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Two-stage Extraction of Milk Fat by Supercritical Carbon Dioxide

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Abstract

To develop milk fat fractions with desirable physico-chemical properties, anhydrous milk fat(AMF) was fractionated by one- and two-stage extractions using supercritical CO_2($SC-CO_2$). Two-stage extraction of AMF was performed by first producing two fractions, an extract and a residue at 40°C/241bar, which were subsequently used as the feed for an extraction at 60°C/241bar and 40°C/345bar, and separated into five and four fractions, respectively, based on extraction time. These fractions were quantified and analyzed for fatty acids and physico-chemical properties. Short-chain(C4~C8) fatty acids in extract fractions from an extract were 200~150% compared with those of the original AMF. Long-chain(C14~C18) fatty acids in extract fractions from a residue were 118~141%. The ratio of unsaturated to saturated fatty acids in the residue fraction was 131%. Melting point ranged from 22 to 43°C, iodine value 21.8 to 36.9, and saponification value 255 to 221 in the extract and residue fractions. $SC-CO_2$ fractionation of AMF by two-stage extraction offers the possibility of developing fractions with discrete fatty acid compositions, and physico-chemical properties such as melting point, iodine value and saponification value.

Key words: milk fat, supercritical CO_2 extraction, fractionation, fatty acids

INTRODUCTION

Milk fat melts over a wide temperature range. At temperatures above +40°C, it exists as a liquid, while at -40°C, it is solid. This is due to variation in molecular weight and the degree of unsaturation of the various triglycerides present in milk fat(1). Although milk fat has excellent properties such as flavor and mouthfeel, its variable physico-chemical properties and its lack of functionality restrict its use in the food industry. There has been much interest on fractionation of anhydrous milk fat(AMF) by supercritical carbon dioxide($SC-CO_2$) to achieve the most desirable physico-chemical properties, particularly spreadability. Previous investigations have concentrated on one-stage extraction by batch and continuous systems (2-9).

The objective of this work was to develop milk fat fractions with distinct physico-chemical properties by two-stage extraction of AMF using $SC-CO_2$ at temperatures of 40 and 60°C, and pressures of 241 and 345bar.

MATERIALS AND METHODS

Commercial grade of butter was converted into AMF by melting at 60°C and filtering through Whatman No. 1 filter paper. AMF was subsequently stored at -20°C for future use.

A batch type supercritical fluid extraction system(Fig. 1) was operated for fractionation of AMF. The extraction vessel(300ml) was heated to the desirous temperature(40 or 60°C), milk fat(20g) was charged into the extraction vessel and then CO_2 was admitted. The system was pressurized to the desirous level(241 or 345bar). Extraction was begun by opening the metering valve and permitting $SC-CO_2$ to flow through milk fat in the extraction vessel. The components of the fat dissolved in $SC-CO_2$ were passed through the metering valve and into the separation vessel. Pressure reduction caused the soluble glycerides to precipitate and collect in the separation vessel. The volume of CO_2 used was determined prior to venting. The collected material in the separation vessel was removed and quantified every hour. At the end of the process, the residue was recovered from the bottom of the extraction vessel. The flow rate of CO_2 was 4L/min. Extract fractions of each step were referred to as E and residues as R.

Two-stage extraction of AMF was performed by first producing two fractions, an extract and a residue at 40°C/241bar. These extract and residue fractions were subse-

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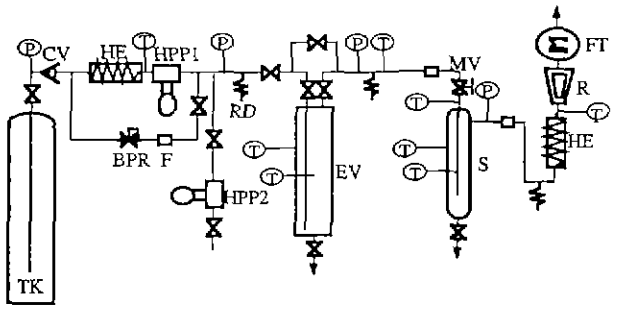

Fig. 1. Flow diagram of supercritical fluid extraction system.
BPR: back pressure regulator, CV: check valve, EV extraction vessel, F: filter, FT: flow totalizer, HE: heat exchanger, HPP: high pressure pump, MV: metering valve, P: pressure gauge, R: rotameter, RD: rupture disk, S: separator, T: temperature indicator, TK: carbon dioxide tank.

quently used as the feed to the extraction vessel maintained at 60°C/241bar and 40°C/345bar, and separated into five and four fractions, respectively, based on extraction time. These fractions were quantified and analyzed for fatty acids and physico-chemical properties.

