Supercritical fluid fractionation of fatty acid ethyl esters from butteroil

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ABSTRACT

Countercurrent supercritical fractionation of the fatty acid ethyl esters from butteroil has been investigated. The main objective of the present study was to obtain extracts rich in short- and medium-chain fatty acid ethyl esters. To that end, transesterification of the original butteroil was used to transform the triacylglycerols into the corresponding fatty acid ethyl esters. Then, several supercritical fluid extractions were carried out at pressures ranging from 8.9 to 18.6 MPa and at 2 different temperatures (48 and 60°C). The flow ratio of CO₂ to butteroil was 15. Composition and yield of short- and medium-chain fatty acid ethyl esters was evaluated at different extraction conditions. Extracts containing ~70% short- and medium-chain fatty acid ethyl esters were obtained at 101 bar and 60°C, and can be used as starting material for the production of highly valuable functional lipids.

Key words: fatty acid ethyl ester, fractionation, short- and medium-chain fatty acid, butteroil

INTRODUCTION

Historically, the goal of agricultural and food research has been to increase yield and productive efficiency, with little focus given to improving the nutrient profile of food products. Mounting research evidence and consumer awareness of the potential health benefits of various microcomponents in foods has given rise to the concept of functional foods and helped create a demand for foods with improved nutrient profiles (Miller, 1999; Lock and Bauman, 2004). Thus, producers and scientists are interested in research and agricultural practices that may improve the nutrient profile of food products (Committee on Opportunities in Agriculture, 2003). One example of this is the dairy industry and previously reported efforts to modify the composition of milk fat (Kaylegian, 1999).

Several attempts of butteroil fractionation have been previously reported (Augustin and Versteeg, 2006).

However, most of these procedures focus on maintaining milk fat identity and the individual fatty acid compositions of all fractions do not vary greatly from the original milk fat.

Supercritical CO₂ extraction may be used in batch or continuous systems to fractionate anhydrous milk fat into fractions with specific properties to enhance its use (Kaufmann et al., 1982; Arul et al., 1987; Bhaskar et al., 1993). The melting properties of the fractions obtained by supercritical CO₂ fluid extraction are not as pronounced as with melt crystallization. Nevertheless, niche applications could be developed if fractions rich in short-chain fatty acids (SCFA) or a solid fraction high in unsaturated fatty acids are required.

There are many studies dealing with the role of medium-chain fatty acids (MCFA) as well as several reviews in this field (Bach and Babayan, 1982; Pfeuffer and Schrezenmeir, 2002; St-Onge and Jones, 2002). Most attention has focused on the potential role of MCFA for weight management (St-Onge, 2005). Several aspects of MCFA metabolism that affect features of the metabolic syndrome such as plasma lipid levels, insulin resistance, inflammatory response, as well as weight management have been also described (Marten et al., 2006).

Recently, it has been proposed that consumption of a functional oil rich in phytosterols and medium-chain triglyceride oil improves plasma lipid profiles (St-Onge et al., 2003). In addition, butyric acid could also have some potential as anticarcinogenic agent although further studies are still necessary (Parodi, 1997, 2005).

Although butteroil is a relatively expensive starting material, other inexpensive natural sources can be used to obtain fractions enriched in MCFA. However, the unique fatty acid composition of butteroil provides an excellent starting material for the production of extracts highly enriched not only in MCFA but also in SCFA.

It should be considered that one-third of milk fat contains one molecule of butyrate located at the sn-3 position, and that more than 50% of total triacylglycerols in butteroil contain at least one SCFA or MCFA (Jensen, 2002). Hence, fractionation of triacylglycerols from butteroil does not permit one to obtain products highly enriched in SCFA and MCFA for nutritional applications. Therefore, in the first step, fatty acid

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residues from milk fat should be transformed into their corresponding fatty acid ethyl esters to facilitate the fractionation of this product based on the different physical and chemical properties of individual fatty acid constituents.

Based on those premises, the aim of the present study is to effectively fractionate butteroil based on the individual fatty acid types included in this fat via countercurrent CO₂ extraction at pressures ranging from 8.9 to 18.6 MPa and at 2 different temperatures (48 and 60°C). Composition and yield of extracts and raffinate were used to determine the best extraction conditions.

