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Improving the Functional Properties of Buffalo Butter Oil Fractions Obtained by Multi-Step Dry Fractionation

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ABSTRACT

Buffalo Milk fat has excellent properties; its variable physicochemical properties and its lack of functionality restrict its uses in food industry. The Multi-step dry fractionation was applied on buffalo butter oil (BO) for enhancement of the nutritional value and functionality at three different temperatures i.e. 35, 25 and 15 °C, three solids (S35, S25 and S15) and three liquid (L35, L25and L15) fractions was gained. LMF (L15and S15) was significantly higher not only in short chain fatty acids (SCFA) but also in long chain unsaturated fatty acids (LCUSFA), whereas, lower in medium saturated chain fatty acids (MCSFA) than those of BO, HMF(S35) and MMF (L25, S25 and L35) were detected. LMF had positive healthy effect of lower oxidative stability index (OSI) and of the highest nutritional (by ω6: ω3 3PUFA) than other fractions. differential scanning calorimeter (DSC) values for both solid and liquid fractions are significantly ($P<0.05$) decreased with decreasing fractionation temperature even in melting or crystallization behaviors. Solid fat content (SFC) was significantly the lowest in LMF at 25^{O}C. The liquid fractions had larger crystals size than those solid fractions and BO. Strong negative correlation between SFC at 20^{O}C and (USFA, SCFA and IV) and strong positive correlation between SFC and (SFA, MCSFA, LCSFA and DSC) were observed. Results will increase the knowledge to improve the processing condition to produce buffalo butter oil fractions with increased functionality and more varieties for more dairy fat products. The chosen dry fractionation temperature resulted in an increase of the functional and nutritional properties of obtained buffalo butter oil fractions.

Keywords: Buffalo butter oil, dry fractionation, Thermal analysis, fatty acids, and crystals morphology

INTRODUCTION

Buffalo milk (BM) is less studied than the other mammal's milk. Buffalo milk is the second most produced milk in the world with 82billion liters produced each year (12.5% of milk produced in the world), after cow's milk (~84% with 551 billion liters)(IDF, 2010). Milk fat composed of saturated fatty acids (~70%) with diversity lengths, and low content of unsaturated fatty acids(~ 30%).On the other hand, milk fat has been characterized as nutritional constraints by dieticians thus it composed of considerable concentration of C14:0 and C16:0 that classified as atherogenic if increasing level from daily intake lead to increased risk of heart disease. Buffalo milk has low cholesterol 8 mg/100 g as compared to cow and goat milk which is 14 and 10 mg/100 g, respectively (Khan,*et al*.,2019).

Furthermore, there are several components in buffalo milk fat that could influence health positively, since high intakes of particular omega-3 fatty acids which includes conjugated linoleic acid (CLA) can assist in the ailment and disorder prohibition and help in maintaining and control of metabolism in humans, manage plasma TG and cardiovascular functions, decreasing and lowering cancer promoting, as well as obstructing tumor growth and metastasis from cancer breasts (Verdurico, *et al*., 2012),so that many European countries dietary guideline emphasis on 0.2g daily intake of omega-3 fatty acids of individuals to prevent cardiovascular disease and brain development and function and depression. On the other hand, milk fat relatively has low concentrations of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids to increase milk fat softness with a low solids content should kept at temperatures up to 37°C, which result in limited involving in dairy industry (Reddy, 2010).

Dry crystallization is more suitable for industrial application due to no risk of solvent residues (Lopez *et al*., 2006). Fractionation of milk fat can significantly alter the fatty acid composition of the fractions as compared to native milk fat (Fatouh, *et al*., 2007).

Dry fractionation/crystallization the most preferred commercially method for dairy fat industry is which is based on the different melting point of the TG molecules in the fat mixture and only indirectly on the melting point of the individual fatty acids, that resulted in mainly three fractions, Low melting fraction (LMF), Medium melting fraction (MMF) and high melting fraction (HMF). However, the fatty acids content of TAGs for each fraction are different, within the HMF the major component is long-chain saturated fatty acids while the low melting fraction is mainly composed of short-chain and unsaturated fatty acids and medium melting fraction contains the long saturated and medium long chain fatty acids (Lopez & Ollivon, 2009).

Fractionation of milk fat and incorporation of selected fractions facilitate the desired changes, control and the improvement of milk fat properties, consequently, the

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novel of dairy products-based milk fat. Utilization of LMF as a food component and even with the great amount of saturated fatty acids, it has been suggested that the high melting fraction might be predominately suitable for encapsulating the most active ingredients in some functional milk fat based products (Relkin, *et al*., 2008; Abd El-Rahman, *et al*., 1998) try to resolve the problem of lack of functional properties of buffalo milk fat, lower solid fat index and higher content of saturated fatty acids may by fractionating the buffalo milk fat into hard and soft fractions. Fatty acid and triglycerides profiles of LMF of milk fat have been investigated and these findings have suggested that therapeutic value of low melting fractions of buffalo milk fat were greater than native milk fat. The thermal behavior of milk fat is usually studied by DSC, which is function of solid/liquid fat ratios at different temperatures, (Gandhi, *et al*., 2013) reported that the good cold spread ability is exhibited in fats that are approximately 30 to 40% solid at 5^{O}C and 7 to 15% solid at 25^{O}C.

The investigation of the thermal and structural properties of buffalo milk fat(BMF) fractions and the formulation of novel dairy products based BMF cannot be achieved without increasing the knowledge on crystallization, melting behaviors and microstructure during storage at 5^{O}C.The crystallization properties of buffalo milk fat and its primary fractions obtained by dry fractionation at different temperature 15-40^{O}C were investigated on slow cooling rate and detailed by Fatouh, *et al*.,(2003), other studies were reporting the characterization of the stearin obtained by thermal fractionation of anhydrous milk fat (Bonomi, *et al*., 2012). Microstructure/morphology (Size - shape - distribution) of fat crystals is often studied by polarized light microscopy. Confocal laser scanning microscopy (CLSM) has been used in a few studies for monitoring microstructure of blends of milk fat (Rousseau, *et al*., 1996). Additionally, the physical properties of milk fat are function of DSC, SFC, and Microstructure/Morphology. The aim of this study is to increase the nutritional value and alter physiochemical properties of buffalo butter oil fractions by using Dry Fractionation.

MATERIALS AND METHODS

Fresh buffalo butter (~82 % fat) obtained from the dairy unit, of Faculty of Agriculture, Cairo University, and stored at -18^{O}C till processing. Nile blue and acrdine orange were obtained from Sigma-Aldrich (USA). Solvents (Hexane, Acetone and methanol) used were analytical grade supplied from Merck (Darmstadt, Germany).

2. Dry fractionation of Buffalo Butter oil

BO was prepared from fresh butter by melting the butter at 80^{O}C for 60min without agitation, then removing the top oil layer by filtering the oil by (Whatman No.1 paper) and drying over anhydrous sodium sulfate under vacuum in a Buchner funnel to remove the ingredients of aqueous phase material. The collected oil was then stored at -18^{O}C until fractionation.

