Isopropanol Fractionation of Butter Oil and Characteristics of Fractions

P.K. Pal, D.K. Bhattacharyya*, and S. Ghosh

Department of Chemical Technology, Oil Technology Division, University of Calcutta, Calcutta - 700 009, India

ABSTRACT: Fractionation of butter oil from isopropanol and characterization of the chemical composition and the melting properties of the fractions obtained have been investigated. Butter oil was fractionated from isopropanol (1:4 wt/vol) at 15 to 30°C. The yields of stearins and oleins were dependent on the temperature employed during fractionation. Thus, 24.8 to 48.9% of stearins and 51.5 to 75.2% of oleins could be obtained as the crystallization temperature varied from 15 to 30°C. The stearin fractions displayed a distinct variation in the fatty acid compositions. The palmitic acid content of the stearin fractions varied from 39.1 to 44.0%, and that of stearic from 15.1 to 16.8%, respectively. The olein fractions contained 43.2% stearic acid, and 2.4 to 2.8% palmitoleic acid (C16:1). The solid fat content values of the stearin fractions obtained were 62–67, 39–50, and 21–25 at 10, 20, and 30°C, respectively. From the results, it is evident that anhydrous milk fat can be fractionated at relatively high temperatures from isopropanol to produce stearin and olein fractions of specific composition and properties.

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KEY WORDS: Butter oil, isopropanol fractionation, milk fat fractions.

Fractionation of milk fat by dry process and from appropriate solvents, including supercritical carbon dioxide, is well documented in the published literature (1–7). The alterations in fatty acids and triglyceride components of the fractions compared to the original milk fat are also quite extensively recorded in the literature (8–12). Dry fractionation of milk fat is a common technique to produce fat fractions with properties and compositions that are suitable for food products. Among the solvents used for fractionation are several bio-renewable solvents like acetone, ethanol, and supercritical carbon dioxide. Isopropanol, which is also a biorenewable solvent, is increasing in importance for vegetable oil extraction, and its use in fractionation of fats is also known. Acetone has advantages over hexane in that higher crystallization temperature and selectivity in separating diacylglycerols are associated with acetone (13). Isopropanol is likely to have similar advantages over hexane. It also has advantages over hexane because of its latent heat of vaporization and flash point (14) during separation and recovery of solvent. Saturated acid-rich glycerides, having both short-chain and long-chain acid moieties, are relatively less soluble in isopropanol than the corresponding glycerides with unsaturated fatty acids. As a result, a sharper fractionation is expected between the saturated acid-rich glycerides and the glycerides with unsaturated fatty acids. Also, it has been reported that isopropanol, as compared to hexane, enables fractionation to be conducted at a comparatively high temperature and with a lower volume of solvent (15). Further, time of crystallization is reported to be reduced (16), and the loss of isopropanol is also less in comparison to *n*-hexane or acetone during recovery by distillation.

In fact, isopropanol fractionation of natural hard fats like sal fat (*Shorea robusta*) and mango kernel (*Mangifera indica*) shows the above advantages in comparison with hexane or acetone (16,17).

Information about single and multiple dry fractionation of milk fat is widely available (18). However, fractionation of milk fat using isopropanol has not been done before. The present study aims at investigating the fractionation of anhydrous milk fat from isopropanol, primarily to get some insight into the separation pattern of the stearins and oleins and their chemical compositions.

MATERIALS AND METHODS

Butter oil (anhydrous milk fat) was purchased from Ghosh Dairy Co. Ltd. (Calcutta, India). Isopropanol (A.R. Grade) was supplied by S.D. Fine Chemicals India Ltd. (Mambai, India).

Fractionation of butter oil (anhydrous milk fat) from isopropanol. The butter oil (250 g) was placed in a 2 L beaker (Borosil) after it was completely melted in a water bath at 50°C. Isopropanol was added (4 mL/g butter oil), and the mixture was kept at this temperature for 10 min. The beaker was placed in a constant-temperature bath and stirred by a low-speed mechanical stirrer for 1 h. The crystallization process was done at different temperatures, *viz*. 15, 20, 25, and 30°C. The solid material was separated from the liquid oil by filtration under vacuum. The fractions were desolventized at 80°C for 1 h at 10 mm Hg pressure, weighed, and stored in a refrigerator.

