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Application of Buffalo Butter Oil Fractions for the Preparation of Modified Spread Butter

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Abstract

Butter oil of buffalo milk has relatively low concentrations of unsaturated fatty acids (USFA), which makes its usage limited in food industries. The goal of this study was to increase the low solids content of modified buffalo butter oil (**BO**) fractions through multi-step dry fractionation at different temperatures (15, 25, and 35 °C) to obtain three solid fractions (**S15**, **S25**, and **S35**) and three liquid fractions (**L15**, **L25**, and **L35**). Some buffalo fractions were reformed to produce modified butter (**MB**) by using various milk fat fractions at ratios of L15:L25:S15 (7:2:1 and 8:1:1) L15:L25:S25 (6:3:1 and 7.5:1.5:1), and L15:S15:S25 (7:2:1) blend for, **MB1**, **MB3**, **MB2**, **MB5**, and **MB4**, respectively. The chemical characteristics of **MB**, such as chemical composition, fatty acids, and solid fat content (SFC) of **MB** were studied. Furthermore, the thermal behavior, texture, and sensory properties were evaluated and compared with those of native **BO**. The collected fraction of **L15** was significantly increased the MUNSFA, PUSFA, ω6:ω3 ratio, iodine value and oxidative stability index (OSI), and decreased the slip melting point (SMP), cholesterol content, atherogenicity index (AI), thermo biogenicity index (TI), firmness and stickiness. The **MB5** sample had the highest overall acceptability by the panelists for both fresh and 90 days stored sample. The **MBS** was contained PUSFA from 3.98 to 4.80 mg/100g which improved the nutritional value and beneficial for health.

Keywords: Buffalo butter oil; multi-step fractionation; fatty acids; oxidative stability

1. Introduction

The buffalo (Bubalus bubalis) is the most considerable milk-producing animal that has a significant contribution to the world's wide milk production, such as in India, Pakistan, China, and Egypt [1]. Buffalo milk was used to produce several products, such as yogurt, butter, cream, and many kinds of cheese [2]. Milk fat is a unique fat; it contains saturated fatty acids of varying lengths and a low content of unsaturated fatty acids, resulting in low spread ability and limited food application [3,4]. For many years, traditional butter has been the preferred spread due to its full flavor, mouth feel, and excellent storage properties. Although the negative health image of fat is associated with the lack of spread ability of butter at low temperature. Thus, several studies have been conducted on the dry milk fat fractionation to overcome these limitations and obtain smoother and more spreadable butter [5, 6, 7]. Also, milk fat fractionation by multi-step dry fractionation can change the fatty acids composition of milk fat [8, 9]. Additionally, the changes in the physical properties of butter that depend on the content of the solid fat, melting point, and crystallization rate are achievable by different methods, such as the thermal fractionation [10], making changes in cooling rate and storage conditions of the sample [11], in addition to cream maturing [12], and the inclusion of low-melting fraction (LMF) in manufacturing butter, which

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decreases the solid fat content at 15 °C and its hardness [13].

Furthermore, making changes in animal feeding can modify the milk fat composition that can improve the spread ability of butter subsequently [14,15]. Several ways can improve the milk fat composition such as blending unsaturated fatty acids [16], milk fat interesterification to obtain a mixture of milk fat and vegetable oil [17], hanging the concentration of unsaturated fatty acids in milk fat through dry fractionation [18], or combining high melting and low melting fractions [4,19]. Low melting fraction (LMF) has an intense butter flavor and can be included into milk powder to make functional improvements. It can also be used in dairy products [20]. On the other hand, the LMF has a positive healthy effect and ω6 and ω3 contents higher than other fractions.

In this context, the aim of this study to fractionate the buffalo butter by multi-step dry fractionation and produce new restructured buffalo butter. The chemical and thermal properties of the resulting modified butter samples were characterized during cold storage for 90 days at 5-10 °C.

2. Materials and Methods

2.1. Materials

2.1.1. Fresh butter

Fresh buffalo butter was made from milk obtained from buffalo heard at Cairo University-Faculty of Agriculture during the winter season of 2021, then stored at -18°C till processing. Commercial butters (CB) were purchased from local market in Giza. All samples were purchased with the same production date and they were wrapped up in dark bags.

2.1.2. Butter milk powder

Butter milk powder (BMP) was purchased from M/S Bob's Red Mill Natural Foods, Milwaukee, USA. The contents of total fat, saturated fatty acids, trans fatty acids, and cholesterol in BMP were 1 g, 0.5 g, 0 g and 10 mg per 100 g sample, respectively. Also, each of protein, carbohydrate and sodium content of BMP were 5 g, 7 g and 85 mg, respectively.

2.1.3. Skim Milk Powder

Low heat skim milk powder was obtained from Dairy America, California-USA. The analytical data of skim milk powder were protein 34%, moisture 4%, fat 1.25%, titratable acidity 0.16%, and solubility index 1.2 mL.

2.1.4. Emulsifier

Food grade Soy lecithin Adlec (E322) was purchased from ADM, Hamburg – Germany. Mono-diglycerides, Grindsted® was procured from DuPont, Denmark.

2.2. Methods

2.2.1. Preparation of buffalo butter oil fractions

Butter oil (BO) was prepared from butter by multi-step dry fractionation according to the method of Amer et al. [21]. Fresh butter was heated at 60°C for 2 h with agitation to obtain BO from top oil layer then stored at -18 °C. BO crystallization was performed by gradual and consecutive cooling procedure described by van Aken et al. [22]. About 3liters of BO were placed in a two-glass double walled tempering beaker (2 liters each) and held at 80 °C for 10 min using water-bath (Memmert, model WB 7, Memmert Gmbh + Co. KG, Germany) without agitation to form crystal nuclei. The sample was then fractionated by multi-step fractionation using an incubator (Low Temp. Incubator, Model DK-LI 010-P, Daiki Scientific CO. Korea). Then, BO was cooled slowly to 25 °C for 24 h, and the resultant solid fraction (S25) was collected from the liquid15 psi under vacuum (Buchner funnel). A similar process was used to collect solid (S35) and liquid fractions (L35) at 35oC. Also, L25 was further fractionated at 15 °C producing liquid (L15) and solid (S15) fractions. Figure 1 shows the multi-step dry fractionation procedure of butter.