Using this methodology, fractions as high as 70% of SCFA and MCFA ethyl esters were obtained. These fractions are intended to be used as starting material for the production of highly valuable functional lipids for nutritional applications.

MATERIALS AND METHODS

Materials

Butteroil was kindly donated by Industrias Lacteas Asturianas S.A. (Reny Picot, Navia, Spain). Carbon dioxide (99.98%) was purchased from AL Air Liquide España S.A. (Madrid, Spain). All solvents used were HPLC grade from Lab-Scan (Dublin, Ireland).

Ethanolysis of Butteroil

Butteroil was mixed with sodium ethoxide (5.25% wt/vol) in absolute ethanol at a ratio of 4 to 1 (vol/vol). The mixture was stirred for 30 min at 60°C and then washed twice with distilled water. The volume used in these 2 washings was half of the volume of butter oil. After the second washing, the mixture was centrifuged at $585 \times g$ for 10 min. Finally, the product of the ethanolysis reaction was dried with sodium sulfate and vacuum filtrated. The composition of the final product is shown in Table 3.

Supercritical Fluid Extraction Equipment and Extraction Method

Figure 1 shows a flow diagram of the countercurrent supercritical fluid extraction system employed in this study. The countercurrent extraction column (316 stainless steel) is 100 cm $\times$ 12 mm i.d. and is packed with Fenske rings (3 $\times$ 0.5 mm). The countercurrent supercritical fluid extraction device also includes 2 separator cells (S1 and S2) of 270 mL capacity each (where a cascade decompression takes place) and a cryogenic trap at atmospheric pressure. Both CO₂ and liquid feed sample were preheated at the exit of their respective

pumps (Dosapro Milton Roy Ibérica, Madrid, Spain) before introduction into the extraction column. All units were equipped with electrical thermostats. The device has computerized programmable logic controller-based instrumentation and a control system with several safety devices including valves and alarms.

During the extraction, a continuous flow of CO₂ was introduced into the column through the bottom. When the operating pressure and temperature were reached, the liquid sample was pumped (100 mL/h) from the top during the entire extraction time (60 min).

The first separator was maintained at 6 MPa and 20°C and the second separator cell was maintained at low pressure and temperature (2 MPa and 10°C). The raffinate and liquid fractions collected in the separators were weighted and analyzed. The material balance closed in all experiments with an inaccuracy <7.4%.

Gas Chromatography

For the analysis of fatty acid ethyl esters, 0.2 μ L of a sample solution at a concentration of $\sim$ 4 mg/mL of chloroform was injected into a Hewlett-Packard 5890 series II gas chromatograph with on-column injection using a 7-m 5% phenyl methyl silicone capillary column (Quadrex Corporation, New Haven, CT; 0.25 μ m i.d.). A section of a deactivated column (12 cm $\times$ 530 μ m i.d.) was used as precolumn. Injector and detector temperatures of 43°C and 360°C, respectively, were used. The temperature program was as follows: starting at 40°C and then heating to 250°C at 42°C/min with a 10-min hold, followed by heating from 250°C to 325°C at 7.5°C/min with a 20-min hold. Helium was used as a carrier gas at a pressure of 0.036 MPa. The peaks were computed using GC ChemStation software (Agilent Technologies, Santa Clara, CA).

RESULTS AND DISCUSSION

Extraction Conditions and Yield

Fatty acid ethyl esters from butteroil were effectively fractionated via countercurrent CO₂ extraction at pressures ranging from 8.9 to 18.6 MPa and at 2 different temperatures (48 and 60°C). These extraction conditions were selected to find a broad range of extraction conditions based on the mass balance between extracts and raffinates; in other words, we explored extraction conditions from those that produce a small percentage of extract to those that produce an almost complete extraction of fatty acid ethyl esters. To that end, the use of different densities of supercritical CO₂ was used. The density of supercritical CO₂ can be changed by varying the pressure and the temperature. The higher

Figure 1. Experimental countercurrent supercritical fluid extraction equipment. The device comprises a 100-cm high column (12 mm internal diameter) packed with Fenske rings, 2 separator cells of 270-mL capacity each (S1 and S2) and a cryogenic trap at atmospheric pressure. Both CO₂ and liquid feed sample were preheated at the exit of their respective pumps before introduction into the extraction column.

the temperature, the lower the density of CO₂ attained. The opposite effect is observed with pressure. The range of densities of CO₂ used in the present study was from 0.3 to 0.7 g/mL.