Analytical procedures. Melting points were determined by the method of the Indian Standard Institution (19). Two clean

*To whom all correspondence should be addressed at Department of Chemical Technology, Oil Technology Division, University of Calcutta, 92, A.P.C. Road, Calcutta–700 009, India. E-mail: dkb@cucc.ernet.in

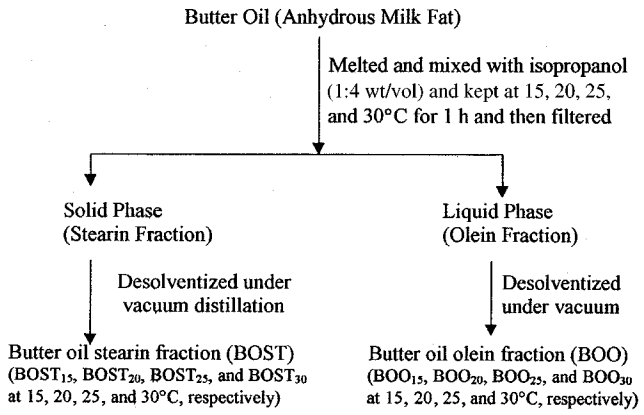
and dry capillary tubes, having dimensions of 50–60 mm length, 0.8–1.1 mm inside diameter, and 1.2–1.5 mm outside diameter were dipped into a dried, clean molten fat sample so that the fat sample rose to 10 mm height in each capillary tube. The samples in the capillary tube were chilled at once by holding a piece of ice at the end of the capillary until the fat solidified, after which the samples were stabilized in a refrigerator at a temperature of 4 ± 1°C for 16 h. The tubes were removed from the refrigerator and attached to a thermometer (with 0.1°C subdivision) in the Thiele's melting point tube containing distilled water (temperature sufficiently below the slip point of the sample). The side tube of the apparatus was heated gently so that the temperature of the water rose by 0.5°C per min. The temperature at which the solidified fat liquefied and started slipping due to hydrostatic pressure was precisely noted and taken as the slip melting point of the sample. The average of the two melting temperatures for the two capillaries was calculated and the same procedure repeated for duplication. Finally, the average of two sets was noted for accurate determination of slip point.

Solid fat content (SFC) was determined with a pulsed nuclear magnetic resonance (NMR) spectrophotometer (Minispec PC 120; Brucker, Germany). Before the analysis, the samples were heated to completely liquify for homogeneity and then the samples were cooled and held at 0°C for 15 min, tempered at 27°C for 30 min, and cooled again and stabilized at 0°C for 24 h. The samples were then equilibrated at the lowest temperature in the temperature range of interest for at least 30 min, and the solid content was measured by pulsed NMR. Samples were then equilibrated at each of the next higher temperatures for SFC measurement.

Fatty acid compositions were determined by a gas–liquid chromatography (GLC) method after conversion into methyl esters (20). The HP-5890A GLC (Hewlett-Packard, Palo Alto, CA) was connected with a HP-3390A data integrator. The GLC was fitted with a glass column (1.83 m × 3.75 mm i.d.), packed with 10% diethylene glycol succinate supported on Chromosorb-WHP (100/200 mesh). The oven temperature was programmed from 140 to 190°C at an increase of 5°C/min. The injector and detector block temperatures were maintained at 230 and 240°C, respectively. IOLAR-2 nitrogen (Bharat Oxygen Limited, Calcutta, India) was used as the carrier gas (flow rate 30 mL/min). The fatty acid esters' peaks were identified and calibrated with standard methyl esters, supplied by Sigma Chemical Company (St. Louis, MO). Data are mean ± standard deviation of three determinations.

