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Physical, chemical and stability properties of buffalo butter oil fractions obtained by multi-step dry fractionation

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Abstract

Chemical, physical and stability properties of buffalo butter oil fractions, obtained by stepwise crystallization at several temperatures between 15 and 40 °C, were investigated. Saponification, iodine, Reichert-Meissl, Polenske and Krishner values of low-melting fractions (LMF) were significantly higher ($P < 0.05$) than high-melting fractions (HMF) or the original butter oil. Cholesterol, fat-soluble vitamins (A, D and E) and lactones tended to concentrate more in LMF than middle-melting fractions (MMF) and HMF. Refractive index and specific gravity of MMF lie between those of LMF and HMF. LMF showed the lowest stability toward thermal oxidation (5.6 h) when compared to MMF (7.7–11.2 h) and HMF (11.1–16.0 h). Rate of hydrolysis of LMF was the highest (7.8 ml of 4 g/l NaOH) among all the obtained fractions, while HMF was the lowest (4.9–1.6 ml of 4 g/l NaOH). Differences in chemical and physical properties of the fractions were attributed to differences in fatty acid composition.

Keywords: Crystallization; Buffalo butter oil; Physico-chemical properties; Stability

1. Introduction

Milk fat is one of the most complex fats found in nature (Amer, Kupranycz, & Baker, 1985). This complexity stems from the extreme diversity of its fatty acids (FA) (e.g., chain length, degree of unsaturation, and branching) and more than 400 of these have been identified recently (Jensen & Newburg, 1995). Milk fat also contains thousands of triacylglycerol (TAG) species. Gresti, Bugaut, Maniongui, and Bezard (1993) quantified more than 200 individual molecular species of even-numbered TAG alone. Due to this complex composition, milk fat has a wide melting range from −40 to 40 °C (Breitschuh & Windhab, 1998).

Dry fractionation has become the commercially used crystallization technology to separate the solid and liquid fractions within butter oil (BO) on the basis of melting point, while maintaining the natural flavour of the original product (Versteeg, 1991). Moreover, the selectivity of this technology allows overcoming the functionality problems as well as seasonal variation frequently encountered in milk fat (Abd El-Rahman, Shalabi, & Kilara, 1998). Dry fractionation creates fractions with a wide variety of different chemical and physical properties. These fractions have found extensive use in food and non-food applications (Kaylegian, 1995).

The significant differences observed earlier between the chemical compositions (FA and TAG) of the various buffalo BO fractions obtained by multi-step dry fractionation (Fatouh et al., 2003) are expected to have considerable impact on the physical and chemical properties of these fractions. The objective of this study was to investigate chemical, physical and stability properties of buffalo BO fractions in an effort to characterize these fractions.

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2. Materials and methods

2.1. Dry fractionation

BO fractions were obtained by the multi-step fractionation procedure previously described by Fatouh et al. (2003). BO was melted at 80 °C for 10 min, then slowly cooled to the fractionation temperature (30 °C). While cooling, BO was continuously stirred at constant 10 rpm. After 9 h, the solid fraction (S30) was separated from the liquid (L30) by vacuum filtration. A similar fractionation process was applied for fractionation of L30 at 20 °C, resulting in solid (S20) and liquid (L20) fractions. Similarly, L20 was further fractionated at 15 °C, producing liquid (L15) and solid (S15) fractions. Following the same procedure, S30 was fractionated at 35 °C, yielding solid fraction (S35) and liquid fraction (L35). Finally, crystallization of S35 at 40 °C generated a solid (S40) and a liquid (L40) fraction.

2.2. Cholesterol

Cholesterol content was determined by the method of Jekel, Vaessen, and Schothorst (1998).

2.3. Reagents

Reagents were saturated KOH in water for saponification, 30 g/l pyrogallol in ethanol as antioxidant during saponification, butylated hydroxytoluence (BHT) (5 mg/ml) as antioxidant in both the internal and cholesterol standards, 5-α cholestane in toluene (100 μg/100 ml) as internal standard, and cholesterol standard in toluene (1 mg/ml).

2.4. Saponification and extraction

About 200 mg butter oil were weighed in a screw-cap tube (25 × 200 mm). One milliliter of internal standard was added, and the tube was flushed with nitrogen for 2 min to prevent oxidation. About 8 ml of pyrogallol solution was added, along with 0.5 ml of KOH solution. The tube contents were mixed using a vortex mixer and then the tube was placed in a water bath at 80 °C for 30 min. After cooling to room temperature, the sample was extracted by adding 20 ml hexane and 12 ml deionized water, then mixing for 5 min. The water and hexane layers were allowed to separate for 15 min. A 10 ml aliquot from the top layer (hexane) was taken, placed in a 20 ml vial and evaporated to dryness by nitrogen. One ml of hexane was added to the vial and the contents were mixed for 3 min. The resultant hexane solution was used for gas chromatograpic (GC) analysis.

2.5. GC analysis

One microliter of the hexane solution was injected into a SAC-5 fused silica capillary column (30 m × 0.20 mm ID × 0.20 μm film thickness) (Supelco, Bellefonte, PA). The column was installed in a HP5980 Series II GC (Hewlett-Packard, San Fernando, CA) apparatus equipped with a flame ionization detector (FID) and on-column injector. Nitrogen was the carrier gas at a flow rate of 20 ml/min with hydrogen and air being supplied to the FID at a flow rate of 33 and 400 ml/min, respectively. The oven temperature was programmed from 155 to 275 °C at 20 °C/min and held for 30 min isothermally at 275 °C. The injector and detector temperature were 270 and 300 °C, respectively.

2.6. Cholesterol standard curve

For the quantification analysis, 1 ml or the internal standard (5-α cholestane in toluene (100 μg/100 ml)) was added to tubes containing 0.63; 1.25; 2.50; 5.0; 10.0; 20.0 mg of cholesterol. Hexane was added to each tube to give a total volume of 100 ml. The contents were mixed using a vortex mixer, and then evaporated to dryness by nitrogen flushing. One milliliter of hexane was added, and then 1 μl of the solution was injected into the GC.