It should be noted that at densities of CO₂ of 0.3 g/mL (8.9 MPa and 48°C and 10.1 MPa and 60°C), approximately 10% (wt/wt) of the material introduced into the column was extracted. On the contrary, no raffinate was obtained using a density of CO₂ of 0.7 g/mL (18.6 MPa and 60°C.)

It should be also noted that the mass balance for the shortest-chain fatty acid ethyl ester (ethyl butyrate) was lower (50–70%) than that of the rest of the fatty acid ethyl esters (~100%). Although the depressurization of the extracts takes place in cascade in 2 consecutive steps (7 MPa and 20°C for the first separation cell and 2 MPa and 10°C for the second separation cell), this fatty acid ethyl ester has a relatively high vapor pressure even at 2 MPa and 10°C, precluding its complete recovery.

Table 1. Yield in weight percentage (% wt/wt) of extracts obtained by supercritical fluid fractionation

Item	Extraction experiment														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Conditions															
Pressure (MPa)	8.9	8.9	9.8	10.5	10.5	11.7	14.2	10.1	10.1	10.1	11.5	11.5	12.9	14.9	18.6
Temperature (°C)	48	48	48	48	48	48	48	60	60	60	60	60	60	60	60
Yield															
C4	65.2	65.6	74.9	46.4	76.5	72.5	81.3	60.8	58.6	59.0	63.1	72.3	75.8	67.4	66.7
C6	69.1	65.6	83.0	70.8	92.0	83.0	87.3	76.6	77.1	56.6	84.1	88.7	92.3	82.5	88.6
C8	41.2	37.8	62.0	88.0	99.1	93.1	96.5	47.3	50.3	35.4	81.3	80.1	99.4	91.8	100.5
C10	23.3	20.1	37.1	87.7	78.9	93.4	97.4	25.3	27.0	20.9	49.3	49.3	87.3	91.9	102.6
C12	13.5	11.7	22.8	64.5	52.6	92.3	98.3	14.3	15.4	10.7	29.8	30.3	60.2	89.9	100.4
C14	7.5	7.9	13.6	45.1	34.4	86.3	97.8	9.2	7.8	5.1	18.3	19.4	40.7	80.1	99.8
C16	4.0	5.4	7.7	31.3	21.3	68.5	97.1	5.3	3.8	2.5	11.9	11.4	26.3	63.0	98.0
C18	2.3	4.1	4.4	21.2	13.2	50.8	96.7	3.2	2.1	1.4	7.5	6.4	17.5	49.1	96.5
C18:1-C18:2	2.6	4.1	4.9	23.5	14.6	55.0	97.2	3.5	2.2	1.6	8.1	7.1	19.1	52.2	97.3

Table 2. Yield in weight percentage (% wt/wt) of raffinates obtained by supercritical fluid fractionation of butteroil

Item	Extraction experiment													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Conditions														
Pressure (MPa)	8.9	8.9	9.8	10.5	10.5	11.7	14.2	10.1	10.1	10.1	11.5	11.5	12.9	14.9
Temperature (°C)	48	48	48	48	48	48	48	60	60	60	60	60	60	60
Yield														
C4	7.9	2.3	10.3	0.0	0.0	0.0	0.0	11.2	0.0	0.0	0.0	0.0	6.7	0.0
C6	13.1	19.5	4.4	0.0	0.0	0.0	0.0	10.5	6.4	21.9	0.0	0.0	0.0	0.0
C8	56.0	58.3	32.7	0.0	0.0	0.0	0.2	49.6	42.4	57.1	11.6	16.8	0.0	0.0
C10	73.9	75.8	60.7	0.0	20.7	0.0	0.3	71.5	68.9	76.6	46.0	48.5	11.7	1.1
C12	83.5	83.7	76.2	30.6	49.8	1.3	0.3	81.3	81.7	89.3	66.5	67.8	38.6	2.9
C14	87.8	86.9	84.5	52.7	67.4	9.1	0.3	86.8	90.8	95.2	77.1	77.7	56.7	14.5
C16	90.8	89.4	90.9	67.4	79.3	29.8	0.4	91.6	94.9	97.6	83.8	86.7	69.2	35.0
C18	94.3	91.9	94.8	76.8	88.4	49.0	0.4	94.2	95.8	100.0	88.7	92.7	77.4	51.4
C18:1-C18:2	93.8	90.9	94.3	75.1	85.4	44.9	0.4	94.2	96.4	97.9	87.6	91.6	76.1	48.3