BO fractionation was carried out by stepwise and successive cooling procedure described by Van Aken, et al, (1999). The multi-step dry fractionation procedure is outlined in the following Fig 1.

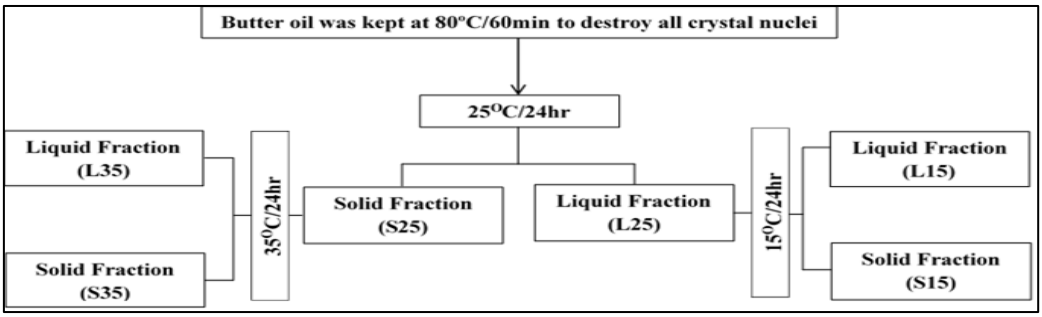

Fig. 1. The multi-step fractionation procedure.

3. Yield and Slip Melting point (SMP)

The yield was calculated from the follow equation = (A/B)*100 as A is the resulted fat fraction, B is the total of origin fat. The SMP was determined according to AOCS, (1998).

4. Determination of Fatty acid composition & Atherogenicity index (AI)

Fatty acid methyl esters (FAME) were prepared by trans-esterification of the oils using sodium-methoxide complex as catalyst according to method Ce 1–62 of the AOCS, (1998). Using a Pasteur pipette, 100 mg (±0.5 mg) of oil was transferred into a screw-cap vial to which was added 5 ml of hexane and vortexed briefly. Sodium methoxide (250 µL) was then added and the mixture vortexed for 1 min followed by addition of 5 mL saturated sodium chloride solution. The mixture was capped, shaken vigorously for15 s and allowed to stand for 10 min. using a Pasteur pipette; the top hexane layer (passed over Na2SO4 granules for 20 min) was injected into a GC system.

The GC system was an Agilent (Agilent 6890N, Network GC-System, and Wilmington, USA) fitted with auto sampler (Agilent 7683-Series), an injector (Agilent 7683-B Series) and flame ionization detector. Separation was carried out on a capillary column DB-Wax (length 30 m, internal diameter of 0.30 mm) with helium as carrier gas at a flow rate of 1 mL/min. Sample was injected at 1 µL in a split ratio of 1:20 and flow rate of 2 the ml/min (at room temperature). The injector and detector temperatures were 250 °C while the initial oven temperature was 100 °C for 2 min, then increased to 230 °C at 5 °C/min. The oven temperature was subsequently held at 230 °C for 10 min. Identification of the fatty acid methyl esters (FAMEs) was achieved by comparing their retention times with those of known standards (37-Component FAME MIX, SUPELCO) and quantified as percent-weight of total fatty of the fatty acid methyl esters (FAMEs) was achieved by comparing their retention times with those of known standards (37-Component FAME MIX, SUPELCO) and quantified as percent-weight of total fatty acids.

Atherogenicity index (AI) resulted from modified calculation of hyper-cholesterolemic fatty acids were calculated by the flowing equation (Ulbricht and Southgate (1991), Kim, et al, (2008).

AI = [C12:0 (W/w,%) + 4 × C14:0 (W/w, %) + C16:0 (w/w, %)] /USFA (w/w, %).

5. Iodine value

Iodine Value was obtained from fatty acid profile by using reacting ratios (calculation factors) between I (iodine) and either the fatty acids bound to a triglyceride or the free fatty acids according to the methods described by Ham et al., (1998) as follow equation:

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

6. Oxidation Stability

Rancimat equipment model 679 (metrohm Ltd. CH-9100 Herisau, Switzerland) was used for determination of an oxidative stability of butter oil and its fractions as described by Mendez, et al., (1996).

7. Determination of Cholesterol

Cholesterol was determinate according to Shin, and Chang, (2001) by HPLC system.

8. Differential scanning calorimetry (DSC)

The melting profile was done by using (Modulated DSC model 60; Shimadzu, Japan). The cooling and heating rate were selected based on reference method by AOCS, (1998).

9. Solid Fat Content (SFC)

SFC of butter oil and fractions were measured with a pulsed nuclear magnetic resonance (pNMR) spectrometer (NMS/120 minispec, BRUKER, USA) operating at 20 MHz SFC was determined according to the method of the AOCS, (1998).

10. Confocal laser scanning microscopy (CLSM)

Microstructure analyses by confocal laser scanning microscopy were followed at described by Wiking, *et al*, (2009).

11. Texture Profile analysis (TPA)

The FTC systems comprising TMS-Pro equipment, with TL-Pro™ software, provide precise force, speed and position for rigorous food texture analysis (Food Technology Corporation, USA) was employed for determination of hardness and adhesiveness of Fat samples at temperatures of 5^{O}C according to Bobe, *et al*., (2003).

12. Statistical analysis

General linear Model (GLM), one-way analysis of variance was used to compare means among groups. Post hoc multiple comparisons were conducted using Tukey's test, with the level of statistical significance taken as $P < 0.05$, using SAS (version 9.4 TS Level 1M3, SAS Institute Inc., Cary, NC, USA). Least squares mean was calculated and presented throughout.

RESULTS AND DISCUSSION

Chemical and physical characteristics of dry fractionation of solid fractions (S35, S25 and S15) and Liquid fractions (L35, L25 and L15) of buffalo butter oil are mentioned in (Table 1). The obtained fractions were categorized into three main groups: LMF included only L15, S15; MMF included L25, L35, and S25, and finally, HMF included S35.

Yield

Data presented in (Table 1) show that the yield of the butter oil (BO) and its fractions by using dry fractionation at different temperature from 35-15^{O}C.The yield of solid and liquid fractions varied with decreasing the crystallization temperature is due to the variation of their fatty acids and TAG composition. However, at 25^{O}C the resulted solids yield was higher than Liquids, which explained by the fatty acid profile (Table 2) as SFA & USFA were formulated (64.54%, 34.60%) respectively. The first obtained yield of S25 fraction forms 54.67 (g/100g) level of the total amount of BO. The S35 and L35 fractions yielded about 22.48and 32.19 from the original S25 fraction yielded, respectively. The higher content of L15 was 66.42% of the original L25 among of liquid fraction. On the other hand, the ratio liquid and solid fraction was 45.33:54.67, 58.88:41.12 and66.42: 33.58 at 25, 35 and 15^{O}C respectively. The results agreed with those finding of Fatouh *et al*., (2003).