2.2.2. Production of modified spread butter

Production of modified spread butter (MSB) was made from different ratio of buffalo butter oil fractions as showed in Fig 2. Five modified butters (MB1 = L15:L25:S25 (6:3:1), MB2=L15:L25:S15 (7:2:1), MB3= L15:L25:S25 (7.5:1.5:1), MB4 = L15:S15:S25 (7:2:1), MB5=L15:L25: S15 (8:1:1) were prepared and compared with buffalo butter (BM) and commercial butter (CB). The overall composition of the high load emulsions included 80% milk fat (anhydrous milk fat or milk fat fractions) with 16-18% water, 0.3 % soy lecithin, 1.2% skim milk powder and 0.3% buttermilk powder to enhance emulsion stability.

Buffalo fractions with the selected ratio was heated to 60 °C, combined by stirring them with a stainless-steel spatula, held for 30 min, then added to a mixture of emulsifiers. The water phase was

prepared by mixed of buttermilk and skim milk powders in water and then emulsified with the heated milk fat fractions for 90 s at low speed in a magnetic starrier (Pyro Multi-magnestir, Lab-Line instruments, Inc. Melrose Park, ILL). The butter emulsion (40°C) was poured into sterilized glass bottles (100 g) and tempered at 5-10 °C for 48 hours.

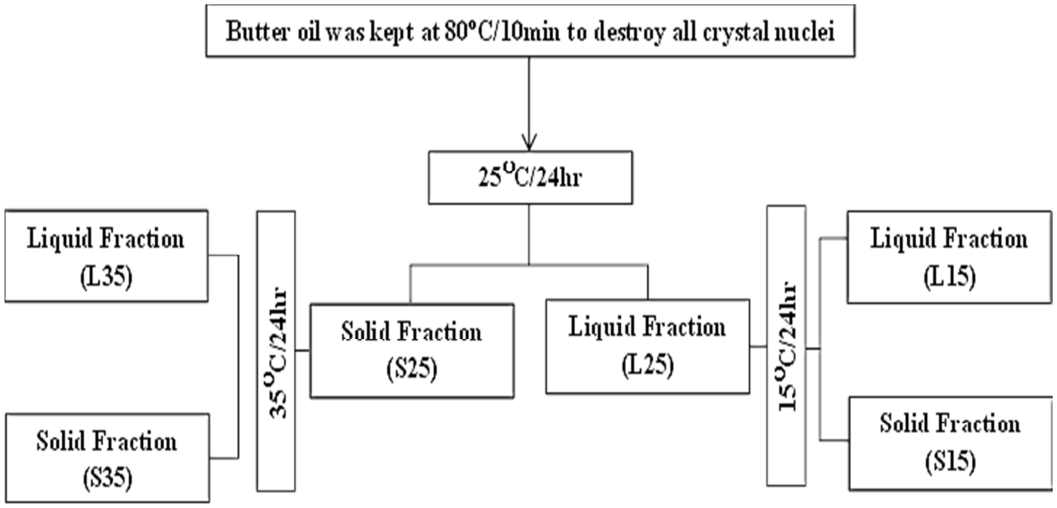


Fig. 1: Schematic diagram for fat fractions by multi-step dry fractionation

2.2.3. Determination of fatty acid atherogenicity index (AI)

Fatty acid methyl esters (FAME) were prepared by trans-esterification of the butter oil and its fractions and butter samples using sodium-methoxide complex as catalyst according to AOCS [23]. About 100 mg of oil was transferred into a screw-cap vial then 5 ml of hexane was added. Sodium methoxide (250 µL) was added, and the mixture was swirled for 1 min followed by addition of 5 mL saturated sodium chloride solution. The mixture was covered with the bottle cap, shaken vigorously for 15 seconds, and then kept at room temperature for 10 minutes. The top hexane layer was injected into the GC.

The GC Agilent instrument (Agilent 6890 N, Network GC-System, and Wilmington, USA), an injector (Agilent 7683-B Series) and flame ionization detector was used. Separation capillary column DB-Wax (length 30 m, internal diameter of 0.30 mm) was carried out with helium as an inert gas at a flow rate of 1 mL/min. The beginning oven temperature was 100°C for 2 minutes before rising to 230°C at a rate of 5°C/min. The injector and detector temperatures were 250°C. The oven temperature was subsequently held at 230°C for 10 min. The identification of fatty acid methyl esters (FAMEs) was achieved by comparing with known standards (37-Component FAME MIX, SUPELCO) and quantified as percent-weight of total fatty acids. Atherogenicity index (AI) of hyper-cholesterolemic fatty acids were calculated by the flowing equation [24, 25].

AI = [C12:0 % + 4 × C14:0 % + C16:0 %] / USFA %

Where:

C12:0; Lauric acid

C14:0; Myristic acid

C16:0; Palmitic acid

USFA: Unsaturated fatty acid

2.2.4. Slip melting point (SMP) of butter

The SMP was measured according to AOAC [26]. Briefly, the completely melted butter oil, fractions and fat are spread into a capillary tube with a capacity of 75 mm in length and 1.2 mm in internal diameter. The tube was incubated at 5-7°C for 16 h to solidify the fat. The tube was attached to a thermometer then suspended in a 600 mL beaker of distilled water. The temperature was decreased to 8–10°C below the slip melting point of the sample then heated to increase the bath temperature. The temperature at which the fat column rose was reported as the SMP.