To check the reproducibility of the methodology used for the fractionation, some of the extraction experiments are depicted also in Tables 1, 2, 3, and 4. Inspection of these experiments (e.g., extractions 1, 2, and 3, or extractions 4 and 5 in Table 3) indicates some fluctuation of the composition and yields, especially for SCFA. In these experiments, the percentage of C4 fluctuates from 18 to 24% in extractions 1, 2, and 3, and from 4 to 9% in extractions 4 and 5. These fluctuations can be attributed to the high volatility of these compounds; they are partially volatilized during the time they are precipitating in the extraction cells.

Another important aspect of fractionation is to determine how the different fatty acid ethyl esters are partitioned between extracts and raffinates. Figure 2 shows the trend of the composition for the different fatty acid ethyl esters versus pressure used in the supercritical CO₂ fractionation. Examination of Figure 2 indicates that approximately 50% of C14 ethyl ester is extracted at 11.5 MPa. To completely extract all C14 ethyl ester, pressures of 15.0 MPa should be used.

On the contrary, C16 ethyl ester is accumulated in the raffinates at pressures lower than 13.0 MPa and

it is effectively extracted at 15.0 MPa. The C18:1 and C18:2 ethyl esters accumulate in the raffinates even at a pressure of 15.0 MPa, which indicates that at these extraction conditions they could be effectively fractionated from shorter-chain fatty acid ethyl esters.

Figure 2 also indicates that at 60°C, the extraction of SCFA and MCFA takes place even at the lowest pressure investigated. However, to efficiently extract these fatty acids, pressure up to 12.9 MPa should be used. Unfortunately, at these extraction conditions, significant amounts of C14 and C16 ethyl esters are also extracted. For that reason, it is necessary to strike a balance between composition and yield to obtain extract highly enriched in SCFA and MCFA ethyl esters and acceptable yields.

Compositions of the Supercritical Fractionation of Butteroil Ethyl Esters

It can be observed in Table 3 that the extracts enriched in SCFA and MCFA ethyl esters are obtained at 8.9 MPa and 48°C (extractions 1 and 2) and 10.1 MPa and 60°C (extractions 8, 9, and 10). At these 2 extrac-

Table 3. Composition (% wt/wt) of fatty acid ethyl esters from original butteroil and extracts obtained by supercritical fluid fractionation

Item	Butteroil	Extraction experiment														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Conditions																
Pressure (MPa)		8.9	8.9	9.8	10.5	10.5	11.7	14.2	10.1	10.1	10.1	11.5	11.5	12.9	14.9	18.6
Temperature (°C)		48	48	48	48	48	48	48	60	60	60	60	60	60	60	60
Composition																
C4	3.2	24.3	22.2	18.3	4.4	9.1	3.5	2.7	19.9	21.6	28.1	12.0	13.8	7.7	3.5	2.2
C6	2.3	18.0	15.5	14.2	4.7	7.7	2.8	2.0	17.5	19.8	18.9	11.2	11.9	6.5	3.0	2.1
C8	1.4	6.5	5.4	6.4	3.5	5.0	1.9	1.4	6.5	7.8	7.1	6.5	6.5	4.3	2.0	1.4
C10	3.4	9.2	7.2	9.5	8.7	9.9	4.8	3.4	8.7	10.5	10.5	9.9	9.9	9.3	5.0	3.6
C12	3.7	5.7	4.5	6.4	6.9	7.2	5.1	3.8	5.3	6.4	5.8	6.5	6.6	7.0	5.3	3.8
C14	11.7	10.1	9.6	12.0	15.3	14.8	15.2	11.9	10.9	10.3	8.7	12.6	13.4	14.9	15.0	12.0
C16	27.7	12.8	15.7	16.2	25.3	21.8	28.6	27.9	15.0	11.9	10.2	19.4	18.7	22.9	27.9	28.1
C18	8.8	2.3	3.7	2.9	5.4	4.3	6.7	8.8	2.9	2.1	1.8	3.9	3.3	4.8	6.9	8.7
C18:1-C18:2	36.9	10.9	15.8	13.7	25.2	19.8	30.6	37.1	13.0	9.3	8.6	17.6	15.5	22.0	30.8	37.1

