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ORIGINAL PAPER

Fractionation of Short and Medium Chain Fatty Acid Ethyl Esters from a Blend of Oils via Ethanolysis and Short-Path Distillation

Luis Vázquez · Casimir C. Akoh

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Abstract The main objective of the present work was to obtain fractions enriched in short (SCFAEE) and medium chain (MCFAEE) fatty acid ethyl esters (FAEE) from a blend of oils. For this purpose, coconut oil and dairy fat were blended and a transesterification of this blend was performed in order to transform the triacylglycerols into their corresponding FAEE. A detailed study of fractionation conditions for FAEE was carried out using short-path distillation. Different conditions were explored such as feed rates and temperatures; operating conditions, such as successive steps or a single step. The composition and yield of SCFAEE and MCFAEE were determined under different distillation conditions. Optimal fractionation of the desired compounds was achieved. High purity of SCFAEE and MCFAEE (94 wt%) was attained with a yield of ~50%. An increased yield of SCFAEE and MCFAEE was achieved (85%) with a purity of 83 wt%. A broad range of high value fractions was obtained depending on the conditions selected. The fractions we obtained can be used as starting materials for the production of functional lipids.

Keywords Short and medium chain fatty acids · Short-path distillation · Fractionation · Fatty acid ethyl esters · Ethanolysis

Introduction

There have been several studies as well as a number of reviews reporting the use of medium chain triacylglycerols (MCTs) and short (SCFA) and medium chain fatty acids (MCFA) [1–3]. MCTs are triacylglycerols with all three positions of the glycerol molecule esterified with C6:0–C12:0 fatty acids, also known as medium chain fatty acids. SCFA, which range from 2 to 6 carbons long, have also been reported to possess bioactive properties. Normally, SCFA and MCFA are minor dietary components. However, consumption of these compounds was reported to provide a rapid source of energy that is readily digested and easily absorbed. MCTs are rapidly absorbed and transported to the mitochondria where they are utilized for fuel and may be less likely stored as body fat. MCFA do not require chylomicron formation and they are transported back to the liver directly by the portal system [4]. MCFA may actually reduce fasting lipid levels more than oils rich in mono- or poly-unsaturated fatty acids and recent studies confirmed the potential of MCFA to reduce body weight and particularly body fat [5]. MCTs are used as a part of the fat blend for liquid diet formulas intended for patients with impaired digestion or diverse medical conditions requiring fluid restriction, such as AIDS, cystic fibrosis, postoperative cancer patients, multiple traumas, burn injury, respiratory distress, hepatic or renal disease [6]. SCFA and MCFA have lower heats of combustion than long chain fatty acids, making them lower in calories. This characteristic has made triacylglycerols enriched in these fatty acids attractive for use in low-calorie products [7].

Butyric acid is a short fatty acid that has been the subject of much attention because of its physiological functions, specially its potential as an anticarcinogenic agent [8]. Butyric acid is a potent inhibitor of proliferation and an inducer of differentiation and apoptosis in a number of cancer cell lines [9]. Several studies have provided multiple lines of evidence that butyrate indeed interferes with the pathogenesis of colorectal cancer [10]. Lauric acid

L. Vázquez · C. C. Akoh (✉)
Department of Food Science and Technology,
University of Georgia, Athens, GA 30602-2610, USA
e-mail: cakoh@uga.edu

(C12:0) has also been investigated due to its bioactive properties. This compound is the main antiviral, antibacterial, substance found in human breast milk. Numerous bactericidal effects have been attributed to the activity of lauric acid and monolaurin [11]. This compound has been shown to be effective against *Staphylococcus aureus*, *Mycobacterium terrae*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Streptococcus agalactiae* and several other groups of streptococci [12]. Some of the viruses inactivated by monolaurin include HIV, measles, Herpes simplex-1, vesicular stomatitis, the visna virus, and the cytomegalovirus [13]. Caprylic acid, capric acid and myristic acid can be virucidal and bactericidal as well [11].

Classical processes such as distillation [14], urea complexation [15], low-temperature crystallization [16], or liquid–liquid extraction [17] have been applied to fractionate numerous lipid samples from different sources. However, newer technologies, such as supercritical fluid extraction [18] or short-path distillation [19] are adequate for the production of food-grade ingredients and offer advantages against conventional methods, mainly due to the greener chemistry and mild conditions involved, enhanced versatility, selectivity and efficiency.

Short-path distillation is a process based on the evaporation of molecules into a substantially gas-free space vacuum. The controlling factor is the rate at which the molecules escape from the heated surface of the distilling liquid and are received by the cooled condenser surface [20]. Hence, short-path distillation offers an alternative method to fractionate lipid samples into a heavy fraction (residue) and a light fraction (distillate) on the basis of volatility. This technology is suitable for the separation, purification, and concentration of thermolabile substances with low vapor pressure. There are many examples of applications related to lipids: fractionation of ethyl esters from marine sources [19], fractionation of milk fat [20], or purification of structured lipids [21].