Fatty acid composition of AMF and its SC-CO_2 fractions were analyzed by a GC fitted with a flame ionization detector(HP 5890 series II, Hewlett Packard, Palo Alto, CA, USA). Fatty acids were converted to methyl esters (10,11) and analyzed using a capillary column, DB™-WAX capillary(30m×0.25mm i.d., Supelco, Inc., Bellefonte, PA, USA). Oven temperature was held at 50°C for 4min, then increased at 10°C/min to 250°C and held for 2min. Injector and detector temperatures were 250 and 300°C, respectively. Carrier gas was nitrogen at 1.3ml/min. Injection volume of sample was 1μl. Split ratio was 1 : 100. Physico-chemical properties such as melting point, iodine value and saponification value were analyzed according to the AOCS methods(10).

RESULTS AND DISCUSSION

AMF was extracted by SC-CO_2 at 40°C/241bar(one-stage extraction trial) and fatty acid composition of the fractions obtained is shown in Table 1. The fractions removed earliest from the extraction vessel(E1) contain more of short-chain fatty acids(SCFA, C4~C8) of milk fat. Fractions(E2, E3) removed later in the process have fatty acid compositions more concentrated in long-chain fatty acids(LCFA, C16~C18). The residual fat(R) remaining in the vessel after extraction is depleted in SCFA,

Table 1. Fatty acid composition(mol%) of AMF and its SC-CO_2 fractions obtained in one-stage extraction at 40°C/241bar

Fractions	AMF	E1	E2	E3	R
Extraction time(hr)	0	1	2	3	3
Fat yield(wt%)	100	10.4	18.7	12.1	58.8
C4 : 0	7.3	12.2	11.6	10.7	5.3
C6 : 0	3.7	4.6	3.7	4.2	2.5
C8 : 0	2.0	2.5	2.5	2.1	1.5
C10 : 0	4.4	4.8	5.1	3.9	3.7
C12 : 0	4.7	4.8	5.4	3.9	4.1
C14 : 0	12.7	12.4	13.8	11.9	12.4
C16 : 0	27.6	27.2	27.1	27.8	28.4
C16 : 1	1.6	1.4	0.9	1.6	1.5
C18 : 0	10.8	8.7	8.8	10.0	12.2
C18 : 1	22.2	18.7	18.3	21.0	25.1
C18 : 2	2.4	2.3	2.3	2.5	2.5
C18 : 3	0.6	0.4	0.5	0.5	0.7

E : extract fraction, R: residue

resulting in higher melting components. Again, the extracted fractions(E1-E3) were slightly reduced in the unsaturated fatty acids. However, fatty acid profiles of the fractions did not show much differences from those of the original AMF, with the extracted fractions being enriched slightly in shorter chain fatty acids and depleted in longer chain fatty acids. SC-CO_2 in one-stage extraction was less able to fractionate milk fat into distinct differences in fatty acid composition among the extracted fractions. A multistage fractionation process should be taken into account.

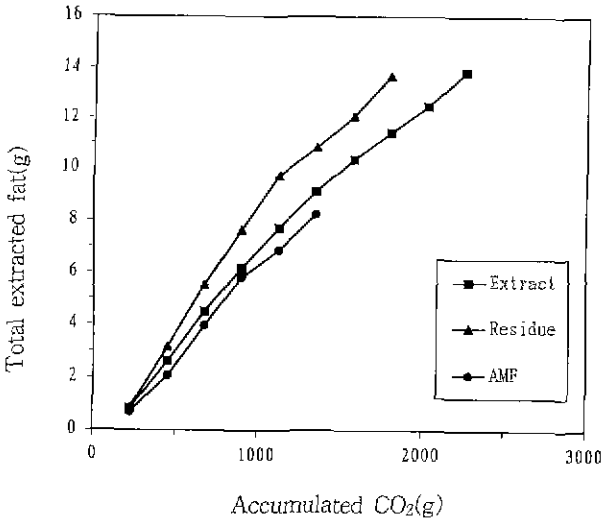

Fig. 2. Extraction curves of AMF at 40°C/241bar, of an extract at 60°C/241bar, and of a residue at 40°C/345bar by SC-CO_2.