RESULTS AND DISCUSSION

The fractionation of butter oil using isopropanol has been carried out at different temperatures, *viz.* 15, 20, 25, and 30°C for 1 h only, as shown in Scheme 1. Fractionation from isopropanol was observed to be rapid and reproducible. The stearin and olein fractions obtained at various temperatures are tabulated in Table 1 along with their yield and melting point. Yields of stearins and oleins obtained depend on the crystallization temperatures. This is corroborated by our previous studies (16,17). The stearin fraction ($BOST_{25}$) at 15°C has a slip point of 36°C and the corresponding olein fraction ($BOST_{15}$) has a slip point of 13°C, which may be explained by the fact that the high-melting glycerides are concentrated relatively more in the stearin fraction and the low-melting glycerides are concentrated in the olein fraction. The concentration of the high-melting glycerides increased in the stearin fraction as the temperature was increased during crystallization from 15 to 30°C.



SCHEME 1

Fatty acid composition of the fractions obtained by fractionating butter oil from isopropanol at various temperatures are shown in Table 2. Both the stearin fractions and the olein fractions show differences in the fatty acid compositions. The stearin shows higher content of short-chain acids and other saturated acids than the oleins. Milk fat, after single-stage dry fractionation, yields stearin and olein in varying amounts depending on temperature of crystallization for a particular time period (18). The characteristics of the stearins and oleins match with the trends observed in the present study using single isopropanol fractionation in respect to yields and melting points. The melting point of the stearin fraction at 15°C ($BOST_{15}$) has increased from original butter oil by 4.1°C due to the increase in both palmitic acid (from 31.7 to 39.1%) and stearic acid (from 14.0 to 21.3%). On the other hand, in the

TABLE 1
Yield and Slip Point of the Butter Oil and Its Fractions Obtained by Isopropanol Fractionation

Butter oil/fractions[a]	Yield (% w/w)	Slip point (°C)
Butter oil (original)	—	31.9 ± 0.21
$BOST_{15}$	48.9 ± 1.7	36.0 ± 0.20
BOO_{15}	51.1 ± 1.7	13.0 ± 0.31
$BOST_{20}$	35.2 ± 1.9	38.4 ± 0.45
BOO_{20}	64.8 ± 1.9	14.0 ± 0.35
$BOST_{25}$	28.5 ± 2.3	40.1 ± 0.51
BOO_{25}	71.5 ± 2.3	19.8 ± 0.25
$BOST_{30}$	24.8 ± 1.3	42.5 ± 0.22
BOO_{30}	75.2 ± 1.3	19.2 ± 0.12

[a]Values are mean ± SD. Refer to Scheme 1 for butter oil fractions.

TABLE 2
Fatty Acid Composition of the Fractions Obtained by Isopropanol Fractions

Butter oil/ fractions[a]	Fatty acid composition (% w/w)										
	$C_{4:0}$	$C_{6:0}$	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
Butter oil (original)	2.8 ± 0.06	2.1 ± 0.12	1.3 ± 0.12	2.0 ± 0.04	1.7 ± 0.21	9.1 ± 0.11	31.7 ± 0.23	2.2 ± 0.22	14.0 ± 0.10	32.4 ± 0.24	0.7 ± 0.03
$BOST_{15}$	3.1 ± 0.12	2.4 ± 0.08	1.8 ± 0.11	2.3 ± 0.12	2.1 ± 0.14	10.4 ± 0.23	39.1 ± 0.54	1.9 ± 0.16	15.1 ± 0.32	21.3 ± 0.27	0.5 ± 0.04
BOO_{15}	2.5 ± 0.18	1.7 ± 0.12	0.8 ± 0.04	1.6 ± 0.19	1.3 ± 0.06	7.5 ± 0.12	24.7 ± 0.44	2.4 ± 0.30	12.6 ± 0.24	43.9 ± 0.74	1.0 ± 0.32
$BOST_{20}$	3.5 ± 0.12	3.1 ± 0.18	2.1 ± 0.26	2.5 ± 0.09	2.4 ± 0.24	11.5 ± 0.12	42.2 ± 0.43	1.4 ± 0.22	15.8 ± 0.35	15.6 ± 0.49	0.4 ± 0.08
BOO_{20}	2.4 ± 0.11	1.7 ± 0.22	0.9 ± 0.16	1.9 ± 0.10	1.4 ± 0.05	8.1 ± 0.12	23.3 ± 0.24	2.7 ± 0.22	13.2 ± 0.44	43.1 ± 0.52	1.2 ± 0.42
$BOST_{25}$	3.2 ± 0.14	3.1 ± 0.16	2.2 ± 0.21	2.8 ± 0.05	2.2 ± 0.20	11.6 ± 0.32	43.4 ± 0.73	1.1 ± 0.32	16.4 ± 0.25	13.5 ± 0.29	0.5 ± 0.01
BOO_{25}	2.5 ± 0.21	1.7 ± 0.32	1.0 ± 0.06	2.0 ± 0.09	1.2 ± 0.09	7.8 ± 0.22	22.2 ± 0.34	2.8 ± 0.32	12.5 ± 0.34	44.5 ± 0.54	1.8 ± 0.62
$BOST_{30}$	3.0 ± 0.14	3.1 ± 0.16	2.5 ± 0.21	3.1 ± 0.05	2.1 ± 0.20	11.9 ± 0.32	44.0 ± 0.73	1.3 ± 0.32	16.8 ± 0.25	11.6 ± 0.29	0.7 ± 0.01
BOO_{30}	2.6 ± 0.11	1.8 ± 0.18	1.2 ± 0.09	2.3 ± 0.14	1.1 ± 0.16	7.5 ± 0.25	21.5 ± 0.38	2.7 ± 0.32	13.1 ± 0.44	44.0 ± 0.34	2.2 ± 0.42