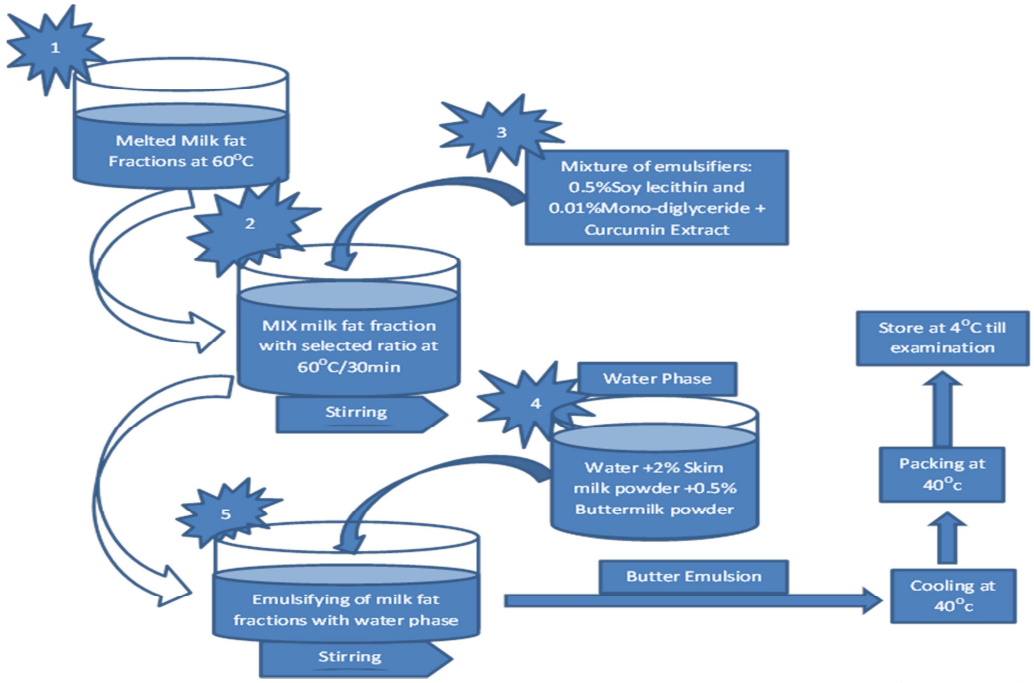


Fig. 2: Cold Spreadable Butter Manufacture

2.2.5. Calculation of iodine value of butter

Iodine Value (IV) was measured according to the methods described by Ham et al.[27] using the following equation:

Calculated IV = (% Tetradecenoic acid * 1.1212) + (% Hexadecenoic acid * 0.9976) + (% Octadecenoic acid * 0.8985) + (% Octadecadienoic acid * 1.8099) + (% Eicosenoic acid * 0.8173) + (% Docosenoicacid * 0.7496) + (% Tetracosenoic acid * 0.6923).

2.2.6. Cholesterol content of butter

Cholesterol level was determined according to Shin and Chang [28] by HPLC system. Agilen MB1260 infinity HPLC Series (Agilent®, USA), equipped with Quaternary pump, a Kinetics XB-C18 column 100 mm x 4.6 mm (Phenomenex®, USA), operated at 35°C. The separation was achieved using isocratic elution with acetonitrile: methanol (3: 1). The injected volume was 201 JL and the Variable Wavelength Detector (VWD) at 205 nm used for measurement.

2.2.7. Oxidation Stability Index (OSI)

Rancimat equipment model 679 (Metrohm Ltd. CH-9100 Herisau, Switzerland) was used for the determination of the oxidative stability of butter oil and its fractions. The evaporative decomposition products (mainly organic acid) are captured and trapped in measuring repositories filled with distilled water (60 ml) and detected with conductivity cells uninterruptedly (conductivity range 25-200 US/cm). Conductivity curves and results were delivered to a built-in printer of the control unit. The induction time was then designated, i.e., the time of the start of the experiment and intersection point, according to Mendez et al. [29].

2.2.8. Solid Fat Content (SFC)

The SFC of butter oil and fractions was determined using a 20 MHz pulsed nuclear magnetic resonance (pNMR) spectrometer (NMS/120 minispec, BRUKER, USA). SFC was determined according to the method of the AOAC [26]. The determination of SFC was performed in the temperature range of 0–45°C at 10°C intervals, and the signal intensities for both sample and reference oils were read.

2.2.9. Differential scanning calorimetry (DSC)

The thermal profile analysis was conducted at Micro Lab, Faculty of Science – Cairo University and heating thermograms were obtained by using DSC (Modulated DSC model 60; Shimadzu, Japan). The instrument was calibrated with deionized water and indium standards. Samples of butter oil (2 ± 0.05 mg) were hermetically sealed in an alodine-coated aluminum pan (Shimadzu, Japan). The sample cell was purged with the gas (25 mL/min) and cooled with N gas (30 mL/min) during the analysis. Samples were restrained at 80 °C for 5 min to melt any

crystals that has formed, cooled to -50°C at 10 °C/min, held for 15 min, and then heated to 60 °C at 10°C /min to obtain a melting profile. The cooling and heating rate were selected based on reference method mentioned by Kaylegian and Lindsay [30].

2.2.10. Determination of texture profile

Food Technology Corporation, USA, used FTCTM systems with TMS-Pro equipment and TL-ProTM software to precisely control force, speed, and position for rigorous food texture analysis. The hardness and stickiness of modified butter spread samples were determined at temperatures of 5°C according to Bobe et al. [14].

2.2.11. Sensory evaluation

The sensory analyses of butter spread were obtained by trained panelists. Parameters, such as color, spreadability, texture, flavor, and overall acceptance were evaluated as described by Gaze et al. [31]. The sensory analyses were carried out at room temperature (25°C). The overall acceptability was expressed as the average of other sensory parameters. The 9-point hedonic scales were used to categorize the sample. On the hedonic scale, 1 indicates extreme dislike and 9 indicates extreme like.

2.2.12. Statistical analysis

Analysis of all data with three replications for each parameter has used a randomized complete block design with one and two factors. The treatment means were compared by the least significant difference (L.S.D.) tested by the assistant program.