Two-stage extraction and fractionation was performed using an extract and a residue obtained in one-stage extraction as the feed at 60°C/241bar and 40°C/345 bar, respectively. Fig. 2 is the extraction curves showing amount of fat extracted as a function of cumulative amount of CO_2 passed through the extraction vessel. The shape of the extraction curves was similar for all cases. As can be anticipated, total extracted fat is enhanced by an increase in consumption of CO_2. For longer extraction time, non-linear profiles can be expected as the more soluble components are depleted. Such extraction behavior has been previously observed for milk fat extraction in SC-CO_2(2-9).

The initial lag period in the extraction curves occurred because CO_2 which was the first to exit the extraction system did not contact milk fat under experimental conditions and carried little solubilized milk fat. Extract loading of the original AMF in SC-CO_2 was 0.645% at 40°C/241bar(ρ=0.873g/cm^3). AMF was divided into two fractions (an extract and a residue) at a weight ratio of 41.2 to 58.8 in one-stage extraction trial. Extract loadings of an extract in SC-CO_2 at 60°C/241bar(ρ=0.775g/cm^3) and of a residue in SC-CO_2 at 40°C/345bar(ρ=0.933g/cm^3) were 0.686 and 0.847% in two-stage extraction trial.

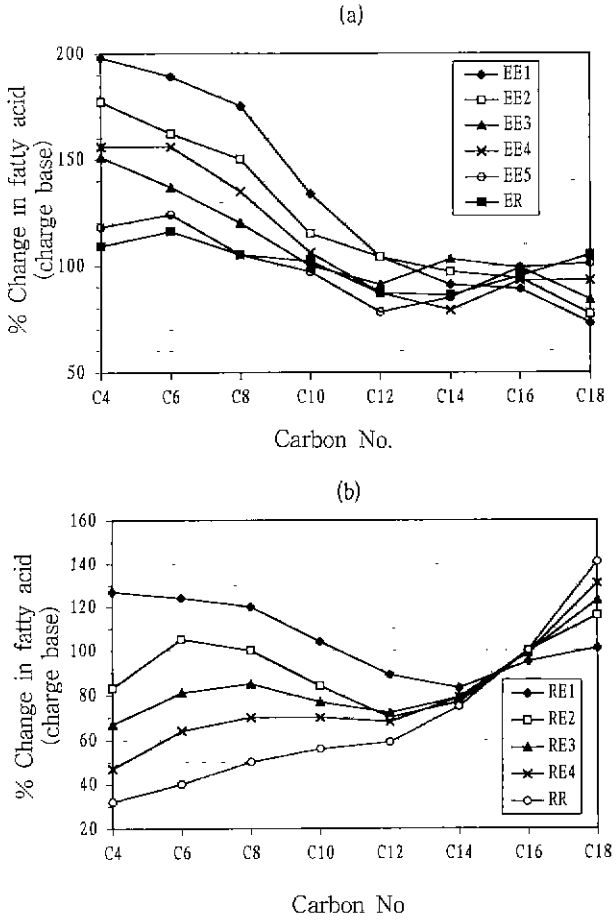

Fig 3. Distribution of fatty acids in the fractions obtained in two-stage extraction trial from an extract(a) and a residue(b).
EE and ER: extract fractions and residue obtained in two-stage extraction trial from an extract in one-stage-extraction trial, RE and RR: extract fractions and residue obtained in two-stage extraction trial from a residue in one-stage-extraction trial.

Fig. 3 shows distribution of fatty acids in the fractions obtained in two-stage extraction trial from an extract(a) and a residue(b) in one-stage extraction trial, respectively in comparison with those of the original AMF. In two-stage extraction trial, the second extraction(EE) from an extract was performed at the same pressure(241bar) as one-stage extraction trial, but at a higher temperature (60°C) in order to remove the high molecular weight triglycerides as the second residue fraction(ER). Likewise, the second extraction from a residue was performed at the same temperature(40°C) as one-stage extraction trial, but at a higher pressure(345bar) in order to remove the relatively low molecular weight triglycerides as the extract fractions(RE). This is possible because the fractionation of milk fat by SC-CO_2 is caused by a large disproportion in solubilities of triglycerides.