[a]Values are mean ± SD. Refer to Scheme 1 for butter oil fractions.

olein fraction (BOO_{15}), palmitic acid and stearic acid have both decreased from 31.7 to 24.7% and 14.0 to 12.6%, respectively, while oleic acid has increased. The fatty acid compositions of the stearin and olein fractions obtained at 30°C and also at 25°C from isopropanol in the present work appear to closely match the pattern composition of fatty acids of the corresponding fractions isolated by dry fractionation technique, as reported by Breitschuh and Windhab (21).

Similarly, the change in slip point of the stearin and olein fractions at 20, 25, and 30°C are mainly due to the change of palmitic, stearic, and oleic acids in the fatty acid moieties of the glycerol backbone.

The SFC values (obtained by NMR) of the stearin fractions obtained by isopropanol fractionation at various temperatures have also been measured and are shown in Table 3. The SFC of the stearin fractions indicated significant values, *viz.* 62–67 at 10°C, 39–51 at 20°C, and 21–34 at 30°C. The stearin fractions obtained at 30, 25, and 20°C are all fairly similar in properties. This suggests that the temperature of fractionation, between 30, 25, and 20°C, does not lead to significant differences in physical characteristics. The stearin fractions, by virtue of their slip melting points and SFC values, can meet the plasticity characteristic requirements for specific applications in food products like shortenings and melange composition.

It is evident that milk fat can be fractionated with the help of a biorenewable polar solvent like isopropanol to readily produce stearin and olein fractions. The fractionation has been achieved at a relatively higher temperature in a period of 1 h. The fractions have potential for utilization in making edible fat products to cater to specific food applications.

TABLE 3
Solid Fat Content of the Stearin Fractions Obtained by Isopropanol Fractionation of Butter Oil (anhydrous milk fat)

Fat/fractions[a]	Solid fat content by NMR (°C)		
	10	20	30
$BOST_{15}$	62.3 ± 0.43	50.1 ± 0.62	33.7 ± 0.49
$BOST_{20}$	67.4 ± 0.65	50.6 ± 0.82	32.5 ± 0.29
$BOST_{25}$	66.9 ± 0.78	50.1 ± 0.62	33.7 ± 0.59
$BOST_{30}$	67.5 ± 0.45	50.7 ± 1.31	33.7 ± 0.64

[a]Refer to Scheme 1 for fat/fractions. Values are mean ± SD. NMR, nuclear magnetic resonance.