SMP was recorded 36.54^{O}C for BO, while its values descended in order as follows: 39.76, 36.30. 32.36 ,26.83 .22.46 and 13.37˚C for S35, S25, L35, L25, S15, and L15, respectively. This might be due to differences in fatty acids composition esterified to milk fat TAG which plays a vital role in melting point of the fat fraction (Fatouh *et al*., 2003 and Wang and Hu ,2017). Based on these melting properties, it could, be divided into LMF with SMP ranged (10-25^{O}C), MMF with SMP ranged (25 - 35^{O}C), while HMF with SMP (35-45^{O}C). Data in Table (1) revealed that S35 were grouped as high melting fraction (HMF), with SMP while S25, L35 and L25 were grouped as medium melting fraction (MMF) with SMP, and finallyS15and L15 were grouped as low melting fraction (LMF). Low-melting fraction (<15^{O}C) characterized with strong butter flavor and could be incorporated into milk powder to improve functionality (Illingfort, 2002). Moreover, various factors are affected during fractionation including the cooling rate, the agitation rate and final temperature which greatly influence the chemical composition and physical attributes of fraction obtained by Ahmed *et al*., (2015).

Iodine Value (IV):

Iodine value was associated with the degree of unsaturation present in oils and fats. Higher the unsaturation more would be iodine which used as mainly of measured the oxidation stability. Iodine value (IV) of BO and its various fractions were shown in (Table 1). It revealed that IV value of the LMF (L15 and S15) was (43,87 and 38.65) higher than other fractions and BO due to decrease melting point (MP) and significantly increase in USFA due to the migration of PUFA &USFA to liquid fractions especially L15., while a situation vice versa in HMF (S35) was.26.73. As far (IV) for MMF (L25, S25 and L35) were 36.19, 28.26 and 33.19 respectively, while (IV) for control sample was 30.94. The obtained results were in agreement with those of Amer, *et al*., (1985) and Fatouh, *et al*., (2005). Slip melting point was inversely related with iodine value (IV).

Cholesterol:

The distribution of cholesterol among various obtained fraction and BO was stated in Table (1). The concentration of cholesterol in liquid fraction was significantly ($P<0.05$) higher than that of solid fractions. About 95.39% of total cholesterol was recorded in L15, whereas, in S15 was 76.45%. The obtained results might

be attributed to the higher affinity of cholesterol for SCFA and USFA which predominated in LMF, as compared with HMF. The proportion content of cholesterol in BO was 280.95 (mg/100g) while cholesterol in LMF was 214.79 - 268.01. Whereas it was 144.15 (mg/100g) in HMF and, its value was 194.25 - 204.93 - 170.76 (mg/100g) in MMF. Cholesterol belongs to unsaponifiable fraction was higher amount of unsaponifiable matter in the low melting point fractions could be justified by the affiliation of cholesterol to the low melting point fractions (Fatouh, *et al*., 2005). Cheddar cheese prepared from low melting point fractions of milk fat had higher content of cholesterol than parent milk fat (Nadeem, *et al*., 2015).

Oxidative Stability Index (OSI):

The OSI values of BO and its various fractions are shown in Table (1). The OSI in, BO was (9.30) the highest among all fractions, and this might be due to its low content of LCUSFA, MUSFA and PUSFA. Significant difference between BO and all studied fractions could also observed. The OSI of obtained fractions revealed that liquid fraction was of low oxidation stability as increasing of IV, PUFA and USFA. These results were previously confirmed with Abd EL-Aziz, (2008).

Fatty Acid Composition and Atherogenicity Index

Table (2) show that the FA composition of solid and liquid fractions. It could be, noticed that the palmitic and stearic content (C16:0 and C18:0) were the most abundant in HMF (S35), being 35.47 and 14.94 mg/100g, respectively, which are of higher melting point at 63 and 71.2^{O}C, whereas, the oleic (C18:1 cis) with melting point 12.8^{O}C ,was of the highest level in LMF (S15and L15) with range between 33.17 – 35.53 mg/100g (Lakshminarayana and Rama-Murthy, 1985).

Table 1. Physical and Chemical properties of Buffalo Butter Oil and its fractions

BO& obtained fractions	Yield%	SMP	IV	OSI	Cholesterol (mg/100g)
BO	100^{A}	36.54^{b}	30.94^{e}	9.30^{a}	280.95^{a}
S35	22.48^{F}	39.76^{a}	26.73^{g}	7.87^{b}	144.15^{g}
S25	54.67^{B}	32.36^{c}	28.26^{f}	7.25^{b}	194.25^{e}
S15	15.22^{G}	22.46^{e}	38.65^{b}	5.59^{d}	214.79^{c}
L35	32.19^{D}	33.13^{c}	33.19^{d}	7.77^{b}	170.76^{f}
L25	45.33^{C}	26.83^{d}	36.19^{c}	6.93^{c}	204.93^{d}
L15	30.11^{E}	13.37^{f}	43.87^{a}	4.65^{e}	268.01^{b}
SE	0.150	0.658	0.119	0.297	0.032
LSD	0.456	1.996	0.362	0.902	0.098

Significantly at a level of 5% of probability (p < 0.05). Means in column with the same letter are not significantly different. *LSD = 1.996 Standard Error (SE) = 0.658. Due to the procedure used for preparing the fractions, the amounts sum up according to S25+L25=100%; S25=S35+L35; L25=S15+L15. L, liquid fraction; S, solid fraction; BO, buffalo butter oil. Numbers following the type of fraction (S or L) correspond to the temperature at which the fraction separated (15–35^{O}C).

Slip melting point values (SMP), IV: Iodine value, OSI :Oxidative Stability Index.

Table 2. Fatty Acid profile of Buffalo Butter Oil, and its fraction and atherogenicity index