Extract fractions(EE1~EE4) from an extract were much enriched in SCFA(Fig. 3(a)), while extract fractions (RE2~RE4) from a residue(Fig. 3(b)) enriched in LCFA in two-stage extraction trial compared with one-stage extraction trial. SCFA in the extract fractions(EE1~EE4) were 200~150% compared with those of the original AMF. In the EE1 fraction, SCFA are twice as high as in the original AMF. This means that each fifth fatty acid in that fraction is SCFA. This is important because the SCFA are the acids using the direct way to the liver via the portal vein(12). LCFA in the fractions(RE2~RR) from a residue were 118~141% compared with those of the original AMF. More SCFA and MCFA shifted towards the extract fractions and more LCFA towards the residue fractions in two-stage extraction trial compared with one-stage extraction trial.

Fig. 4 shows long-chain saturated and unsaturated fatty acid profiles of SC-CO_2 fractions in comparison with those of the original AMF. Extract fractions(EE1~EE4) from an extract were much depleted in long-chain saturated and unsaturated fatty acids, while extract fractions(RE2~RE4) from a residue were much enriched in long-chain unsaturated fatty acids in two-stage extraction trial compared with one-stage extraction trial. The

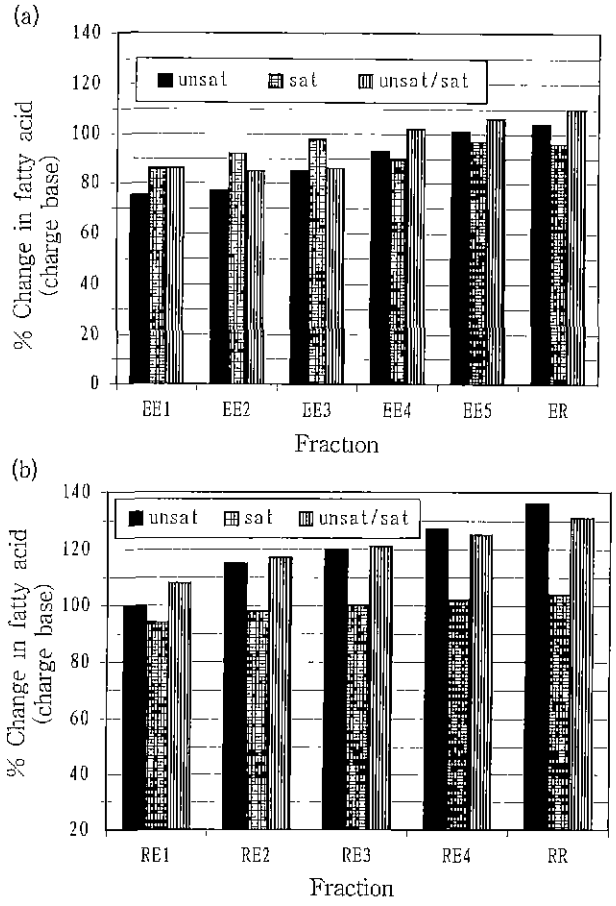

Fig. 4. Distribution of long-chain saturated and unsaturated fatty acids in the fractions obtained in two-stage extraction trial from an extract(a) and a residue(b).
EE and ER: extract fractions and residue obtained in two-stage extraction trial from an extract in one-stage extraction trial, RE and RR: extract fractions and residue obtained in two-stage extraction trial from a residue in one-stage extraction trial.

unsaturated fatty acids were primarily retained in the later fractions and the residue. The ratio of long-chain unsaturated to saturated fatty acids(unsat/sat) in the residue fraction(RR) was 131% compared with those of the original AMF. Therefore, SC-CO_2 primarily separates fatty acids based on molecular size and not based on degree of saturation in the extraction of AMF.

In the range of temperature and pressure studied, fractionation of milk fat in SC-CO_2 followed the expected trends, based on fatty acid composition. Similar trends have been shown previously for fractionation of milk fat by SC-CO_2(2-9). However, SC-CO_2 fractionation of AMF by two-stage extraction studied in this research offers the possibility of altering the fatty acid compositions in the fractions by concentrating SCFA and MCFA in the extract fractions and maximizing long-chain unsaturated fatty acids in the residue fractions.