3. Result and discussion

3.1. Fatty acid composition of modified butter (MBs)

Buffalo milk fat fractions were selected for incorporation into new modified butter spread based on their unsaturated fatty acids and SFC content. LMF was significantly the lowest SFC at 25°C and this result is in agreement with Fatma et al. [9]. The fatty acid profile of buffalo milk butter (BMB), commercial butter (CB) and modified butter (MBs) are compared in Table (2). The fatty acid content was influenced ($P \leq 0.05$) by different ratios of butter oil fractions that have been used in the manufacture of butter. As observed, the unsaturated fatty acid (USFA content) markedly increased in all butter products with a concurrent decrease in its saturated fatty acid (SFA) content. On the other hand, short-chain fatty acids (SCFA) are composed of Butyric, Caproic, Caprylic, and Capric acids. SCFA content is one of the reasons that milk fat has its plasticity and may help to increase the spreadability of MBs therefore the SCFA has low melting points. Butyric acid is a unique FA of butter, it has a bioactive activity which has been known to reduce blood cholesterol levels and bowel infectious disease [32]. Butyric fatty acid content in MBs treatments ranged from 4.52 to 6.46% and 1.65 to 2.22%, respectively, and was arranged in the order of MB5> MB2> MB4> MB3> MB1 for both treatments [33]. Increasing the ratio of L15 fraction results in an increase of SCFA and butyric acid of MBs. Referring to fatty acid profile of butter oil and its fraction in previous study [9], reported that LMF has significantly ($P<0.05$) the highest SCFA and C4:0 content (6.01 – 6.52% and 2.13 – 2.37 mg/100g fat respectively) compared to the BMF and rest of the fractions. These results are also in agreement with Shin et al. [34] and Abdeldaiem et al. [35].

In addition, the most predominant SFA in all treatments, including the control was palmitic acid (C16:0) followed by stearic acid (C18:0), and then myristic acid (C14:0), whereas the most predominant USFA in all treatments was oleic acid (C18:1) followed by linoleic acid (C18:2) and linolenic acid (C18:3) as show in Table 1. All experimentally modified butter (MBs) becomes enriched with oleic acid (C18:1) which is beneficial for anti-cholesterolic effect when it is used in food and dairy products. Table 1 shows that the incorporation of mixed butter oil fractions in butter made (MBs) has significantly reduced the content of hypo-chlosterolemic fatty acid such as lauric acid, myristic acid and palmitic acid in descending order in MB5, followed by MB4, MB2 and MB1 lastly MB3. An increased palmitic acid concentration combined with a decrease in SCFA that led to lower spreadability [36]. Additionally, Richmond [37] reported that myristic, lauric and palmitic acids are regarded as artherogenic fatty acids among all the available sources of dietary lipids. On the other hand, the increment of USFA was 9.93, 21.34, 14.98, 21.34 and 23.55% for MB1, MB2,MB3, MB4 and MB5 respectively compared with BMF. The highest content of monosaturated fatty acid (MUSFA) was observed in MB5 followed by MB2, MB4 and MB3 lastly MB1. Diets rich in MUSFA consumed either from plant or animal sources have shown positive effect on insulin sensitivity and

cardiovascular health [38]. Moreover, the polyunsaturated fatty acids (PUSFA) also significantly increased in all treatment MBs which ranged from 3.98 to 4.80 mg/100g, compared with BMF (as control 2.81 mg/100g) which is a good nutritional value [39,40]. The desaturation index C14:1/C14:0 ratio, C16:1/C16:0 and spread ability index C18:1/C16:0 ratio increased by increasing the level of low melting fractions (L15 and S15) particularly in MB5, MB2 and MB4 treatments. The C18:1 yield increased linearly, as well as increasing LMF led to an increase in the quantity of total PUFA, particularly C18:2 and C18:3. These results agree with data revealed by Hurtaud et al. [41]. The highest ω6: ω3 ratio was recorded in all MBs when compared to the control treatments (BMF or CB). MBs ranged from 1.59:1 to 1.98:1, while BMF and CB were1.13:1 and 0.66:1 respectively. That plays an important role in the prevention of cardiac, hepatic, hypotensive, allergic, and diabetic diseases [42]. A ratio of ω6: ω3 4:1 was associated with a prevention of cardiovascular diseases with 70% decrease in total mortality [43].

The reduction percentage of artherogenic index (AI) was found to be 14.96 in MB1, 27.73 in MB2, 21.53 in MB3, 24.81 in MB4 and 30.65 in MB5 when compared with BMF which is due to higher content of USFA in butter that contains the low melting fractions with different ratio. These results are in the same line with Kim et al. [25] who reported that the structured butter blend showed the lowest AI as compared with pure butter fat and butter-canola oil blend. The AI indicated the relationship between the anti-altherogenic properties and fatty acids that is reflecting the inhibition of plaque aggregation and the level of cholesterol, esterified fatty acids and phospholipids [44]. On the other hand, the reduction percentage of TI in modified butter was 20.58, 31.91, 26.79, 29.11 and 34.59 for MB1, MB2, MB3, MB4 and MB5 respectively, with compared to the control. From these results could be conducted that the nutritional value of modified butter was improved by using low melting fractions obtained by multi-step fractionation.

3.2. Chemical characteristics of modified butter spread

Chemical characteristics including slip melting point (SMP), iodine value (IV), cholesterol, and oxidative stability index (OSI) of new modified butter spread can be seen in Table 2 and Figure 1. The results showed a significant ($P \leq 0.05$) effect of blending different ratios of buffaloes' butter oil fractions on the chemical properties of MBs. The highest SMP was 36.10°C in BMF and the lowest SMP was found in MB5, MB2 and MB4 being 21.73°C 22.40°C and 22.97°C respectively (Fig. 3), and these were attributed to the higher concentration of C 18:1 that has a melting point of about 16°C [45]. Also, there is not significantly different of SMP were observed between MB1 and MB3 (24.64°C and24.93°C, respectively). These results agree with those reported by Fereidoon [46] and Nadeem et al. [47] which the melting point is based on the content of SFA. The fat has high melting point are generally regarded as unhealthy, as compared to those have lower melting points [48].

Because of auto-oxidation, significant changes can take place in IV. IV of BMF and CB showed the lowest value than all MBs. The lowest IV was observed in MB1 by 32.78 mg/100g, while the highest value was 39.97 mg/100gfor MB5 compared with other treatments. By combining of major amount of low melting fractions in modified butter positively respond towards increasing of IV and was due to the enhancement of USFA [46] and these results agree with Nadeem et al. [47].

Cholesterol content was significantly affected by the source of butter treatments. All treatments had lower cholesterol as compared with both BMF and CB samples. The lowest cholesterol content was in MB1 (155.32 mg/100g) and the highest was in MB5 (167.81 mg/100g). The decrease in cholesterol content with fatty acids (SFA) with increasing the poly unsaturated fatty acids (PUSFA) in all experimental butter which decreases in the intake of fat rich dairy products is recommended [49].