2.7. Fat-soluble vitamins (A, D and E)

2.7.1. Sample preparation and saponification

Approximately, 15 ml of sample were placed in a 125 ml actinic volumetric flask and 1.5 ml of ethanolic pyrogallol solution (100 g/l) were added. The flask was placed in an ice bath and 15 ml of ethanolic KOH solution (prepared by dissolving 70 g KOH in 155 ml ethanol and 25 ml distilled water) were added. The headspace was flushed with N_2 gas for 2 min. Sample saponification was performed for 18 h in a shaking water bath at 40 °C, except for S35 and S40; saponification was performed at 60 °C due to their high slip melting points.

2.7.2. Liquid extraction of saponified samples

The contents of the saponification flask were transferred to a separatory funnel and the saponification flask was washed with 15 ml distilled water, 5 ml ethanol and 45 ml hexane, in order, and transferred to the separatory funnel which was vigorously shaken for one min and layers were allowed to separate. The aqueous layer was further extracted, twice with 45 ml hexane. About 50 ml KOH (5 g/100 ml) were added to the hexane extracts (total of three extractions) and the contents were vigorously shaken for 1 min and the layers were allowed to separate. The hexane extract was washed with 100 ml of ethanol:water 55:45 (v/v) and the aqueous phase was discarded.

2.7.3. Evaporation/solid phase extraction

Hexane extract was passed through sodium sulfate and rotary-evaporated (35 °C) to almost complete dryness; then the final volume of the extract was made up to 10 ml with hexane.

2.8. Vitamins A and D analysis

Vitamins A and D were determined by HPLC (FDA, 1995). A Shimadzu LC-10AS liquid chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD), equipped with a Shimadzu SPD-10 AV UV–VIS spectrophotometric detector (Shimadzu Scientific Instruments, Inc., Columbia, MD), was used to determine the contents of vitamins A and D in butter oil and its fractions. For vitamin A, a Whatman Partisil ODS-3 column (250 mm × 4.6 mm × 5 μm) (Whatman Inc., Clifton, NJ) was used while, for vitamin D, a Vydac C18 reversed phase column (250 mm × 4.6 mm × 5 μm) (The Separations Group, Hesperia, CA) was used. Both columns were equipped with a Whatman guard column (Whatman Inc., Clifton, NJ). For vitamin A, the isocratic mobile phase was methanol:water (90:10 v/v) with a flow rate of 1.5 ml/min, while, for vitamin D, the isocratic mobile phase was acetonitrile:methanol (90:10 v/v) with a flow rate of 1.5 ml/min. A silica extract-clean column (Alltech Inc., Deerfield, IL) was used for purifying the sample extract before determining vitamin D. Injection volumes were 20 and 50 μl for vitamins A and D, respectively. Vitamin A and D contents were calculated by using the peak area, compared between standard and the sample at 325 and 254 nm, respectively. Peak integration was performed using a Shimadzu CR-501 Chromatopac (Shimadzu Scientific Instruments, Inc., Columbia, MD).

For vitamin A chromatographic analysis, 0.45 ml of sample extract was evaporated to dryness with N_2 gas and 0.45 ml of mobile phase was added before injecting into the HPLC. For vitamin D chromatographic analysis, the remainder of the hexane extract (9.55 ml) was evaporated to 1 ml with N_2 gas and passed through the silica cartridge. The collected eluent was evaporated to dryness and 0.45 ml of mobile phase was added before injecting into the HPLC.

2.9. Vitamin E analysis

Vitamin E content was determined by the method of Ye, Landen, Lee, and Eitenmiller (1998). The normal phase high pressure liquid chromatography (HPLC) used consisted of a Shimadzu LC-6A pump (Shimadzu Scientific Instruments, Inc., Columbia, MD) equipped with a Shimadzu RF-10A spectroflurometric detector (Shimadzu Scientific Instruments, Inc., Columbia, MD), and a 250 mm × 4 mm × 5 μm Lichrosorb Si60 column (Hibar Fertigsaule RT., Darmstadt, Germany) which was equipped with a guard column, packed with Perisorb A 30–40 μm (Hibar Fertigsaule RT., Darmstadt, Germany). The isocratic mobile phase contained 0.9 % (v/v) isopropanol in *n*-hexane. The mobile phase was filtered using a 0.22 μm nylon membrane filter (MSI Inc., Westboro, MA) and degassed by a sonicator (Cole-Parmer Instrument Co., Vernon Hills, IL). The mobile phase flow rate was 1.0 ml/min. A 0.5 g sample was weighed in a 25 ml actinic volumetric flask and the volume was made up by hexane-BHT solution (1 g BHT/l) and 20 μl of this solution were injected into the column. Vitamin E content of the samples was calculated using the peak area compared with vitamin E standard area at 290 nm. Peak integration was performed using a HP 3392 A (Avondale, PA) integrator.