Nomination	Control	Solid Fraction			Liquid Fraction			*SEM	**LSD
		S35	S25	S15	L35	L25	L15		
C4:0	1.80^{c}	1.37^{ef}	1.28^{f}	2.13^{b}	1.63^{d}	1.46^{e}	2.37^{a}	0.042	0.127
C6:0	1.26^{c}	0.90^{e}	0.68^{f}	1.40^{b}	1.14^{d}	0.86^{e}	1.65^{a}	0.030	0.091
C8:0	0.64^{b}	0.46^{c}	0.49^{c}	0.87^{a}	0.56^{bc}	0.78^{a}	0.83^{a}	0.032	0.099
C10:0	1.42^{b}	1.03^{d}	1.08^{d}	1.61^{a}	1.30^{c}	1.48^{b}	1.67^{a}	0.034	0.104
C12:0	2.36^{a}	1.68^{d}	1.68^{d}	2.04^{b}	1.86^{c}	1.85^{c}	2.06^{b}	0.044	0.134
C14:0	11.05^{a}	10.37^{c}	10.68^{b}	10.46^{bc}	10.62^{bc}	10.10^{d}	9.14^{e}	0.082	0.251
C14:1ω5	0.64^{a}	0.45^{b}	0.46^{b}	0.66^{a}	0.61^{a}	0.73^{a}	0.69^{a}	0.041	0.127
C15:0	1.70^{a}	1.02^{bc}	0.97^{c}	1.14^{b}	1.07^{bc}	1.05^{bc}	1.10^{bc}	0.050	0.153
C16:0	30.42^{c}	35.47^{a}	34.70^{b}	25.06^{e}	30.34^{c}	28.87^{d}	23.30^{f}	0.136	0.415
C16:1ω7	2.08^{e}	0.65^{f}	0.70^{f}	3.49^{b}	2.64^{c}	2.32^{d}	3.63^{a}	0.045	0.183
C17:0	0.64^{d}	1.95^{ab}	2.06^{a}	1.57^{c}	1.86^{b}	1.87^{b}	0.49^{e}	0.048	0.145
C18:0	13.20^{c}	14.94^{a}	14.18^{b}	8.63^{f}	11.96^{d}	10.84^{e}	7.17^{g}	0.042	0.130
C18:1Cis	24.94^{f}	23.81^{g}	25.31^{e}	33.12^{b}	27.66^{d}	30.53^{c}	35.35^{a}	0.059	0.179
C18:1Trans	3.10^{d}	2.89^{e}	2.40^{f}	4.14^{b}	3.36^{c}	2.96^{de}	4.58^{a}	0.045	0.138
C18:2 Cis	1.51^{b}	0.89^{cd}	1.00^{c}	0.83^{d}	1.00^{e}	0.65^{e}	1.99^{a}	0.039	0.120
C18:2 Trans	0.56^{c}	0.22^{d}	0.63^{bc}	0.84^{a}	0.20^{d}	0.65^{b}	0.87^{a}	0.027	0.082
C18:3Cis	1.78^{c}	0.93^{e}	0.89^{e}	0.55^{f}	1.29^{d}	1.96^{b}	2.21^{a}	0.058	0.177
C20:0	0.04^{c}	0.08^{c}	0.08^{c}	0.53^{a}	0.08^{c}	0.09^{bc}	0.13^{b}	0.015	0.047
Not Identified	0.75^{c}	0.83^{abc}	0.88^{a}	0.88^{a}	0.86^{ab}	0.92^{a}	0.76^{bc}	0.033	0.100
USFA	34.60^{e}	29.84^{g}	31.28^{f}	43.63^{b}	36.74^{d}	39.80^{c}	49.32^{a}	0.113	0.345
SFA	64.54^{c}	69.28^{a}	67.88^{b}	55.44^{f}	62.40^{d}	59.25^{e}	49.91^{g}	0.144	0.437
SCFA	5.13^{c}	3.77^{e}	3.54^{e}	6.01^{b}	4.62^{d}	4.58^{d}	6.52^{a}	0.051	0.155
MCFA	15.75^{a}	13.52^{c}	13.79^{bc}	14.3^{b}	14.15^{b}	13.73^{c}	12.98^{d}	0.071	0.216
LCSFA	44.31^{c}	52.44^{a}	51.01^{b}	35.79^{e}	44.24^{c}	41.67^{d}	31.10^{f}	0.127	0.387
MUFA	30.75^{e}	27.80^{g}	28.86^{f}	41.41^{b}	34.26^{d}	36.54^{c}	44.25^{a}	0.096	0.293
PUFA	3.84^{b}	2.04^{f}	2.41^{de}	2.22^{ef}	2.48^{d}	3.25^{c}	5.06^{a}	0.086	0.262
PUFA:SFA	0.05^{b}	0.02^{d}	0.03^{c}	0.04^{c}	0.03^{c}	0.05^{b}	0.10^{a}	0.001	0.005
ω6:ω3	0.85^{BC}	0.97^{B}	1.01^{B}	1.51^{A}	0.78^{C}	0.33^{D}	0.91^{BC}	0.056	0.170
(AI)	2.33^{C}	2.73^{A}	2.63^{B}	1.69^{F}	2.12^{D}	1.88^{E}	1.37^{G}	0.011	0.034

Significantly at a level of 5% of probability (p < .05), Means in rows with the same letter are not significantly different.

BO = Buffalo Butter Oil, S = Solid fraction, L = Liquid fraction separated at temperature from 35-15^{O}C.SCFA (C4-C10) = Short chain Fatty acids, MCFA (C12-C15) = Medium Chain Fatty Acids, LCSFA (C16-C20) = Long chain saturated fatty acids, MUFA= Mono unsaturated fatty acids, PUFA = Poly unsaturated fatty acids, ω6: ω3 = (Omega 6: Omega 3 Ratio), Atherogenic Index = AI

*** (SEM) = Standard Error ** (LSD) = Least Significant Difference.**

By decreasing fractionation temperature, it decreased significantly ($P \leq 0.05$) the total saturated fatty acids (SFA) and also decreasing medium and long chain saturated fatty acids (MCFA and LCSFA) as Lauric and myristic acids which are responsible for the atherogencity, while the proportion level of (USFA),however, significantly increased ($P<0.05$) with the decrease of the temperature of fractionation process, that plays an important role in the physical and nutritional functionality of modified milk fat. On the other hand, the positive impact of mono and poly unsaturated fatty acids (MUFA and PUFA) significantly ($P \leq 0.05$) increased at $25^{O}C$ and $15^{O}C$, which resulted in the increasing omega ratio (n-6/n-3PUFA) and the decrease of atherogenicity index (AI). Thus, mono and poly unsaturated fatty acids, especially, (Oleic, Linoleic and linolenic fatty acids) lowering the bad health effect of myristic, palmitic and stearic fatty acids which they increased the AI values. Concerning, LMF (S15 and L15) and (SCFA) and (LUCFA) contents which enriched and reduced in (LCSFA) among all the fractions obtained. These results are in agreement with those reported by Kim, *et al*., (2008) and Abd El-Rahman *et al*., (1998), Amer *et al*., (1985), and Fatouh *et al*., (2003). The highest omega6: omega3 ratio in S15 was observed among the rest fractions due to the migration of the palmitic and stearic acids into the solid phase during crystallization. It could be concluded that using dry fractionation could able to modify milk fat, which of adversely effect on their physical chemical properties and therapeutic role. Eventually, the chosen dry fractionation temperature resulted in the increase of the functional and nutritional properties of obtained fractions at ($25^{O}C$ and $15^{O}C$), which increase the involving of milk fat in dairy products and other industries.