The most important factor influencing the shelf life of oils and fats is their oxidative stability index (OSI). As IV, PUFA, and USFA levels increased, the OSI of the resulting fractions showed that the liquid fraction had limited oxidative stability [9]. By incorporation of the various ratios of milk fat fractions in modified butter spread which significantly improved of OSI. The MB5 had the highest OSI value (34.81/ h.). These results are due to the higher source of antioxidants activity of low melting point fractions than the buffalo's butter oil [50]. The increments of OSI of MBs were 51.12, 52.05, 57.60, 60.79 and 62.51% for MB1, MB3, MB4, MB2 and MB5 respectively compared to CB.

Table 1

Fatty acids composition of butter control (BMF & CB) and modified butter spread (MBs) made with different ratio buffaloes butter oil fractions (mg /100g)

FA	BMF	CB	MB 1	MB 2	MB3	MB 4	MB 5	**LSD
C4:0	1.84bc	1.91abc	1.65c	2.16ab	2.07ab	2.15ab	2.22a	0.364
C6:0	1.25a	1.22a	0.73b	1.31a	1.29a	1.36a	1.41a	0.342
C8:0	0.83ab	0.93a	0.64c	0.77bc	0.70bc	0.75bc	0.84ab	0.160
C10:0	1.60bc	1.26d	1.49cd	1.82a	1.73ab	1.77ab	1.97ab	0.281
C12:0	2.32b	2.75a	2.41c	2.24bc	2.31b	2.29b	2.06c	0.207
C14:0	11.61ab	11.73a	11.24bc	10.70d	10.96cd	10.81cd	10.59d	0.436
C14:1	0.63ab	0.32c	0.23c	0.73ab	0.56b	0.73ab	0.80a	0.189
C15:0	1.49c	0.58d	2.94a	2.39b	2.49ab	2.36b	2.07b	0.522
C16:0	33.10a	33.69a	29.72b	28.23cd	28.64c	28.55cd	27.95d	0.689
C16:1	1.56bc	0.85d	1.26cd	2.27a	1.93ab	2.17a	2.45a	0.557
C17:0	0.84c	0.84c	1.74a	1.40b	1.65ab	1.37b	1.05c	0.312
C17:1	0.28cd	0.83a	0.17d	0.40bc	0.24d	0.31cd	0.51b	0.144
C18:0	13.16a	13.77a	11.34b	10.53c	10.87bc	10.63c	10.22c	0.660
C18:1C	21.46d	22.06d	24.13c	25.53a	24.55bc	25.11ab	25.57a	0.787
C18:1T	4.53a	4.20b	4.61a	4.54a	4.57a	4.55a	4.50ab	0.314
C18:2 C	1.22d	0.56e	2.06c	2.56ab	2.14bc	2.28bc	2.83a	0.452
C18:2 T	0.52a	0.61a	0.65a	0.56a	0.61a	0.61a	0.53a	0.132
C18:3C	1.06bc	0.87c	1.26ab	1.35ab	1.36ab	1.29ab	1.44a	0.328
C20:0	0.04b	0.81ab	0.14a	0.06ab	0.10ab	0.12ab	0.05b	0.083
NI	0.58bc	0.88abc	1.46a	0.45c	1.27ab	0.84abc	0.91abc	0.736
USFA	31.29d	30.32d	34.40c	37.97a	35.98b	37.97a	38.66a	1.021
SFA	68.12a	68.79a	64.06b	61.64d	62.84c	62.20cd	60.47e	0.868
SCSFA	5.53bc	5.33c	4.52d	6.06ab	5.80abc	6.05ab	6.46a	0.665
MCSFA	16.06b	15.39c	16.82a	16.07B	16.32ab	16.20b	15.53c	0.508
LCSFA	47.16b	48.39a	42.94c	40.24e	41.28d	40.68de	39.28e	0.907
MUSFA	28.48e	28.27e	30.41d	33.48ab	31.86c	32.88b	33.86a	0.863
PUSFA	2.81c	2.04d	3.98b	4.48ab	4.12b	4.19ab	4.80a	0.650
AI	2.74b	2.89a	2.33c	1.98ef	2.15d	2.06de	1.90f	0.115
TI	8.21b	9.05a	6.52c	5.59ef	6.01d	5.82de	5.37f	0.412
PUSFA/ SFA	0.04d	0.02e	0.06c	0.07ab	0.06bc	0.06bc	0.07a	0.010
C14:1/C14	0.05b	0.02c	0.02c	0.06ab	0.05b	0.06ab	0.07a	0.018
C16:1/C16	0.04c	0.02d	0.04cd	0.08ab	0.06b	0.07ab	0.08a	0.018
C18:1/C16	0.64e	0.65e	0.81d	0.90ab	0.85c	0.87bc	0.91a	0.035
C18:2/C18:3	1.13/1bc	0.66/1b	1.66/1a	1.89/1a	1.57/1ab	1.81/1a	1.98/1a	0.495

Significantly at a level of 5% of probability ($p < .05$), Means in rows with the same letter are not significant. ** (LSD) = Least Significant Difference, BF =butter fat made milk fat, CB= Commercial butter, USFA = Unsaturated Fatty acids, SFA = Saturated Fatty acids, SCSFA (C4-C10) = Short Chain Saturated Fatty acid, MCSFA (C12-C15) = Medium Chain Saturated Fatty Acids, LCSFA (C16-C20) = Long chain saturated fatty acids, MUSFA = Monounsaturated fatty acids, PUSFA = Poly unsaturated Fatty acids,C18:2/C18:3 = Omega 6: Omega 3. Thrombogenic index = (TI), Atherogenic index = AI, Desaturation index C14:1/C14:0 ratio, C16:1/C16:0. MB1=L15:L25:S25 (6:3:1), MB2=L15:L25: S15 (7:2:1), MB3= L15:L25: S25 (7.5:1.5:1), MB4= L15:S15: S25 (7:2:1), MB5=L15:L25: S15 (8:1:1).