2.10. Lactones analysis

Lactone contents BO and its fractions were determined by the method of Ellis and Wong (1975). A 10 g sample was ground on to 35 g Celite 545 in a mortar and packed into a chromatographic column above 20 g anhydrous Na_2SO_4 and 6 g acidic Al_2O_3. The column was eluted with acetonitrile until 20 ml were collected. The acetonitrile extract was reduced to 0.5–1.0 ml under a stream of N_2. Acetonitrile was removed from above the fat and the fat was successively extracted with two 1 ml quantities of fresh acetonitrile. The combined acetonitrile extracts were evaporated under N_2 to a volume of 0.5–1.0 ml and acetonitrile was transferred to a small column containing 1 g acidic Al_2O_3. The trace amount of fat remaining in the tube was extracted twice with 1 ml fresh acetonitrile, which was also transferred to the small alumina column. The column was finally eluted with a further 1 ml of acetonitrile and the combined eluate from the column was reduced to 1 ml for GC analysis. One μl of the eluate was injected into a SAC-1 fused silica capillary column (30 m × 0.25 mm ID × 0.25 μm film thickness) (Supelco, Bellefonte, PA). Peak identities were established by mass a spectrometer, HP 5970 Series Mass Selective Detector coupled to a HP5980 Series II GC (Hewlett-Packard, San Fernando, CA) equipped with a FID and on-column injector. Helium was the carrier gas at a flow rate of 10 ml/min with hydrogen and air being supplied to the FID at flow rates of 30 and 350 ml/min, respectively. The oven temperature was programmed as follows: initially held at 100 °C for 5 min, and then increased at a rate of 5 °C/min until a temperature of 220 °C was reached and held at this temperature for 20 min. The injector and detector temperatures were 280 °C. Peak areas were integrated by using HP3365 Series II Chem Station software and compared with those obtained from standards (δ-10, δ-12 and δ-14) to obtain lactones content.

2.11. Rate of hydrolysis

Rate of hydrolysis was determined by the method of Marchis-Mouren, Sarda, and Desnuelle (1959), as modified by Lakshminarayana and Rama-Murthy (1986). The method involved incubating the enzyme lipase type II (lipase porcine pancreas) and the substrate emulsion at 37 °C and pH 9.1. Lipolysis was followed by continuously titrating the liberated fatty acids and maintaining the pH. The substrate emulsion was prepared by dispersing 20 ml of butter oil or its fraction in 180 ml of 100 g/l acacia gum in water at 10 °C, followed by homogenization for 10 min using a laboratory homogenizer (Polytron PT 1200, Kinematica, AG, Switzerland). Twenty-five milliliters of the emulsion were tempered at 37 °C in a 100 ml tempering beaker. A pH stat titrator (Brinkman, Westbury, NY) was used. The titrator electrode was immersed in the emulsion and the pH was brought up to 9.1 by adding, with stirring, a few drops of 4 g/l NaOH. 50 μl of enzyme preparation (20 g/l) were added. The FA which were liberated by the enzyme tended to lower the pH, but the optimum pH was maintained by adding 4 g/l NaOH for 20 min.

2.12. Oil stability index

The induction period of oxidation of BO and its fractions was determined by the AOCS method Cd 12b-92 (AOCS, 1998), using the oxidative stability instrument (Omnion Inc., Rockland, MA). The method included passing a stream of purified air through the sample (5 g), which was held in thermostatted bath (110 °C). The effluent air from the sample was then bubbled through a vessel containing deionized water, which was continually monitored for its conductivity by the instrument software. The effluent air (swept from the oxidized sample), containing volatile organic acids (mainly formic acid), tended to increase the water conductivity as oxidation proceeded. Oil stability index was defined as the point of maximum change of the rate of oxidation. This time-based end-point was calculated by the tangent intersection.

2.13. Iodine value

Iodine value was determined by the AOCS method Cd 1-25 (AOCS, 1998). Approximately, 0.5 g sample (dissolved in 15 ml CCl_4) was mixed with 25 ml Wijs solution (IC1) to halogenate the double bonds. After storing the mixture in the dark for 30 min, excess IC1 was reduced to free I_2 in the presence of 20 ml of KI (100 g/l) and 100 ml distilled water. Free I_2 was measured by titration with 24.9 g/l $Na_2S_2O_3 \cdot 5H_2O$ using starch (1.0 g/100 ml) as an indicator. IV was calculated as cg I_2 adsorbed/g sample.

2.14. Saponification value

Saponification value was determined by the AOCS method Cd 3-25 (AOCS, 1998). Fifty milliliters alcoholic KOH (40 g/l) was added to approximately 5.0 g sample and the mixture was gently boiled until the sample was completely saponified. Excess KOH was titrated with HCl. (43.01 ml/l) in the presence of phenolphthalein indicator (1 g/100 ml). Saponification value was expressed as mg of KOH required to saponify 1 g sample.

2.15. Reichert-Meissl, Polenske and Kirschner values

These values were determined by the AOCS method Cd 5-40 (AOCS, 1998). After saponification of the sample (about 5.0 g) with 20 ml glycerol–soda solution (20 ml of 500 g/l NaOH mixed with 180 ml glycerol), the soap solution was diluted with 135 ml distilled water and acidified with 6 ml H_2SO_4 (200 ml/l). The volatile fatty acids were distilled and the insoluble fatty acids were separated from the soluble by filtration. The aqueous solution of the soluble acids and the ethanolic solution of the insoluble acids were then titrated with 4 g/l of NaOH in the presence of phenolphthalein indicator (1 g/100 ml). Reichert-Meissl, Polenske and Kirschner values were expressed in terms of ml of 4 g/l NaOH required to neutralize the fatty acids obtained from distillation of 5 g of sample under the specific conditions of the method.

2.16. Refractive index (RI)

RI was determined by AOAC method 41.1.07 (AOAC, 2000) using an Abbe' refractometer (Bausch & Lomb, Salt Lake, UT).

2.17. Specific gravity

Specific gravity was determined gravimetrically by the pycnometer method (AOAC method 920.212 (AOAC, 2000)).

2.18. Replication and statistical analysis

The fractionation process was triplicated at each fractionation temperature (15, 20, 30, 35 and 40 °C) and duplicate analyses were performed on each replicate, except fat-soluble vitamins and lactones, where a representative sample was analyzed. Analysis of variance was performed by the SAS General Linear Methods procedure (SAS, 1994). Differences were considered significant at $P < 0.05$.

2.19. Chemicals

All chemicals used were of analytical grade except that solvents were HPLC grade. The chemicals were

purchased from the following sources: Sigma Chemicals Co. (St.Louis, MO), Aldrich Chemical Co. Inc. (Milwaukee, WI), Fisher Scientific (Fair Lawn, NJ), and J.T. Baker (Phillipsburg, NJ).