Differential Scanning Calorimeter (DSC):

DSC gives information about thermodynamic of the primary crystallization of fat. For crystallization process, there are positive correlation between initial (T_{onset}), off-set (T_{end}) temperatures and the change in heating capacity (enthalpy ΔHC (j/g)) for both solid and liquid fractions respectively. The crystallization thermograms recorded by using DSC on cooling BO and its various fractions at $10^{O}C$/min from 50 to $-40^{O}C$ are shown in (Fig. 2 and Table 3). The DSC curves recorded upon cooling BO consists of two observed peaks. The onset temperature (T_{onset}) of the minor first peak was at $20.42^{O}C$, which is related to the appearance of α-Crystals. Whereas, the separate crystallization of high melting TAG, the second crystallization peak was displayed between 18.96 (TC1) to $-27.00^{O}C$ (Tend) due to the crystallization of lower melting TAG with formation of β\ crystals; (Dimick *et al*., 1996, Fatouh *et al*., 2003 and Lopez & Ollivon, 2009). Regarding, S35 two main exothermic peaks were recorded which shifted significantly towards high temperature as compared to BO and other fractions. The first sharp peak at higher exothermic temperature (T_{onset}) in cooling of S35 was displayed 33.79, which could attributed to the highest content of LCSFA ,especially, palmitic and stearic (Table 2) The second major abroad crystallize peak at maximum temperature (TC2) was recorded at 4.31 and for S35, respectively, due to crystallize action in α or β\ forms (Fatouh *et al*., 2005; Lopez *et al*., 2006). The DSC exothermic peak during cooling S15 and L15 showed one abroad exothermic signal between (T_{onset}) 16.76 to (T_{end}) $-30.43^{O}C$, and 6.95 to $-32.54^{O}C$ respectively. These results could attribute to the highest proportion of SCFA in both fractions which led to shift the lower crystallization temperature (Lopez *et al*., 2006). In respect to the initial temperature of crystallization recorded for BO T_{onset} ($20.42^{O}C$) whereas, was intermediate between solid fraction (S35, S25 and S15). As seen in (Fig. 2 and Table 3) the DSC melting curves of BO clearly showed three distinctive major endothermic peaks. Due to three major groups of TAG melting, the first endothermic peak displayed between (T_{onset}) $-30.51^{O}C$ to (Tm1) $11.54^{O}C$ due to the melting of α-Crystals. The second peak appeared from (Tm1) $11.54^{O}C$ to (Tm2) $20.00^{O}C$ with pronounced peak at $16.72^{O}C$. The third peak displayed abroad shoulder peak from $20.00^{O}C$ to (Tm3) $34.81^{O}C$ corresponded to melting of α-crystals After that temperature fat appeared liquid due to that the TAG in the liquid are ordered in Lamella (Lopez *et al*., 2006). In sold fraction (S35 and S25) the thermograms illustrated three peaks like the BO but the proportion of these peaks were different. The third endothermic peak was significantly ($P<0.05$) recorded to shift towards the highest melting temperature with abroad shoulder to the right side that its values was $45.09^{O}C$ and $38.03^{O}C$ for S35 and S25, respectively. These results were due to the higher melting temperature of these fractions, which contained (LCSFA) C16:0 (palmitic acid) and C18:0 (Stearic) than BO. The S15 and L15 fraction show only one endothermic peak between -30.51 to $18.30^{O}C$ and -17.60 to $10.55^{O}C$, respectively, and then they completely melted and presented the lowest melting point compared to the BO and the rest of fractions (Walstra, 1984), this is might be a results of the enrichment of low melted fractions with USFA rich in C18:1 (Oleic acid), C18:2 (Linoleic acid) and C18:3 (Linolenic acid) with melting points 16, -5 and $-12^{O}C$, respectively, and also in SCFA such as C4:0 (Butyric acid), C6:0 (Caproic acid) and C8:0 (Caprylic acid). It could be observed in (Table 3) That the wide range of Tm1 for solids and liquids and the decrease of the ΔH might be due to the action of dry fractionation that altered the composition of TAG in each fraction which affected the crystallization temperature and polymorphism, whereas the low melting fatty acids need low heat energy ,which might be behind the release of latent heat. In case of melting it is clearly observed that ΔHm for BO (72.87 J/g) was higher than HMF (S35:69.66), MMF (S25: 68.85, L35: 44.24 and L25:38.89) and LMF (S15: 43.94 and L15:36.78). The ΔHc for BO was 68.70 J/g in crystallization behavior and it thus had intermediate values between all fractions which ranged from 36.78 J/g and J/g for L15 and S35 respectively, which come in agreement with Truong *et al*., (2014) and Lopez & Olivine, (2009). Shapes of the melting and crystallization thermo grams differ because the crystallization of fats is mainly dependent on kinetic effects such as super saturation, super cooling and diffusion, whereas the dynamic melting of fats is largely independent of such solution effects.

Table 3. Thermal Parameters of crystallization, melting and their enthalpy of Buffalo Butter Oil and its fractions

Crystallization					
Solid Fractions	**Temperature**				**Enthalpy ΔHC (J/g)**
	T_{onset}	**T1**	**T2**	**T_{end}**	
BO	20.42^{C}	18.96^{C}	-6.49^{C}	-27.00^{C}	68.70^{D}
S35	33.79^{A}	30.71^{A}	0.82^{A}	-13.81^{A}	83.69^{C}
S25	25.04^{B}	22.31^{B}	4.31^{B}	-27.51^{D}	86.37^{B}
S15	16.71^{D}	5.04^{D}	…	-30.43^{E}	97.17^{A}
Liquid Fractions					
L35	13.81^{E}	-12.19^{G}	…	-24.10^{B}	58.23^{E}
L25	12.04^{F}	-4.17^{E}	…	-31.90^{F}	37.90^{F}
L15	6.95^{G}	-9.01^{F}	…	-32.54^{G}	36.01^{G}
SE	0.055	0.044	0.029	0.037	0.036
LSD	0.166	0.136	0.102	0.115	0.111

Melting						
Solid Fractions	Temperature					Enthalpy ΔHm (J/g)
	T_{onset}	T1	T2	T3	T_{end}	
BO	-30.51^{F}	11.54^{D}	16.72^{D}	34.80^{C}	38.53^{C}	72.87^{A}
S35	-17.50^{B}	9.73^{G}	15.66^{A}	45.09^{A}	48.80^{A}	69.66^{B}
S25	-27.11^{E}	11.13^{E}	16.33^{E}	38.03^{B}	43.31^{B}	68.85^{C}
S15	-30.51^{F}	15.39^{A}	18.30^{B}	…	25.29^{E}	43.97^{E}
Liquid Fractions						
L35	-23.32^{D}	14.77B	30.15^{A}	…	34.81^{D}	44.24^{D}
L25	-15.80^{A}	14.58C	17.05^{C}	…	25.10^{F}	38.89^{F}
L15	-17.60^{C}	10.55^{F}	…	…	16.71^{G}	36.78^{G}
SE	0.025	0.030	0.033	0.037	0.033	0.031
LSD	0.076	0.093	0.104	0.129	0.102	0.095

