture of all samples except samples 5 and 6 was in international standard range. Peroxide and iodine values of all samples were in a range of international standard limit. The predominant fatty acids are palmitic, oleic, myristic and stearic acids. The results obtained from the microbiological analysis show no microbial load and no detection of coliform bacteria. Therefore, the microbiological quality of the commercial cows' butter that collected from the market was satisfactory.

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