3. Results and discussion

3.1. General

The obtained fractions were categorized into three main groups: LMF included only L15 (Slip melting point (SMP) 12.6 °C); MMF included L20, L30, L35, S15 and S20 (SMP 24.2, 25.1, 29.5,25.9 and 29.8 °C, respectively), and finally, HMF included L40, S30, S35 and S40 (SMP 36.5, 37.1,41.6 and 45 °C, respectively).

3.2. Chemical properties

3.2.1. Fatty acids

Milk fat is legally defined by characteristics such as Reichert-Meissl and Polenske values (de Man & Finoro, 1980). These traditional methods (in conjunction with modern methods, such as GC) are still considered a useful tool in understanding the nature of fats and oils. Iodine value, for instance, is still used as an ingredient specification in the edible oils industry (Kaylegian & Lindsay, 1995).

Table 1 depicts the fatty acid characteristics of the various fractions obtained by multi-step dry fractionation. From the data it is noticed that, saturated fatty acids (SFA) were the most abundant in all fractions. On increasing the fractionation temperature (15–40 °C), SFA gradually increased while unsaturated fatty acids (USFA) gradually decreased. USFA content of LMF (36.2 mg/100 mg) was significantly higher ($P < 0.05$) than the original BO, MMF and HMF, while HMF showed the lowest content of USFA (21.0–26.7 mg/100 mg). Conversely, SFA content of HMF (73.3–79.0 mg/100 mg) was significantly higher ($P < 0.05$) than those of LMF and BO (63.8 and 70.7 mg/100 mg, respectively). Unlike LMF, HMF were enriched in long chain saturated fatty acids (LCSFA) (52.1–61.0 mg/100 mg) and reduced in both long chain unsaturated fatty acids (LCUSFA) and short chain fatty acids (SCFA). FA composition of MMF lies between those of HMF and LMF. Our findings are in agreement with the general trend obtained by Grall and Hartel (1992).

3.2.2. Saponification value (SV)

SVs of the obtained fractions are presented in Tables 2 and 3. The data revealed that, significant differences ($P < 0.05$) were found between LMF (236 mg KOH/g) and the inherent BO (229 mg KOH/g). However, L20, L30 and L35 did not show any significant differences ($P < 0.05$) when compared to BO, while S15 and S20 were significantly lower ($P < 0.05$) than BO. It was noticed that HMF were significantly lower ($P < 0.05$) than BO. SVs of L40, S30, S35 and S40 were 222, 211, 206 and 203 mg KOH/g, respectively. In general, fats containing SCFA exhibit SV higher than those composed entirely of long chain fatty acids (Sonntag, 1982). The trend found in this study was similar to that in other studies. Abd El-Rahman et al. (1998) found that SVs of

Table 1
Fatty acid characteristics of the various fractions (mg/100 mg)[A]

	SFA[B]	USFA[B]	SCFA[C]	MCFA[D]	LCSFA[E]	LCUSFA[F]
Liquid fractions						
BO	70.7 ± 1.0^{b}	29.3 ± 1.0^{bc}	4.7 ± 1.2^{bc}	17.1 ± 0.6^{ab}	48.9 ± 1.5^{b}	29.3 ± 1.2^{bc}
L15	63.8 ± 0.9^{c}	36.2 ± 0.9^{a}	6.7 ± 0.9^{a}	18.0 ± 0.5^{a}	39.2 ± 0.5^{d}	36.2 ± 0.9^{a}
L20	69.6 ± 1.3^{b}	30.4 ± 0.8^{b}	6.4 ± 1.0^{a}	17.4 ± 0.6^{ab}	45.7 ± 0.3^{c}	30.4 ± 0.8^{b}
L30	69.7 ± 0.8^{b}	30.3 ± 0.4^{b}	6.0 ± 0.5^{ab}	17.3 ± 0.6^{ab}	46.4 ± 0.8^{c}	30.3 ± 0.4^{b}
L35	71.2 ± 0.2^{b}	28.8 ± 0.1^{c}	5.5 ± 0.2^{abc}	17.1 ± 0.1^{ab}	48.6 ± 0.1^{b}	28.8 ± 0.2^{c}
L40	73.3 ± 0.4^{a}	26.7 ± 0.4^{d}	4.4 ± 0.6^{c}	16.4 ± 0.3^{b}	52.1 ± 0.8^{a}	26.7 ± 0.4^{d}
Solid fractions						
BO	70.7 ± 1.0^{d}	29.3 ± 1.0^{a}	4.7 ± 1.2^{ab}	17.1 ± 0.6^{a}	48.9 ± 1.4^{e}	29.3 ± 1.2^{a}
S15	70.5 ± 0.5^{d}	29.5 ± 0.5^{a}	5.4 ± 0.7^{a}	16.5 ± 0.4^{ab}	48.6 ± 0.6^{e}	29.5 ± 0.6^{a}
S20	72.4 ± 0.3^{c}	27.6 ± 0.3^{b}	4.6 ± 0.3^{ab}	17.1 ± 0.1^{a}	50.8 ± 0.2^{d}	27.6 ± 0.3^{b}
S30	73.4 ± 0.1^{c}	26.6 ± 0.1^{b}	4.3 ± 0.3^{abc}	16.4 ± 0.2^{b}	52.8 ± 0.4^{c}	26.6 ± 0.1^{b}
S35	76.7 ± 1.0^{b}	23.3 ± 1.0^{c}	3.7 ± 0.7^{bc}	16.1 ± 0.2^{b}	57.0 ± 1.5^{b}	23.3 ± 1.0^{c}
S40	79.0 ± 0.3^{a}	21.0 ± 0.3^{d}	3.2 ± 0.4^{c}	14.8 ± 0.3^{c}	61.0 ± 0.3^{a}	21.0 ± 0.3^{d}

Different letters within the same column are significantly different ($P < 0.05$).

[A] Means ± SD, $n = 3$ (Fatouh et al., 2003).