Significantly at a level of 5% of probability (p < .05), Means in rows with the same letter are not significantly different.
BO = Butter Oil, S = Solid fraction, L = Liquid fraction separated at temperature from 35-15^{O}C.
*** (SE) = Standard Error** **** (LSD) = Least Significant Difference.**

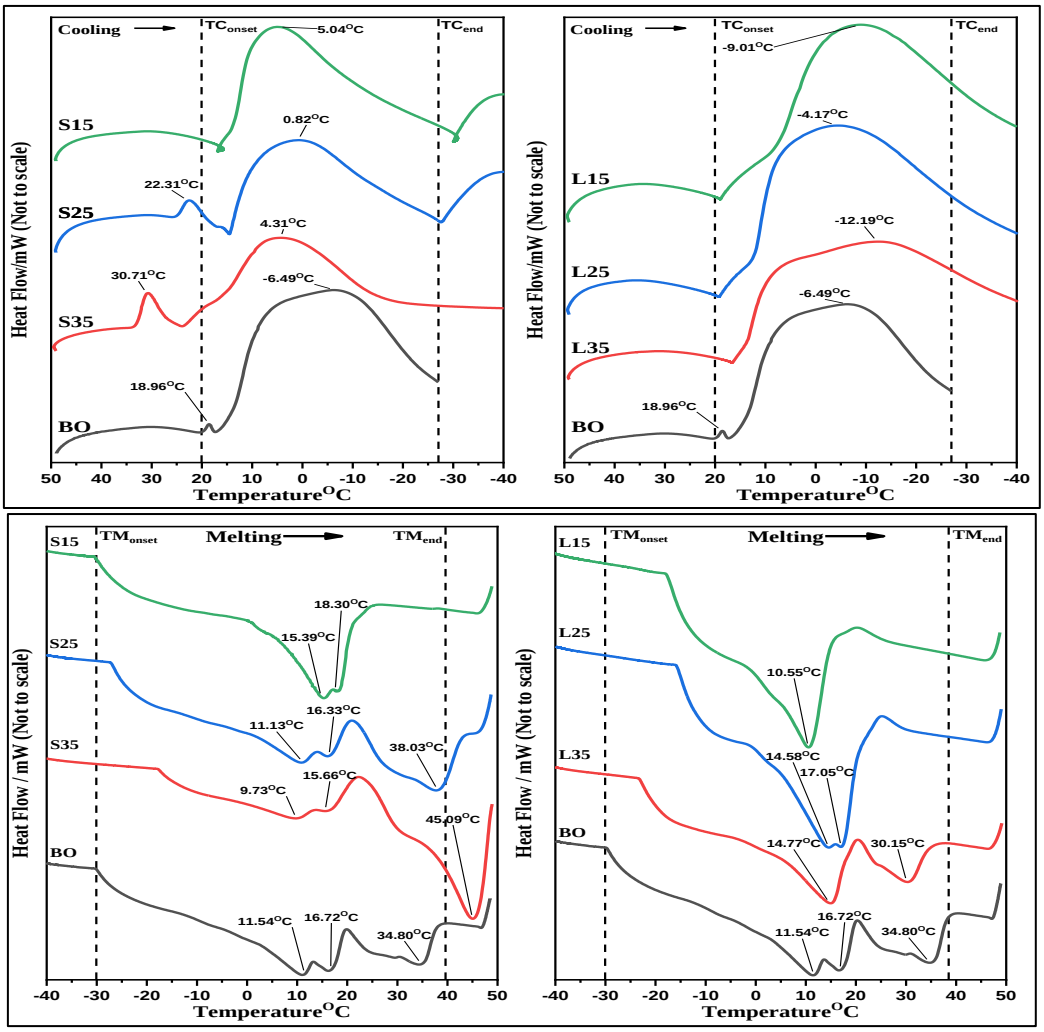

Fig. 2. DSC Curves of native buffalo milk fat and its fractions

Solid fat content SFC:

The SFC is one of the major factors affecting the physical and rheological properties of the product and predicting the behavior of the final product during storage and transport (Liang *et al*., 2008). The differences in SFC for solid and liquid fractions are attributed to the process of dry fractionation, as modification has been altered the TAG/fatty acid profile for each fraction as solid fractions are characterized by high SFA which contains high proportions of (Myrstic – Palmitic – Stearic). The SFC decreased with the decrease of the temperature fractionation for producer of six fractions by using dry fractionation. Results for solid fat contents related to the heating thermograms are presented in (Table 4). At 10^{O}C, S35, S25 & L35 are of the greatest level of SFC and L15 is of the least. The highest SFC showed in HMF followed by MMF and BO and lastly LMF, respectively. As expected, at 0^{O}C both of L15 and L25 fractions were of the lowest SFC among all the obtained fractions being (34.35 and 51.60 g/100g), respectively due to the higher proportion of SCFA and lower LCSFA content table (2). The highest SFC in S35 and S25 at0^{O}C and all measured temperature until 40^{O}C, then completely melting was observed as compared to BO and the rest fractions which might due to the higher level of LCSFA and number of carbon in long chain TAG as well as a higher melting point that indicating their potential to have a waxy mouth-feel (Dimick *et al*., 1996), As for, SFC values in L35 and L25 decreased with the decrease of the temperature. From these results indicated that the differences between the SFC values of all fractions were due to the differences in fatty acid composition between samples which it affected in their melting points and enthalpies. These results are confirmed with Amer, *et al*. (1985); Abd El-Rahman, *et al*., (1997) and Fatouh, *et al*., (2003).

Table 4. Solid fat content (g/100 g) of Buffalo Butter Oil and its fractions

Temperature ^{O}C	BO	Solid Fractions			Liquid Fractions			SE±	LSD
		S35	S25	S15	L35	L25	L15		
0	61.16^{c}	69.21^{a}	65.11^{b}	58.16^{d}	62.45^{c}	51.60^{e}	34.35^{f}	0.533	1.618
10	46.88^{d}	63.74^{a}	59.32^{b}	44.56^{e}	53.96^{c}	28.88^{f}	3.60^{g}	0.300	0.910
15	26.54^{d}	55.67^{a}	38.87^{b}	36.62^{c}	38.27^{b}	19.74^{e}	0.17^{f}	0.308	0.935
20	21.79^{c}	46.36^{a}	32.35^{b}	5.40^{e}	22.07^{c}	7.88^{d}	0^{f}	0.290	0.882
25	14.89^{c}	37.97^{a}	24.96^{b}	1.31^{f}	13.10^{d}	2.92^{e}	0^{g}	0.134	0.407
30	7.89^{b}	23.58^{a}	19.45^{a}	0.93^{b}	4.73^{b}	0^{b}	0^{b}	1.824	5.532
35	2.90^{c}	19.89^{a}	11.12^{b}	0.22^{de}	0.61^{d}	0^{e}	0^{e}	0.108	0.329
40	0.83^{c}	5.03^{a}	3.81^{b}	--	--	--	--	0.043	0.131
45	--	--	--	--	--	--	--	--	--

Significantly at a level of 5% of probability (p < .05), Means in rows with the same letter are not significantly different.
BO = Buffalo Butter Oil, S = Solid fraction, L = Liquid fraction separated at temperature from 35-15^{O}C.
***Standard Error (SE)** ****Least Significant Difference (LSD)**

CLSM micrographs:

It was clearly observed that the morphology of fat crystals very highly affects the physical and microstructure of high-fat products and also the product quality such as spreadability, hardness and softness. The microstructure and fat crystals distributions are presented in (Fig. 3).