Table 2

Chemical characteristics and oxidative stability of modified butter spread (MBs) made with different ratio buffaloe's butter oil fractions

	BMF	CB	MB1	MB2	MB3	MB4	MB5	LSD
IV	30.10^{d}	26.54^{e}	32.78^{c}	36.18^{b}	34.83^{bc}	35.23^{bc}	39.97^{a}	2.66
Cholesterol (mg/100g)	226.93^{b}	279.49^{a}	155.32^{f}	159.38^{d}	157.27^{ef}	158.14^{de}	167.81^{c}	1.967
OSI (hour)	21.72^{d}	21.42^{d}	32.37^{c}	34.04^{ab}	32.57^{c}	33.76^{b}	34.81^{a}	0.876

Significantly at a level of 5% of probability ($p < .05$), Means with the same letter are not significantly different. ** (LSD) = Least Significant Difference, BMF =butter milk fat made milk fat, CB= Commercial butter, IV = Iodine Value, OSI = Oxidative Stability Index, MB1=L15:L25: S25 (6:3:1), MB2=L15:L25: S15 (7:2:1), MB3= L15:L25: S25 (7.5:1.5:1), MB4= L15:S15: S25 (7:2:1), MB5=L15:L25: S15 (8:1:1).

Table 3

Differential scanning calorimetry melting peak maximal temperatures and enthalpies of modified butter spread (MBs) made with different ratios of butter oil fractions

MBS	Melting temperature °C					Enthalpy ΔH_m (J/g)
	T_{onset}	Tm_1	Tm_2	Tm_3	T_{end}	
BMF	-7.31±0.26^{g}	5.92±0.01^{g}	10.64±0.05^{f}	28.97±0.12^{c}	35.29±0.16^{b}	36.60±0.04^{b}
CB	2.88±0.13^{b}	11.21±0.14^{b}	17.04±0.01^{c}	35.12±0.01^{a}	39.66±0.23^{a}	38.65±0.02^{a}
MB 1	3.63±0.10^{a}	11.48±0.02^{a}	20.73±0.12^{a}	32.43±0.04^{b}	35.33±0.34^{b}	33.40±0.10^{c}
MB2	-2.71±0.14^{d}	6.50±0.03^{e}	17.20±0.01^{b}	…..	20.39±0.02^{e}	25.34±0.01^{f}
MB 3	-4.31±0.21^{f}	7.94±0.01^{C}	11.32±0.04^{e}	22.70±0.02^{d}	29.67±0.02^{c}	30.87±0.22^{d}
MB 4	-3.01±0.01^{e}	7.76±0.02^{d}	11.26±0.10^{e}	22.21±0.01^{e}	29.34±0.01^{d}	26.68±0.24^{e}
MB 5	-2.14±0.05^{c}	6.22±0.04^{f}	16.41±0.03^{d}	…...	19.92±0.02^{f}	22.67±0.14^{g}
LSD	0.099	0.082	0.098	0.132	0.097	0.187

Significantly at a level of 5% of probability ($p < 0.05$). Means ± SD with the same letter are not significantly different. ** (LSD) = Least Significant Difference; BMF: butter milk fat; CB: Commercial butter; MB1: L15:L25: S25 (6:3:1), MB2: L15:L25: S15 (7:2:1), MB3: L15:L25: S25 (7.5:1.5:1), MB4: L15:S15: S25 (7:2:1), MB5: L15:L25: S15 (8:1:1).

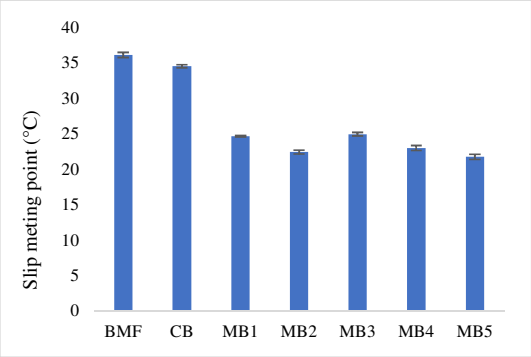


Fig. 3: Slip melting point of modified butter spread (MBs)

3.3. Solid Fat Content (SFC)

Regarding the SFC at 0, 10, 20, 30 and 40°C in BF, CB, modified butter shows that SFC of both BF and CB was found to be higher than all experimental treatments (Fig. 4). It could be observed that the lowest amount of SFC in MB5 among all modified butter being 30.67% at zero °C which is due to its lowest content of SFA and LCSFA. As increasing the temperature, the SFC of all treatments was sharply decreased, particularly at 30°C that ranged from 0.16 to 0.013% then completely melting at 40°C, this is related to the number and structure of fat crystals present and to the interactions between the crystals. The functional properties of fat are largely determined by their SFC at a specific temperature or series of temperatures. A major portion of the structure of the food may depend on the solids contributed by the fat [51].

3.4. Thermal behavior

DSC is a useful tool to determine the final melting temperature and initial crystallization of fat as well as following polymorphic evolutions. The variation in

melting behavior is indicated by the high molecular weight TAGs in the various milk [52, 53].