[B] SFA, saturated fatty acids; USFA, unsaturated fatty acids; L, liquid; S, solid; BO, butter oil. Numbers following the type of fraction correspond to the temperature at which the fraction separated (15–40 °C).

[C] SCFA; short chain fatty acids ($C_{4:0}$–$C_{8:0}$).

[D] MCFA; medium chain fatty acids ($C_{10:0}$–$C_{14:0}$).

[E] LCSFA; long chain saturated fatty acids ($C_{16:0}$–$C_{18:0}$).

[F] LCUSFA; long chain unsaturated fatty acids ($C_{18:1}$–$C_{18:3}$).

Table 2
Chemical and physical properties of the various liquid fractions[A]

	BO	Fraction[B]				
		L15	L20	L30	L35	L40
Iodine value[C]	282 ± 0.5^{c}	35.0 ± 1.0^{a}	29.6 ± 0.6^{b}	28.8 ± 0.1^{bc}	27.9 ± 0.2^{c}	25.7 ± 0.3^{d}
Saponification value[D]	229 ± 0.7^{b}	236 ± 0.8^{a}	229 ± 1.5^{b}	228 ± 1.0^{b}	227 ± 1.5^{b}	222 ± 0.7^{c}
Reichert-Meissl value[E]	34.1 ± 0.3^{c}	38.5 ± 0.2^{a}	37.4 ± 0.4^{b}	37.0 ± 0.6^{b}	34.8 ± 0.5^{c}	30.2 ± 0.5^{d}
Polenske value[E]	1.7 ± 0.4^{b}	2.4 ± 0.2^{a}	2.1 ± 0.2^{ab}	2.0 ± 0.2^{ab}	1.9 ± 0.5^{ab}	1.7 ± 0.3^{b}
Kirschner value[E]	20.7 ± 1.6^{b}	23.4 ± 0.8^{a}	22.2 ± 0.8^{ab}	21.7 ± 0.7^{ab}	21.4 ± 1.9^{ab}	20.7 ± 1.2^{b}
Cholesterol[F]	280 ± 2.1^{c}	359 ± 3.5^{a}	297 ± 7.0^{b}	287 ± 4.9^{c}	285 ± 1.6^{c}	264 ± 1.8^{d}
Vit. A[G]	7.8	13.5	8.9	8.8	8.5	6.7
Vit. D[G]	0.03	0.06	0.05	0.04	0.03	0.02
Vit. E[G]	10.1	12.8	11.3	11.1	10.3	9.5
Lactones (total)[H]	16.8	22.1	18.6	17.6	16.0	14.9
δ-10	3.0	3.7	2.7	2.6	2.2	1.9
δ-12	6.3	8.6	7.2	6.8	6.4	6.0
δ-14	7.5	9.9	8.7	8.3	7.4	6.9
Specific gravity[I]	0.9157 ± 0.0003^{b}	0.9176 ± 0.0005^{a}	0.9148 ± 0.0003^{c}	0.9148 ± 0.0003^{c}	0.9031 ± 0.0004^{d}	0.9014 ± 0.0003^{e}
Refractive index (n_D^{40})	1.4522 ± 0.0001^{c}	1.4538 ± 0.0001^{a}	1.4524 ± 0.0001^{b}	1.4524 ± 0.0000^{b}	1.4522 ± 0.0001^{c}	1.4520 ± 0.0000^{d}

Different letters within the same row are significantly different ($P < 0.05$).
[A] Means ± SD, $n = 3$.
[B] L, liquid fraction; BO, butter oil. Numbers following the type of fraction correspond to the temperature at which the fraction separated (15–40 °C).
[C] cg I_2 adsorbed/g.
[D] mg KOH/g.
[E] ml of 4 g/l NaOH required to neutralize the fatty acids obtained from distillation of a 5 g sample under the specific conditions of the method.
[F] mg/100 g.
[G] μg/g.
[H] μg/g.
[I] At 25 °C.

Table 3
Chemical and physical properties of the various solid fractions[A]

	BO	Fraction[B]				
		S15	s20	S30	S35	S40
Iodine value[C]	28.2 ± 0.5^{a}	28.4 ± 0.0^{a}	26.7 ± 0.4^{b}	25.4 ± 0.8^{c}	22.8 ± 0.9^{d}	20.8 ± 0.5^{e}
Saponification value[D]	229 ± 0.7^{a}	220 ± 1.0^{b}	212 ± 1.4^{c}	211 ± 2.4^{c}	206 ± 4.0^{d}	203 ± 1.4^{d}
Reichert-Meissl value[E]	34.1 ± 0.3^{b}	36.7 ± 0.2^{a}	32.0 ± 0.2^{c}	30.1 ± 0.7^{d}	26.3 ± 1.5^{e}	23.6 ± 0.5^{f}
Polenske value[E]	1.7 ± 0.4^{a}	1.7 ± 0.3^{a}	1.5 ± 0.2^{a}	1.5 ± 0.1^{a}	1.3 ± 0.1^{ab}	1.0 ± 0.1^{b}
Kirschner value[E]	20.7 ± 1.6^{a}	20.6 ± 1.0^{a}	19.9 ± 0.6^{a}	19.7 ± 0.2^{a}	19.1 ± 0.5^{ab}	18.0 ± 0.4^{b}
Cholesterol[F]	280 ± 2.1^{b}	299 ± 4.5^{a}	263 ± 3.2^{c}	255 ± 2.7^{d}	239 ± 2.6^{e}	215 ± 4.9^{f}
Vit. A[G]	7.8	7.4	6.6	5.7	5.2	5.1
Vit. D[G]	0.03	0.03	0.02	0.02	0.01	0.01
Vit. E[G]	10.1	10.8	9.7	9.1	7.6	7.1
Lactones (total)[H]	16.8	16.2	14.7	11.5	8.9	6.6
δ-10	3.0	2.8	2.2	2.2	1.1	0.9
δ-12	6.3	6.8	6.7	5.0	3.5	2.2
δ-14	7.5	6.6	5.8	4.3	4.2	3.5
Specific gravity[I]	0.9157 ± 0.0003^{a}	0.9045 ± 0.0007^{b}	0.9023 ± 0.0009^{c}	0.9021 ± 0.0007^{c}	0.8984 ± 0.0001^{d}	0.8949 ± 0.0004^{e}
Refractive index (n_D^{40})	1.4522 ± 0.0001^{a}	1.4522 ± 0.0001^{a}	1.4520 ± 0.0000^{b}	1.4520 ± 0.0001^{b}	1.4517 ± 0.0001^{c}	1.4516 ± 0.0001^{c}