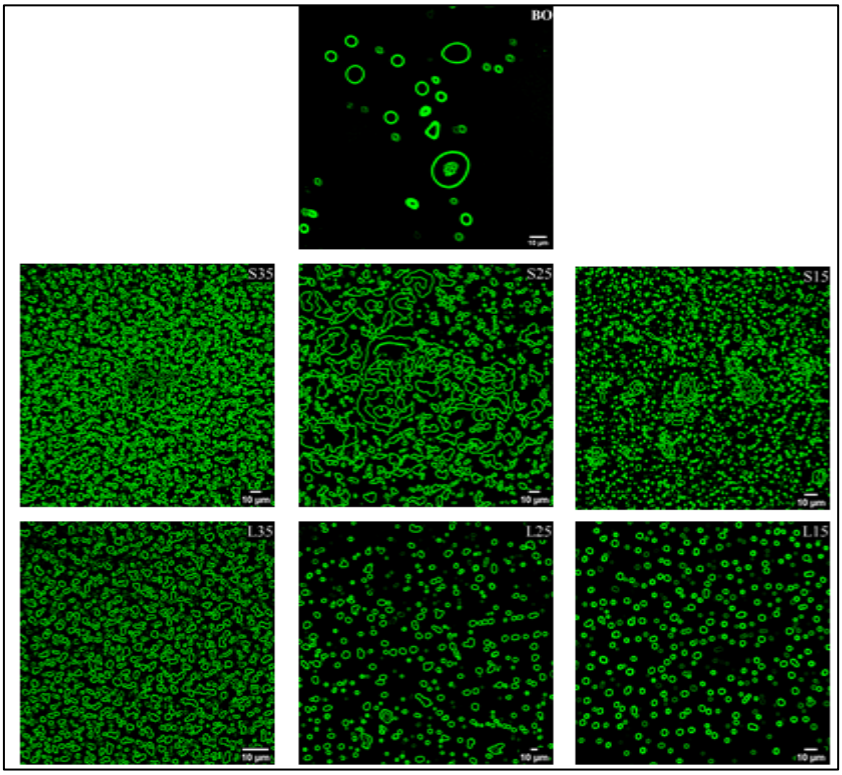

Fig. 3. CLSM micrographs of Buffalo Butter Oil and its fractions

Buffalo butter oil image (Fig. 3) reveals that the natural butter oil is of a well-defined crystal with clear solidified TAGs. A wider crystal's size distributions could also be observed in buffalo butter oil sample, than all buffaloes butter oil fractions. Buffalo butter oil characterized well-defined crystals borders; with crystal size distribution (from 1 to 100µm2) in area and small crystal size tend together under the force of Vander Waals due to different FAs composition, SFA 64.54% and USFA 34.60%. Liquid fractions (L15, L25 and L35) had larger average crystal's size (38.02, 22.27 and 8.67µm2) respectively, than solid fractions (S15, S25 and S35) with average crystal size (74.2829.02, 17.98 and 4.24µm2) respectively in (Fig. 4). Also, the fat clusters became denser and closer in solid fractions than liquid fractions. The more brightness, great size, high distribution and well defined border refer to the low SFC, high level of USFA, LCUSFA and SCFA (Table 2 and 3) which possessing double bound that resulted in the concave shape of fatty acid, subsequently, affecting on the reflecting illumination, excitation of dyes and the irregular border compared to SFA. On the other hand, Shimomura, *et al.*,(2018) reported that slow crystallization result in the irregularly less dense spherulites and large size crystals due to occurring of nucleation and growth in same time which causing broad size distribution. Generally, with decreasing fractionation temperature and SFC%, the fat clusters became denser and wider in size and largely dispersed. The crystals of solid fraction are more irregular, crowded, and aggregated crystals than liquid fractions which characterized with more regular, less dense and aggregation. As for the fractionation at 15^{O}C, it is well appeared that the fractionation of buffalo butter oil under 15^{O}C led to the desperation of crystals, more defined crystal shape than fractions collected under 25^{O}C or 35^{O}C which were irregular, crowded and aggregated may be due to the high SFC%, while morphology of S15 represents some aggregates and denser than L15. These results are in agreement with Shimomura, *et al.*, (2018).