The DSC melting curves have been recorded for all the MBs treatments and two controls samples (BF & CB) from -20 to 50°C at 10°C/min-1 are reported in Table (3) and Figure (5). Both BMF and CB have a broad melting range due to the complexity of its triglyceride composition. The melting curve of BMF and CB recorded three typical endothermic peaks corresponding to LMF, MMF and HMF fractions. These three fractions were exhibited in temperature during heating of LMF fraction from -7.31°C to 5.95°C, 2.88°C to 11.21°C, MMF fraction from 10.00°C to 28.00°C, from 17.04°C to 35.12°C and HMF fraction from 28.10°C to 35.29°C and 35.12°Cto 39.66°C for BMF and CB samples respectively, as shown in Table (3) and Figure (5). These results have been confirmed by a previous study [9]. The DSC of MBs blend has been found to be significantly influenced by continuous incorporation of LMF level. The endothermic peak of MB2 and MB5 recorded a major two melting peaks with shifted towards lower temperature except the MB1. The DSC of MBs blend has been found to be significantly influenced by continuous incorporation of LMF level. The endothermic peak of MB2 and MB5 recorded a major two melting peaks with shifted towards lower temperature except the MB1 and MB3. The first temperature peak (TM1) displayed as to be melting (α form) at 6.50°C in MB2 at 6.22°C in MB5, while the second peak (TM2) and MB3. The first temperature peak (TM1) displayed as to be melting (α form) at 6.50°C in MB2 at 6.22°C in MB5, while the second peak (TM2) recorded as to 17.20°C in MB2 and at 16.41°C in MB5 which corresponding to melting of β form [54]. The Tend of both MB2 and MB5 butter were recorded at 20.39°C and 19.92°C respectively. Similar endothermic peaks of both MB2 and MB5 were observed due to including the same various fractions with L15:L25:S15 at a ratio of 7:2:1 in MB2 and 8:1:1 in MB5 blend. An increasing of the L15 resulted in the melting point to shift towards lower temperature. These results might be due to increasing in unsaturated fatty acids (Table 1), which have melting points of 16°C, -5°C and -12°C respectively, and in SCFA such as butyric acid (C4:0), Caproic acid (C6:0) and Caprylic acid (C8:0) which having melting points of -8°C, -4°C and 16°C respectively. In addition, it is likely caused by the presence of phospholipids in the fat globule membrane [19].

It has been observed a significant difference for MB1, MB3 and MB4 in melting profile as compared to other samples which attributed to the present of L15:L25: S25 at ratio (6:3:1) and (7.5:1.5:1) in MB3 and L15:S15:S25 (7:2:1) in MB4 blend.

As for, enthalpy ΔHC (J/g) it was significantly decreased in MB2, MB4 and MB5 respectively, whereas the BMF and CB recorded 36.60 (J/g) and 38.65 (J/g), respectively. Except for the combination of both L15 and S15 fractions in MB5 blend caused the decrease of ΔHm (J/g) which can be directly related to higher level of USFA, MUFA and PUFA (Table 1). These findings are in accordance with [55, 56].

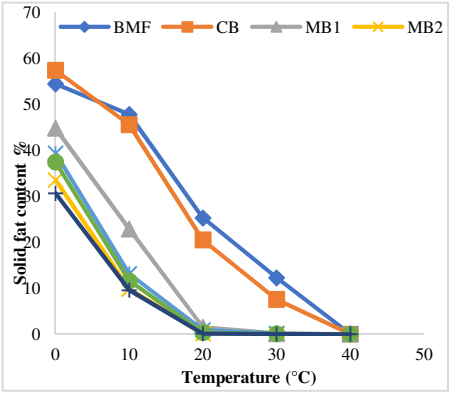


Fig. 4: Solid fat content (g/100 g) of modified butter spread (MBs) made with different ratio butter oil fractions

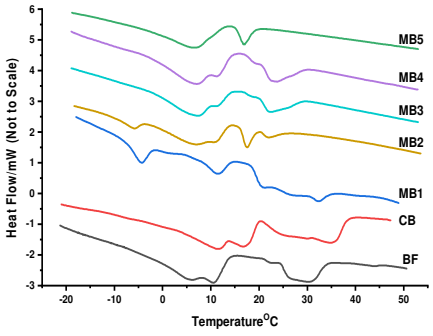


Fig. 5: DSC melting curves of BMF, CB and modified butter spreads.

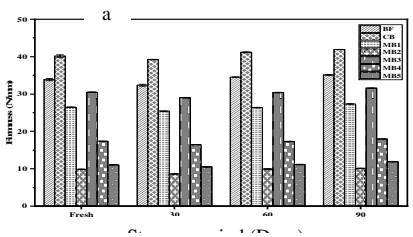
3.5. The firmness and stickiness of new modified Butter

Butter hardness and spread ability are correlated with the crystal network in the liquid phase. The balance between the crystallised and liquid phases determines how spreadable butter is; the greater the crystallised fat, the less spreadable butter [57]. Also, the main parameter that determines the spread ability of fat is firmness that has a great influence on consumer acceptance. The effects of incorporation of the various ratios of milk fat fractions on the consistency and firmness of the new modified butter spread are shown in Figure (6). The firmness of BMF and CB were highest at zero time being (33.85 and 40.18 N/mm) respectively, while the MB5 was recorded the significantly ($P \leq 0.05$) lowest value (9.86 N/mm) followed by MB2 and MB4 finally MB3 and MB1 in fresh. Significant differences were observed in all treatments for firmness, which were due to the incorporation of different ratios of butter oil fractions, especially the increasing levels of both L15 and S15. These results are in agreement with the results of Buldo and Wiking [10]. The composition of the fatty acids in modified butter spread has an impact on its hardness value. Additionally, the inclusion of olein rich in unsaturated fatty acids resulted in a reduction in butter's hardness rating [58]. The highest proportion of L15 markedly increased the USFA, MUSFA and PUSFA in MBS compared to control which inhibits the formation of crystals. Sensory stickiness (sticky mouthfeel) was hypothesized as a result of viscoelastic and adhesive properties of a foodstuff [59]. However, processing conditions also have an influence on the rheological characteristics of the product for example agitation of butter cause a decrease in the stickiness without changing the SFC [60].

Butter's hardness and polymorphism stability are affected by the kind of fat crystal. Crystals may have different shapes (α, β′ and β), and the polymorphism is an irreversible, time-dependent and temperature-dependent process, in which the crystal changes from the less stable (α) to the most stable form (β). Fats with β′ crystals are softer, which is a desirable characteristic in butter and margarines [61]. Figure (6, b) showed that the stickiness of BMF and CB were the lowest at zero time (-9.33 and -11.89 N/mm, respectively), while the MB5 was significantly ($P \leq 0.05$) recorded the lowest value of -9.89 N/mm followed by MB2 and MB4 finally MB3 and MB1 in fresh. This is due to the composition of the solids and fatty acids of the modified butter which had higher USFA took longer to crystallize and lower solid fat content [58]. From Figure (6) the firmness and stickiness of all treatments and control were gradually decreased during the cold storage period till the end storage period (90 days).