Different letters within the same row are significantly different ($P < 0.05$).
[A] Means ± SD, $n = 3$.
[B] S, solid fraction; BO; Butter oil. Numbers following the type of fraction correspond to the temperature at which the fraction separated(15–40 °C).
[C] cg I_2 adsorbed/g.
[D] mg KOH/g.
[E] ml of 4 g/l NaOH required to neutralize the fatty acids obtained from distillation of 5 g sample under the specific conditions of the method.
[F] mg/100 g.
[G] μg/g.
[H] μg/g.
[I] At 25 °C.

LMF, anhydrous milk fat (AMF) and very high-melting fraction (VHMF) were 245, 240 and 234 mg KOH/g, respectively. Norris, Gray, McDowell, and Dolby (1971) reported SVs of 228–231, 226–228 and 225–226 mg KOH/g for liquid fraction, AMF and solid fraction, respectively.

3.2.3. Iodine value (IV)

IVs of the various fractions (Tables 2 and 3) revealed substantial alterations in the chemical composition of the resultant fractions caused by multi-step fractionation. IV of L15, which was entirely liquid at room temperature, was the highest among all the obtained fractions (35.0 eg I_2 adsorbed/g), while S40, which was solid with a waxy appearance, was the lowest (20.8 cg I_2 adsorbed/g). L 20 and S20 (29.6 and 26.7 cg I_2 adsorbed/g, respectively) were the only MMF fractions that showed significant differences ($P < 0.05$) when compared to the original BO (28.2 cg I_2 adsorbed/g). However, IVs of HMF were significantly lower ($P < 0.05$) than those of LMF and BO. IVs of L40, S30 and S35 were 25.7, 25.4 and 22.8 cg I_2 adsorbed/g, respectively. The higher IV of LMF is readily explained by the chemical composition (Table 1). L15 was enriched in USFA (36.2 mg/100 mg), But depleted in SFA (63.8 mg/100 mg) and vice versa in HMF. The trend of the data confirmed other reported studies. Amer et al. (1985) found that IVs of BO samples obtained from winter butter were 33.1, 30.3 and 24.7 cg I_2 adsorbed/g for L19, BO and S29, respectively. Sherbon, Dolby, and Russel (1972) reported IVs of 33.3, 31.3 and 29.6 eg I_2 adsorbed/g for L28, AMF and S28, respectively.

3.2.4. Reichert-Meissl value (RM)

RM values of BO and its fractions are illustrated in Tables 2 and 3. RM value of L15 was significantly higher ($P < 0.05$) than BO, MMF and HMF. As expected, no significant differences ($P < 0.05$) in RM value were found between L20 and L30, since the FA composition of these fractions showed identical profiles (Fatouh et al., 2003). Likewise, the differences between L35 and the inherent BO were not significant ($P < 0.05$). RM values of BO and L35 were 34.1 and 34.8 ml of 4 g/l NaOH, respectively. However, when BO was compared to S15 and S20, significant differences ($P < 0.05$) were found. As the fractionation proceeded toward higher temperatures, RM value decreased. RM values of S30, S35 and S40 were 30.1, 26.3 and 23.6 ml of 4 g/l NaOH, respectively. By virtue of the FA composition of the fractions (Fatouh et al., 2003), SCFA ($C_{4:0}$–$C_{8:0}$) were more concentrated in LMF than HMF. The results in this study are in agreement with the general trend observed from previous studies. de Man and Finoro (1980) found that the range of RM values of liquid and solid fractions, obtained by melt crystallization at 25–32 °C, were 30.3–33.5 and 25.0–25.7 ml of 4 g/l NaOH, respectively. Lakshminarayana and Rama-Murthy (1985) reported RM values of 30.9, 28.6,29.7, 31.4 and 33.2 ml of 4 g/l NaOH for buffalo milk fat, S31, S23, S15 and L15, respectively.

3.2.5. Polenske value (PV)

PVs of buffalo BO and its fractions are given in Tables 2 and 3. Significant differences ($P < 0.05$) were found between BO and LMF. PV of BO and L15 were 1.7 and 2.4 ml of 4 g/l NaOH, respectively. Conversely, when compared to MMF, no significant differences ($P < 0.05$) were found between BO and MMF. Similarly, no significant differences ($P < 0.05$) were found between BO and HMF except S40 (1.0 ml of 4 g/l NaOH). The obtained results are consistent with the changes in FA composition observed early (Fatouh et al., 2003) which indicated a higher content of $C_{8:0}$ and $C_{10:0}$ in LMF than HMF. de Man and Finoro (1980) reported that the ranges of PV in AMF, LMF and HMF were 1.88; 1.9–2.1 and 1.3–1.8 ml of 4 g/l NaOH, respectively. Lakshminarayana and Rama-Murthy (1985) found PVs to be 1.2, 1.0, 1.28,1.39 and 1.5 ml of 4 g/l NaOH for buffalo milk fat, S31, S23, S15 and L15, respectively.