Table 2. Selected physico-chemical properties of the fractions obtained in two-stage extraction from an extract at 60°C/241bar

Fractions	Fat yield (wt%)	Melting point (℃)	Iodine value	Saponification value
AMF	–	37	31.0	242
TE	100	33	27.2	237
EE1	13.1	22	21.8	255
EE2	17.9	25	22.8	256
EE3	14.7	27	21.4	244
EE4	11.5	32	26.5	246
EE5	12.2	33	30.3	236
ER	30.7	35	31.3	233

EE and ER : extract fractions and residue obtained in two-stage extraction trial from an extract(TE) in one-stage-extraction trial

Table 3. Selected physico-chemical properties of the fractions obtained in two-stage extraction from a residue at 40°C/345bar

Fractions	Fat yield (wt%)	Melting point (℃)	Iodine value	Saponification value
AMF	–	37	31.0	242
TR	100	39	21.1	221
RE1	15.8	36	28.9	213
RE2	22.4	36	32.1	234
RE3	15.9	39	34.0	228
RE4	14.4	40	36.3	227
RR	31.5	43	36.9	221

RE and RR : extract fractions and residue obtained in two-stage extraction trial from a residue(TR) in one-stage-extraction trial

Table 2 and 3 show selected physico-chemical properties of AMF and its SC-CO_2 fractions obtained in two-stage extraction trial. Due to the fractionation effect of milk fat by SC-CO_2, physico-chemical properties of the fractions are expected to be changed. Melting point ranged from 22°C in EE1 to 43°C in RR, showing a difference of 21°C compared with 37°C in the original AMF. The earlier fractions removed from the extraction vessel, which were enriched in SCFA and depleted in LCFA, were made up of low melting components of milk fat. This trend was reversed for the later fractions and the residue left in the extractor. Iodine values, reflecting the degree of unsaturation of fats, ranged from 21.8 to 36.9 compared with 31.0 in the original AMF. The iodine value of the fractions increased as the extraction proceeded. This is in agreement with the changes in unsaturated fatty acid composition shown in Fig. 4. Saponification

values, varing inversely with molecular weight, ranged from 255 to 221 compared with 242 in the original AMF. Saponification value decreased as the extraction proceeded. This agreed with the trends observed in the melting point.

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REFERENCES

1. Rajah. K. K. : Milk fat products. *J. Soc. Dairy Technol.*, **39**, 104(1986)
2. Arul, J., Boudreau, A., Makhlouf, J., Tardif, R. and Sahasrabudhe, M. R. : Fractionation of anhydrous milk fat by supercritical carbon dioxide. *J. Food Sci.*, **52**, 1231(1987)
3. Kankare, V., Antila, V., Harvala, T. and Komppa, V. : Extraction of milk fat with supercritical carbon dioxide. *Milchwissenschaft*, **44**, 407(1989)
4. Bradley, Jr. R. L. : Removal of cholesterol from milk fat using supercritical carbon dioxide. *J. Dairy Sci.*, **72**, 2834 (1989)
5. Rizvi, S. S. H , Lim, S., Nikoopour. H , Singh, M. and Yu, Z. Supercritical fluid processing of milk fat. In "*Engineering and Food*" Spiess, W. L. E. and Schubert, H. (eds.), Elsevier Applied Science, New York, Vol. 3, p.145(1989)
6. Hammam, H., Soderberg, I. and Sivik, B. : Physical properties of butter oil fractions obtained by supercritical carbon dioxide extraction. *Food Sci. Technol.*, **93**, 374(1991)
7. Chen, H., Schwartz, S. J. and Spanos, G. A. · Fractionation of butter oil by supercritical carbon dioxide. *J. Dairy Sci.*, **75**. 2659(1992)
8. Yoon, J., Hartel. R. W. and Wang, Y-C. · Analysis of butterfat extraction and fractionation using supercritical carbon dioxide. *J. Food Proc. Preservation*, **17**, 471(1993)
9. Lim, S. and Rizvi, S. S. H. : Continuous supercritical fluid processing of AMF in a packed column. *J. Food Sci.*, **60**, 889(1995)
10. A.O.C.S. : *Official Methods and Recommended Practices of the American Oil Chemists' Society*. 4th ed., American Oil Chemists' Society, Champaign. Illinois, Cc1-25, Cd1-25, Cd3-25(1990)
11. A.O.A.C. : *Official Methods of Analysis of AOAC International*. 16th ed., AOAC International, Arlington, Virginia, 33.6.13(1995)
12. Timmen, H., Frede. E. and Precht, D. : Characterization of short and long-chain butterfat fractions obtained with SC-CO_2. In "*Milk Fat and its Modification*" Marcuse, R. (ed.), Scandinavian Forum for Lipid Research and Technol., Goteborg, p.92(1984)

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