3.6. Sensorial characteristics of modified butter

The effects of the application of various ratios of the butter oil fractions on the sensory attributes of flavor, color, texture, spread ability and overall acceptability of modified butter are presented in Figure (7). The texture and sensorial characteristic of modified butter were affected by the composition of fatty acids [14]. Fresh MBs had higher sensory scores than both BMF and CB. No significant differences were observed for the sensory evaluation of MB5, MB2 and MB4 treatments which gained the highest scores in flavor, color, texture, spreadability and overall acceptability. As expected, the MB5 butter has gained the highest scores of sensory attributes. It was followed by MB2 and MB4 finally MB3 and MB1 in fresh. These results indicated that butter having high amount of low melting fraction to improve the sensory attributes as compared with control samples. The using of various milk fat fractions to make butter can positively affect the nutritional and flavor of the product as aromatic compounds and fat-soluble vitamins are concentrated by multi step fractionation [50, 62, 63, 64]. On the other hand, the sensory score was lower in MB1 and MB3 due to the highest proportion of L25 and S25 as compared with other treatments. During cold storage, the sensory score in both control and all the treatments gradually decreased until 60 days then sharply decreased at the end storage period (90 days).



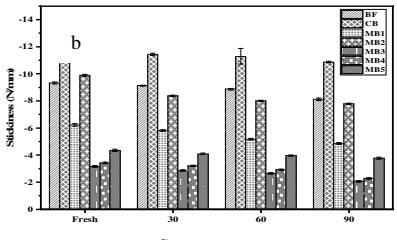


Fig. 6: Firmness (N/mm) (a) and Stickiness (N/mm) (b) of new modified butter spread (MBs) made with different ratio butter oil fractions.

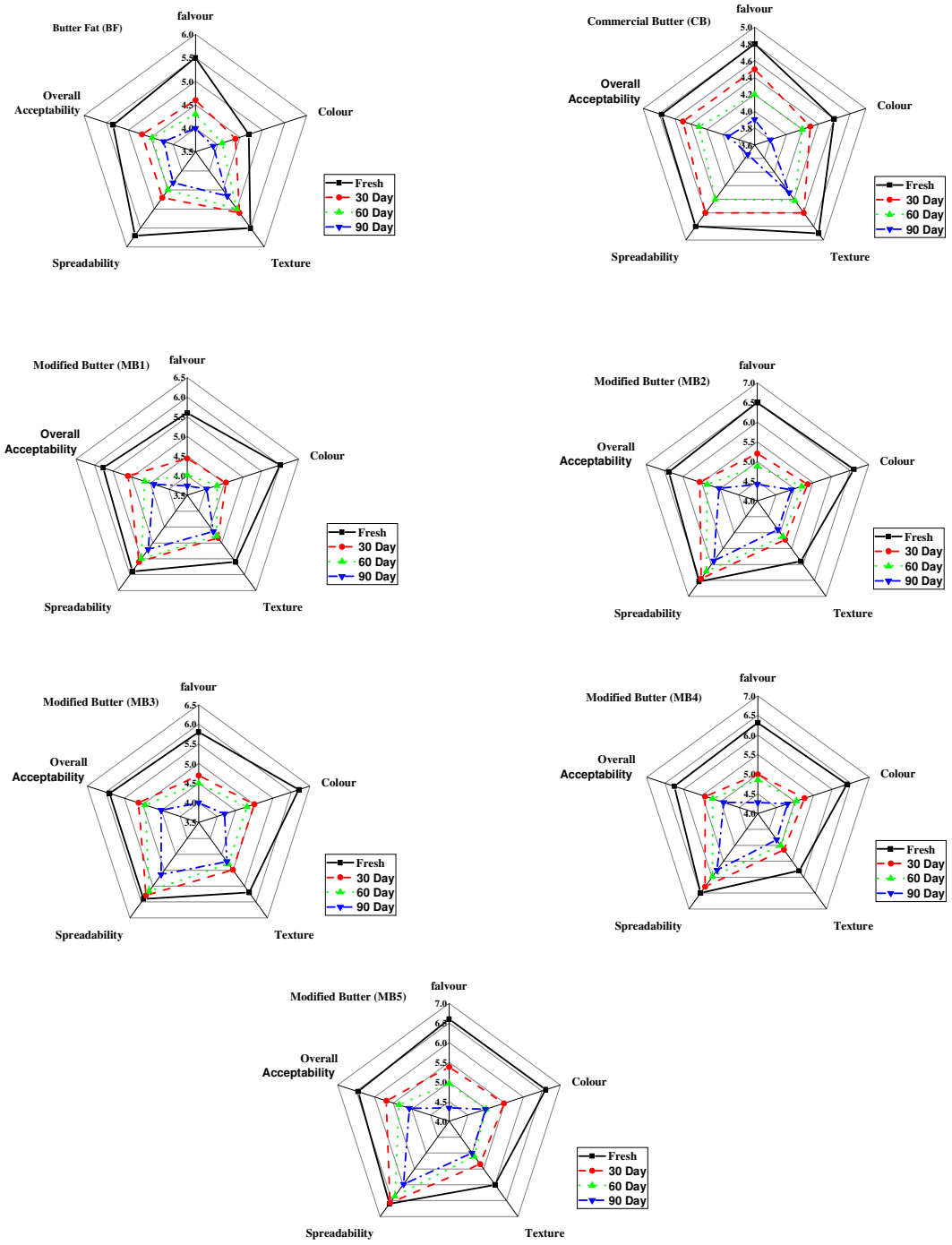


Fig. 7: Sensory evaluation of BMF, CB and modified butter spreads during cold storage at 5°C

4. Conclusion

In this study, using dry fractionation was the most cost-effective and technologically advanced approach to produce modified butter. The production of buffalo butter oil (BO) and solid fractions was higher than the yield of liquid fractions. Also, based on the results obtained in the present study it can be concluded that modified butter containing L15 and S15 can be improved the fatty acids composition, solid fat content, thermal analysis, texture, and sensory attributes. The crystallization and melting behavior of modified butters were better than BMF and CB. This suggests that the dry fractionation temperature resulted in an increase of the functional and nutritional properties of obtained new healthy spreadable butter.

5. Conflicts of interest

The authors declare no conflicts of interest.

6. Formatting of funding sources

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