3.2.6. Krischner value (KV)

The same trend that was found in PV was also found in KV. Tables 2 and 3 show that L15 was significantly higher ($P < 0.05$) than BO and HMF. KVs of L15 and BO were 20.7 and/23.4 ml of 4 g/l NaOH, respectively. KVs of MMF were in the range of 22.2–19.9 ml of 4 g/l NaOH which was not significant ($P < 0.05$) as compared to BO. Likewise, the differences between KV of HMF and BO were not significant ($P < 0.05$) except for S40, which showed the lowest KV among all obtained fractions (18.0 ml of 4 g/l NaOH). Based on FA composition of the fractions (Fatouh et al., 2003), $C_{4:0}$ was more concentrated in LMF as a result of the selectivity of the multi-step fractionation process. This trend concurs with Abd El-Rahman et al. (1998) who reported KVs of 28.7, 23.6 and 14.8 ml of 4 g/l NaOH for LMF, AMF and VHMF.

3.2.7. Cholesterol

Tables 2 and 3 show the cholesterol distribution among the various fractions. In general, cholesterol tended to concentrate more in the liquid fractions than solid fractions. LMF showed significantly higher ($P < 0.05$) cholesterol content than the original BO, MMF and HMF. L15 contained 359 mg cholesterol/100 g sample compared to 280, 263–297 and 215–264 mg/100 g for BO, MMF and HMF, respectively. In view of the chemical composition of the various fractions (Table 1), the higher concentration of cholesterol in LMF may be attributed to the high affinity of cholesterol for SCFA and USFA which predominated in LMF as compared to

HMF, which were enriched in long chain saturated fatty acids (Arul, Boudreau, Makhlouf, Tardif, & Grenier, 1988). The results obtained in this study are in agreement with general trends observed in previous studies of melt crystallization. Bhaskar, Rizvi, Bertoli, Fay, and Hug (1998) reported cholesterol contents of 273, 217 and 454 mg/100 g for AMF, AMF 45, and AMF 10, respectively. Norris et al. (1971) found that the range of cholesterol contents of three different batches of milk fat and liquid and solid fractions was 230–250, 250–260 and 220–230 mg/100 mg, respectively.

3.2.8. Fat-soluble vitamins (A, D and E)

Tables 2 and 3 reveal the fat-soluble vitamin (A, D and E) contents of butter oil and its fractions. Multi-step dry fractionation resulted in a noticeable increase (5.7 μg/g) in vitamin A content of LMF as compared to the original BO (7.8 μg/g). With increasing the SMP of the fractions, vitamin A content exhibited a gradual decrease. MMF and HMF contents of vitamin A were in the ranges 8.9–6.6 and 6.7–5.1 μg/g, respectively. Vitamin D content of BO as well as all obtained fractions was very low. L15 and BO contained 0.06 and 0.03 μg/g, respectively, while S40 was almost depleted of vitamin D (0.01 μg/g). Tocopherol followed the same trend as both vitamins A and D. L15 was the highest (12.8 μg/g), followed by MMF (11.3–9.7 μg/g), which gradually decreased with increasing SMP of the fractions to reach the lowest value (7.1 μg/g) among all obtained fractions for S40. The tocopherol results are supported by the fact that, tocopherol content tends to be high in butter fat containing high amounts of polyunsaturated fatty acids (Money, Rammell, & Catthew, 1976). Norris et al. (1971) found that vitamin A contents of liquid fraction, milk fat and solid fraction were in the ranges 9.6–10.0, 8.4–9.2 and 6.6–8.2 μg/g fat, respectively. Arora and Rai (1998) reported a higher content of tocopherol in goat milk fat liquid fractions (38.0–50.2 μg/g) than solid ones (23.5–27 μg/g).

3.2.9. Lactones

Lactones are considered important constituent of the unique buttery flavour of milk fat. The aromas of butter oil and its fractions were determined for the three most important delta (δ) lactones, namely δ-10, δ-12 and δ-14 (Kankare & Antila, 1989). Data (Tables 2 and 3) revealed that, lactones were concentrated more in the liquid fractions than the solid ones, following the same trend as both cholesterol and fat-soluble vitamins. An increase of 5.3 μg/g of lactones was found in L15 as compared to the original BO (16.8 μg/g). L20 and L30 exhibited a slight increase in their lactone contents when compared to BO. However, L35, S15 and S20 exhibited a slight decrease in their lactone contents (16.0, 16.2 and 14.7 μg/g, respectively) when compared to BO. It was observed that, lactone concentration substantially decreased with increasing SMP of HMF. For instance, S35 (SMP 41.6 °C) and S40 (SMP 45.0 °C) contained 8.9 and 6.6 μg/g, respectively which were lowest among all the obtained fractions. Walker, Cant, and Keen (1977) reported that the enrichment of lactones in LMF is inversely proportional to the exclusion of these compounds from HMF during the fractionation process. Moreover, owing to the low melting point and/or the greater polarities of lactones, they remain preferentially dissolved in LMF rather than HMF. The existence of lactones in HMF may be a result of entrapment of some low melting triacylglycerol in HMF during separation of the fractions. Ellis and Wong (1975) found that lactone contents of commercial butter samples were 12–30 μg/g.

3.3. Physical properties

3.3.1. Refractive index

RI of BO and its fractions are presented in Tables 2 and 3. The greater the degree of unsaturation, the higher was the RI. L15 content of USFA (Table 1) was the highest among all obtained fractions (36.2 mg/100 mg). Consequently, RI of L15 (1.4538) was significantly higher ($P < 0.05$) than the original BO (1.4522) which has a lower content of USFA (29.3 mg/100 mg). As anticipated from their identical chemical compositions (Table 1), no significant differences ($P < 0.05$) were found between RI of L20 and L30. However, when L2 and L30 were compared to L35, significant differences ($P < 0.05$) were found. RI values of L20, L30 and L35 were 1.4524, 1.4524 and 1.4522, respectively. Sequential crystallization resulted in concentrating SFA in HMF. L40, S30, S35 and S40 contained 73.3, 73.4, 76.7 and 79.0 mg/100 mg of SFA, respectively, compared to 70.7 mg/100 mg in BO (Table 1). As a result, RI of HMF was significantly lower ($P < 0.05$) than BO. RI values of L40, S30, S35 and S40 were 1.4520, 1.4520, 1.4517 and 1.4516, respectively. Abd El-Rahman et al. (1998) reported RI values of 1.4543, 1.4524 and 1.4517 for LMF, AMF and VHMF, respectively.