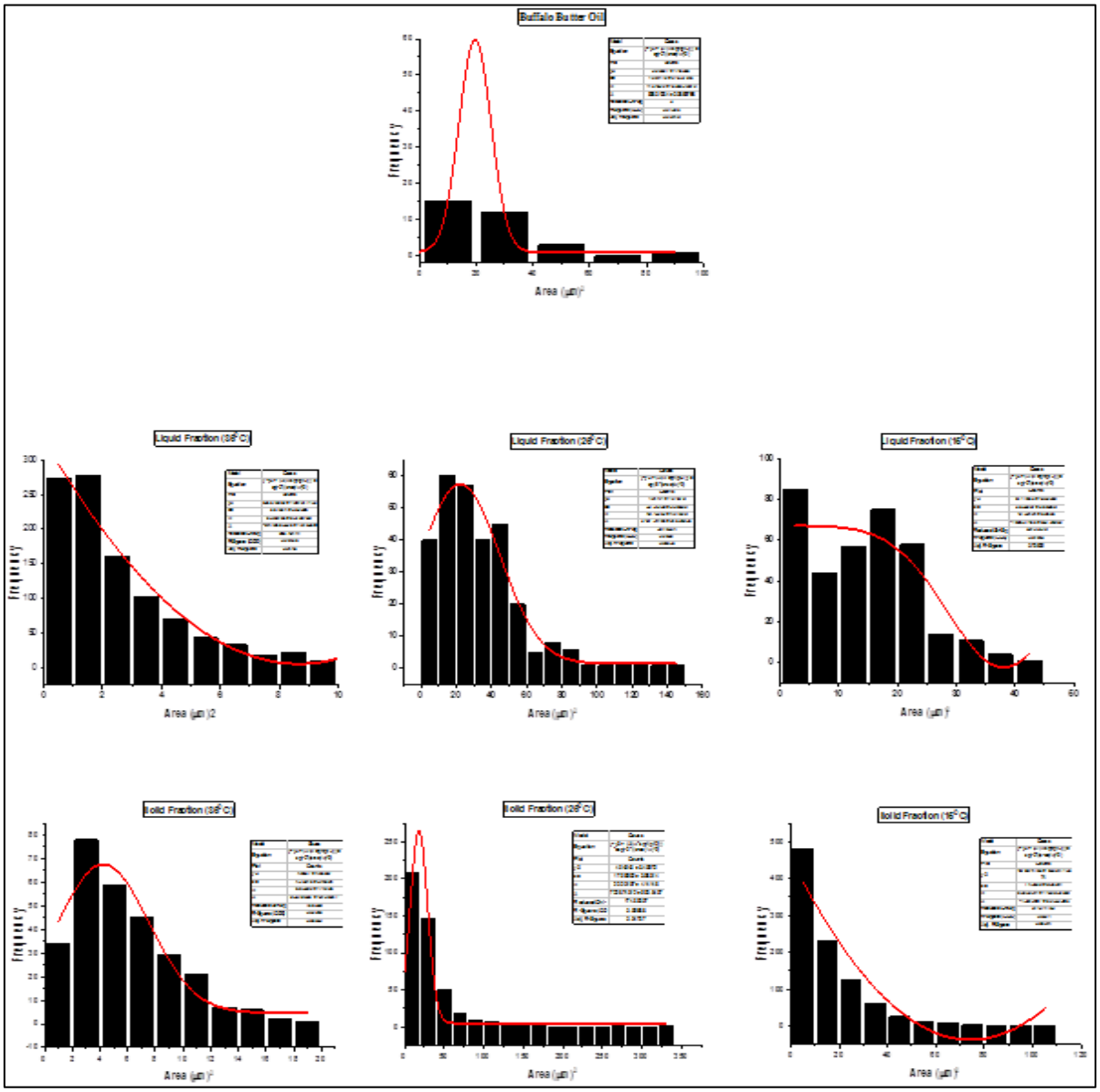

Fig. 4. Fat crystal size distribution of Buffalo Butter Oil and its fractions

Texture Profile Analysis (TPA):

Table S6 shows that the both firmness and adhesiveness were of the similar trend in which they ordered in descending as follow: HMF > MMF > LMF. As previously mentioned, fat droplet size, cooling rate, different crystallization temperature, SFC, SFA and USFA all are considered factors impacting the firmness of fat fractions. The HMF and MMF had the greatest firmness at all fractionation temperatures due to the increased in SFC and SFA compared to the BO and LMF. which agreed with Shimomura, *et al*., (2018).

Correlation among SFC and (USFA, SCFA, MCFA, LCSFA, DSC and IV)

This is matching with correlation between SFC, DSC, USFA and IV (Table S5). There is strong negative correlation between SFC at 20OC and (USFA, SCFA and IV) and strong positive correlation between SFC and (SFA, MCFA, LCSFA and DSC). Our data is in respect agreed with Shimomura, *et al*., (2018).

Table 5. Correlations among Solid Fat Content and functional parameters.[1]

Functional Property	USFA	SAF	SCFA	MCFA	LSCFA	IV	DSC_{onset}
SFC	-0.94	0.94	-0.85	0.09	0.94	-0.94	0.92
	0.002	0.001	0.016	0.850	0.001	0.001	0.003

[1]Top number is the Pearson correlation coefficient and the bottom number is the probability that the correlation differs from zero.

Table 6. Texture profile analysis (TPA) of buffalo butter oil and its fractions

Sample	Firmness(N)	Adhesiveness(N)
Butter Oil	7.21^{c}±0.01	17.68^{b}±0.3
S35	10.79^{a}±0.15	18.10^{a}±0.01
S25	7.87^{b}±0.11	16.76^{c}±0.15
S15	3.37^{e}±0.09	4.62^{f}±0.22
L35	7.22^{c}±0.43	16.12^{d}±0.19
L25	5.40^{d}±0.24	15.56^{e}±0.37
L15	0.88^{f}±0.12	1.37^{g}±0.09

Significantly at a level of 5% of probability (p < .05), Means in rows with the same letter are not significantly different. BO = Butter Oil, S = Solid fraction, L = Liquid fraction separated at temperature from 35-15^{O}C.

CONCLUSION

The use of dry fractionation is the most economic and technological method. The yield of buffalo butter oil (BO) and the solid were higher than liquid fractions. Oxidative stability of fractions obtained decreased with decreasing SMP. The SMP value was inversely related with iodine value (IV). The concentration of cholesterol in liquid fraction was significantly (P<0.05) higher than that of solid fractions. The highest omega6: omega3 ratio in S15 was observed among the rest fractions. There is strong negative correlation between SFC at 20^{O}C and (UFA, SCFA and IV) and strong positive correlation between SFC and (SFA, MCFA, LCSFA and DSC) eventually, the chosen dry fractionation temperature resulted in the improve of the functional and nutritional properties of obtained fractions.

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تحسين الخواص الوظيفية لزيت الزبد الجاموسي بطريقة البللورة متعددة المراحل

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تهدف هذة الدراسة إلي إستخدام البللورة متعددة المراحل لزيت الزبد الجاموسي بغرض تحسين الخواص الوظيفيه لتلائم إستخدامه لكثير من مصانع الأغذيه والالبان . ففي هذة الدراسة تم تطبيق طريقه البللوره علي درجات حرارة مختلفه (٣٥ و٢٥ و١٥°م) وتم الحصول علي ثلاث شقوق دهنيه صلبة (S35,S25,S15) وثلاث شقوق دهنيه سائله (L35,L25,L15) وفيما يلي أهم النتائج المتحصل عليها:شقوق الدهن منخفضة الانصهار (L15,S15) تميزت بارتفاع قيم الايودين والكولسترول و الاحماض الدهنية قصيرة السلسله SCFA و الاحماض الدهنيه طويله السلسله الغير مشبعه LCUSFA ولكن إنخفض كلا من معدل ثبات الأكسدة OSI و الاحماض الدهنيه متوسطة السلسله MCSFA بالمقارنه بالشقوق الأخري و BO، كما لوحظ إرتفاع معنوي ما بين النسبه الاوميجا ٦ والاوميجا ٣.لوحظ إنخفاض معنوي لقيم التحليل الحراري للماسح الضوئي DSC لكلا من الشقوق الصلبه والسائله بإنخفاض درجات حرارة البللورة أثنا عملتي التجميد والانصهار .أظهرت النتائج إرتفاع معنوي في قيم جوامد الدهن الصلبه SFC للشقوق الصلبه عن السائله.علي ضوء هذة الدراسه بالميكرسكوب الالكتروني المجهري أظهرت النتائج أن الشقوق السائله بللورتها كبيرة الحجم بالمقارنة بالشقوق الصلبه وBO.من هذة الدراسة ينصح بإستخدام طريقة البللورة متعددة المراحل لزيت الزبد الجاموسي BO لما لها من خواص وظيفية محسنة للشقوق مما يؤدي الي إستخدامها في عديد من المنتجات الغذائيه واللبنية.