Figure 2. Composition of fatty acid ethyl esters in the raffinates versus pressure at 60°C for the different fatty acid ethyl esters from butteroil.

tion conditions (CO_2 density 0.3 g/mL), ~20% of C4 was obtained in the extracts. Considering the original percentage of C4 (3%) in butteroil, it can be seen that this fatty acid ethyl ester is effectively fractionated.

The composition of extracts and raffinates are shown in Table 3 and 4 respectively. At the lowest density of CO_2 studied (0.3 g/mL), only ~10% of short-chain fatty acid ethyl esters were found in the raffinates.

To calculate the extent of the fractionation obtained, it is very useful to use the concept of enrichment factor. This factor, obtained as the ratio of composition in the extract versus composition in the original butteroil ethyl ester mixture, was found to be >7 for C4 and C6 ethyl ester. This result indicates the efficiency of the fractionation carried out and also that similar frac-

tionation could not be reached if the original butteroil in triacylglycerol form is used. By this methodology, it is possible to obtain extracts as rich as 70% (wt/wt) of SCFA and MCFA ethyl esters with an adequate percentage of recovery (greater than 80%).

CONCLUSIONS

The present study describes a very useful methodology for the fractionation of fatty acid ethyl esters from butteroil. Transformation of triacylglycerols into their corresponding fatty acid ethyl esters permits one to obtain extracts highly enriched in SCFA and MCFA ethyl esters (up to 70%) with good yields (greater than 80%). Very mild extraction conditions were found to

Table 4. Composition (% wt/wt) of raffinates obtained by supercritical fluid fractionation of butteroil

Item	Extraction experiment													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Conditions														
Pressure (MPa)	8.9	8.9	9.8	10.5	10.5	11.7	14.2	10.1	10.1	10.1	11.5	11.5	12.9	14.9
Temperature (°C)	48	48	48	48	48	48	48	60	60	60	60	60	60	60
Composition														
C4	0.3	0.1	0.4	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.3	0.0
C6	0.3	0.5	0.1	0.0	0.0	0.0	0.1	0.3	0.2	0.5	0.0	0.0	0.0	0.0
C8	0.9	0.9	0.5	0.0	0.0	0.0	0.7	0.8	0.7	0.9	0.2	0.3	0.0	0.0
C10	2.9	3.1	2.4	0.0	1.0	0.0	2.7	2.8	2.7	2.9	2.0	2.1	0.6	0.1
C12	3.6	3.7	3.3	1.8	2.5	0.2	3.4	3.5	3.4	3.6	3.2	3.1	2.2	0.3
C14	11.9	12.0	11.6	10.1	10.9	3.5	11.8	11.8	12.1	12.2	11.7	11.3	10.5	5.0
C16	29.2	29.4	29.7	30.6	30.4	26.9	29.7	29.5	30.0	29.7	30.0	30.0	30.3	28.3
C18	9.6	9.6	9.8	11.0	10.7	14.0	9.8	9.6	9.6	9.6	10.1	10.1	10.7	13.1
C18:1-C18:2	40.1	39.7	41.0	45.3	43.5	53.9	40.9	40.3	40.5	39.6	41.7	42.1	44.2	51.8

be effective for fractionation of SCFA and MCFA ethyl esters (8.9 MPa and 48°C). At these extraction conditions, enrichment factors for C4 and C6 ethyl ester were found to be greater than 7. Density of supercritical CO₂ plays an important role in the degree of fractionation of the different fatty acid ethyl esters from butteroil. It is possible to establish the extraction conditions to fractionate the fatty acid ethyl esters as a function of their chain length to obtain different extracts and raffinates with differing compositions and nutritional value.

The present methodology is intended to be used to isolate fractions enriched in SCFA and MCFA ethyl esters for the production of functional lipids. To improve the economical feasibility of the process, the raffinates obtained in the present study could be also used for other industrial applications such as biodiesel.

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