3.3.2. Specific gravity (sp gr)

SP gravities of BO and its fractions are given in Tables 2 and 3. It is noticed that, sp gr decreased with increasing SMP of the fractions, which may be ascribed to the decrease in the USFA content (Formo, 1979). Sp gr of L15 (SMP 12.6 °C) was significantly higher ($P < 0.05$) than BO (SMP 34.7 °C). Sp gr values of L15 and BO were 0.9176 and 0.9157, respectively. Moreover, the differences between BO and MMF (SMP 24.2–29.8 °C), as well as between BO and HMF (SMP 36.5–45 °C), were also significant ($P < 0.05$). Sp gr of MMF was in the range of 0.9148–0.9023, while sp gr of HMF was in the range of 0.9014–0.8949. The trends found in our results are in agreement with data obtained from

other studies. Badings, Schaap, de Jong, and Hagedoorn (1983) reported sp gr values (at 35 °C) of 0.9084–0.9092, 0.9074 and 0.9052–0.9089 for liquid fractions, cow's milk fat and solid fractions obtained from pasture feeding, respectively, while, Lakshminarayana and Rama-Murthy (1985) found that sp gr values (at 30 °C) of L15, buffalo milk fat and S31 were 0.977, 0.965 and 0.9406, respectively.

3.4. Hydrolytic and oxidative stability properties

3.4.1. Oil stability index (OSI)

OSI values of BO and its fractions are presented in Table 4. Chemical composition of the fractions has a great influence on their susceptibility to oxidation. In general, the higher the degree of unsaturation, the lower the OSI. L15 content of USFA (36.2 mg/100 mg) was the highest among all the obtained fractions; thus it is expected to undergo a faster rate of oxidation. OSI of L15 (5.6 h) was significantly lower ($P < 0.05$) than BO, MMF and HMF. Table 1 shows that, no significant differences ($P < 0.05$) were found between USFA content of the original BO and MMF, with the exception of S20. This similarity in the chemical composition is reflected in the OSI. The differences between BO and MMF (except S20) were also not significant ($P < 0.05$). OSI values of BO, L20, L30, L35 and S15 were 8.2, 7.7, 7.9, 9.6 and 8.0 h, respectively. Conversely, OSI of HMF were significantly higher ($P < 0.05$) than BO, which was consistent with the significant differences ($P < 0.05$) found between USFA content of HMF and BO (Table 1). OSI of HMF was in the range of 11.1–16.0 h.

3.4.2. Rate of hydrolysis

Rates of hydrolysis of BO and its fractions are depicted in Table 4. Data reveal that, with increasing SMP of the fraction, the rate of hydrolysis decreased. L15 (SMP 12.6 °C) was significantly higher ($P < 0.05$) than the inherent BO (SMP 34.7 °C) in the rate of hydrolysis. L15 consumed 7.8 ml of 4 g/l NaOH as compared to 5.4 ml of 4 g/l NaOH for BO. MMF (SMP 24.2–29.8 °C) rate of hydrolysis lies between that of LMF and HMF. Rate of hydrolysis of MMF was in the range of 6.8–3.2 ml of 4 g/l NaOH, while for HMF the range was 4.9–1.6 ml of 4 g/l NaOH. Lakshminarayana and Rama-Murthy (1986) reported that, the physical state of the fat greatly influences the rate of hydrolysis, because lipase action is inhibited when the fat is in the solid state. Based on chemical composition (Table 1), the great resistance exhibited by HMF (SMP 36.5–45.0 °C) toward lypolysis may be attributed to its significantly higher ($P < 0.05$) content of SFA compared to LMF. Patel, Fox, and Tarasuk (1968) reported that lipase hydrolyzed SCFA triglycerides faster than long chain fatty acid triglycerides. The results found in this study are similar to those obtained by Lakshminarayana and Rama-Murthy (1986).

Table 4
Stability of the various fractions[A]

Fraction[B]	Oxidative stability index[C]	Rate of hydrolysis[D]
Liquid fractions		
BO	8.2 ± 0.5^{bc}	5.4 ± 0.4^{c}
L15	5.6 ± 1.2^{d}	7.8 ± 0.1^{a}
L20	7.7 ± 1.0^{c}	6.8 ± 0.3^{ab}
L30	7.9 ± 1.1^{c}	6.7 ± 0.2^{b}
L35	9.6 ± 0.2^{b}	5.4 ± 1.0^{c}
L40	11.1 ± 0.8^{a}	4.9 ± 0.9^{c}
Solid fractions		
BO	8.2 ± 0.5^{d}	5.4 ± 0.4^{a}
S15	8.0 ± 0.8^{d}	4.7 ± 0.1^{b}
S20	11.2 ± 2.3^{c}	3.2 ± 0.1^{c}
S30	12.7 ± 0.9^{bc}	3.0 ± 0.7^{c}
S35	14.6 ± 0.8^{ab}	2.2 ± 0.2^{d}
S40	16.0 ± 1.2^{a}	1.6 ± 0.3^{d}

Different letters within the same column are significantly different ($P < 0.05$).

[A] Means ± SD, $n = 3$.

[B] L, liquid fraction; S, solid fraction; BO, Butter oil. Numbers following the type of fraction correspond to the temperature at which the fraction separated (15–40 °C).

[C] h.

[D] ml of 4 g/l NaOH.

4. Conclusion

The present study has shown that, multi-step dry fractionation of BO is an effective tool for yielding several fractions which differ markedly in their properties. Moreover, dry fractionation improved the stability of HMF as a result of the change in the chemical composition. The absence of organic solvents and the reasonable cost of processing make dry fractionation the method of choice for the food industry. Milk fat-based products, such as dairy, bakery and confectionery products, are candidates for the potential utilization of the resultant fractions.

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