REFERENCES

1. Chen, P.C., and J.M. deMan, Characterization of Milk Fat Fractions Obtained by Fractional Crystallization from Acetone, *J. Dairy Sci. 49*:612–616 (1966).
2. Schaap, J.E., H.T. Badings, D.G. Smidt, and E. Frede, Differences in Butter Fat Crystals. Crystallization from Acetone and from Melt, *Neth. Milk Dairy J. 29*:242–252 (1975).
3. Larsen, N.E., and E.G. Samuelsson, Some Technological Aspects on Fractionation of Anhydrous Butter Fat, *Milchwissenschaft 34*:663–665 (1979).
4. Arul, J., A. Boudreau, R. Makhlouf, R. Tardif, and M.R. Sahasrabudhe, Fractionation of Anhydrous Milk Fat by Supercritical Carbon Dioxide, *J. Food Sci. 52*:1231–1236 (1987).
5. Kankare, V., and V. Antila, Physical and Chemical Characteristics of Milk Fat Fractions, *Fat Sci. Technol 90*:171–174 (1988).
6. Shishikura, A., K. Fujimoto, T. Kaneda, K. Ara, and S. Saito, Modification of Butter Oil by Extraction with Super Critical Carbon Dioxide, *Agric. Biol. Chem. 50*:1209–1215 (1986).
7. Rizvi, S.S.H., Supercritical Fluid Processing of Milk Fat, *News Letter of Northeast Dairy Foods Research Centre 3*:1–5 (1991).
8. Norris, R., I.K. Gray, A.K.R. McDowell, and R.M. Dolby, The Chemical Composition and Physical Properties of Fractions of Milk Fat Obtained by a Commercial Fractionation Process, *J. Dairy Res. 38*:179–191 (1971).
9. Amer, M.A., D.B. Kuprancyz, and B.E. Baker, Physical and Chemical Characteristics of Butterfat Fractions Obtained by Crystallization from Molten Fat, *J. Am. Oil Chem. Soc. 62*:1551–1557 (1985).
10. Bhaskar, A.R., S.S.H. Rizvi, C. Bertoli, L.B. Fay, and B. Hug, A Comparison of Physical and Chemical Properties of Milk Fat Fractions Obtained by Two Processing Techniques, *Ibid. 65*:1249–1264 (1988).
11. Hartel, R.W., Application of Milk Fat Fractions in Confectionary Products, *Ibid. 73*:945–953 (1996).
12. Richardson, T., Studies on Milk Fat and Milk Fat Fractions, in *Dairy Lipids and Lipid Metabolism*, edited by M.F. Brink and D. Kritchevsky, AVI Publishing, Westport, 1968, pp. 4–14.
13. Willner, T., W. Sitzmann, and E.W. Miinch, Production of Cocoa Butter Replacers by Fractionation of Edible Oils and Fats, in *Proceedings of the World Conference on Edible Fats and Oils Processing: Basic Principles and Modern Practices*, edited by D.R. Erickson, American Oil Chemists' Society, Champaign, 1990, pp. 239–245.
14. Lusas, E.W., L.R. Watkins, and K.C. Rhee, Separation of Fats and Oils by Solvent Extraction: Non-Traditional Methods, *Ibid.*, pp. 64–70.
15. Mohamed, H.M.A., The High-Melting Glycerides in Cotton seed Oil Stearin, *Fett/Lipid 101*:20–24 (1999).
16. Mondal, K., D.K. Bhattacharyya, and M.M. Chakraborti, Prepa-

ration of Potentially Valuable Confectionary Fats from Sal Fat by Isopropanol Fractionation, *J. Oil Tech. Assoc. India 31*:115–118 (1999).
17. Mondal, K., D.K. Bhattacharyya, and M.M. Chakraborti, Fractionation of Mango Kernel Fat from Isopropanol, *Ibid. 31*:61–63 (1999).
18. Kellens, M., Developments in Fractionation Techniques, in *Treatise in Fats, Fatty Acids and Oleochemicals*, edited by O.P. Narula, Industrial Consultants, India, 1997, p. 15.
19. *Indian Standard Methods of Sampling and Tests for Oils and Fats (Revised)*. 4th Reprint, May 1975, Indian Standard Institution, New Delhi, 1964, IS:548 (Part - I) p. 33.
20. Metcalfe, L.D., and A.A. Schmitz, The Rapid Preparation of Fatty Acid Esters for Gas Chromatographic Analysis, *Anal. Chem. 33*:363–364 (1961).
21. Breitschuh, B., and F.J. Windhab, Parameters Influencing Crystallization and Polymorphism in Milk Fat, *J. Am. Oil Chem. Soc. 75*:897–898 (1998).

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