SCFA are commonly found in bovine milk and butter fat, whereas coconut and palm kernel oils are the main sources of MCFA. Appreciable amounts of SCFA and MCFA (C4–C10) are present in milk fat, contributing up to 10 wt% of the fatty acid composition [20]. Coconut oil and certain coconut products contain approximately 50 wt% lauric acid and approximately 6–7 wt% capric acid [11]. Direct fractionation of triacylglycerols from samples such as butteroil has not yielded very good results of products highly enriched in SCFA and MCFA. For that reason, other authors have reported the ethanolysis of raw material which facilitated the subsequent fractionation of the fatty acid ethyl esters (FAEE) [18].

In the present study, dairy fat and coconut oil were blended (1:1, vol/vol) to obtain a suitable raw material composed mainly of triacylglycerols rich in butyric, caproic, caprylic, capric and lauric acid residues. In this study, the composition of the original blend of oils was transformed by converting the triacyglycerols into FAEE. In the second step, we studied the fractionation of the FAEE produced using short-path distillation. The fractionation was based on the different volatility of FAEE and mainly on their different chain lengths. Composition and yield of distillates and residues were determined in order to describe the process and to determine the most important conditions. The main aim of this study was to obtain a distillate or fraction enriched in SCFAEE and MCFAEE. The fractions selected can be used as starting materials in subsequent processes to produce structured lipids, nutraceuticals, functional foods, or other value-added products, such as antimicrobials and emulsifiers.

Materials and Methods

Materials

Coconut oil (purity, 99.9 wt%) (sample #56216) was purchased from ADM (Quincy, IL). Anhydrous milk fat (purity, 99.9 wt%) (lot #9079-67) was purchased from Dairy Farmers of America (Kansas City, MO). Sodium ethoxide, 21% (w/w) in ethanol was purchased from Alfa Aesar, A Johnson Matthey Company (Ward Hill, MA). Absolute ethanol used in the transesterification reaction was purchased from Decon Labs Inc. (King of Prussia, PA). NaCl used for washing, chloroform, 2-propanol and diethyl ether were obtained from J.T. Baker (Phillipsburg, NJ). HPLC grade hexane and ethyl acetate were purchased from Fisher Scientific (Pittsburgh, PA). Formic acid was purchased from EMD (San Diego, CA). Caproic acid ethyl ester, palmitic acid ethyl ester, and stearic acid ethyl ester were obtained from Sigma Chemical Co. (St. Louis, MO). Caprylic acid ethyl ester and butyric acid ethyl ester were obtained from the Aldrich Chemical Company (Milwaukee, WI). Anhydrous sodium sulfate was purchased from Fisher Scientific (Pittsburgh, PA).

Ethanolysis of Oil Blend

Dairy fat and coconut oil were blended at a ratio of 1:1, vol/vol. Then, a transesterification reaction by ethanolysis of the oil blend was carried out. The methodology used to produce the starting material for Study 1 was based on the method described by Torres et al. [18]. According to this method, the final product obtained was 91% (w/w) in FAEE, with approximately 9% (w/w) of acylglycerols remaining. However, this methodology was optimized to obtain a product with improved results. The optimized procedure is as follows: The reaction was performed in a

1-L cylindrical vessel. 500 mL of the oil blend were mixed with sodium ethoxide (2.625%, w/v) in absolute ethanol at a ratio of 4:2 (vol/vol) (2.25 fold molar excess of ethanol). The mixture was heated at 60 °C with mechanical shaking for 40 min. The product was washed twice in order to completely remove the remaining ethanol, glycerin or any other polar compounds. First washing was done with a NaCl solution (0.5 M), and the second with distilled water. Separation of two phases was done using a separatory funnel (2-L capacity) and centrifugation was not necessary. The volume utilized in each washing was half of the volume of oil utilized. Finally, the product of the ethanolysis reaction was dried over anhydrous sodium sulfate and vacuum filtered.

Using our optimized methodology, the purity of FAEE in the final product was improved [18], containing only 1.7% (w/w) of acylglycerols. FAEE and acylglycerols content were determined by GC and HPLC, respectively. This product was used as the starting material for Study 2. It should be mentioned that the amount of absolute ethanol can be reduced to a ratio, oil/EtOH, of 4:1.5 (vol/vol) (1.68-fold molar excess of ethanol), while still obtaining a final product with similar levels of purity in FAEE. However, a more difficult separation of phases was observed under these conditions. This drawback can also be solved by increasing the concentration of NaCl in water during washings.

Short-Path Distillations

The product of chemical ethanolysis was effectively fractionated by short-path distillation at different temperatures yielding a distillate and a retentate or residue. Short-path distillations were carried out with a KDL-4 (UIC Inc., Joliet, IL) unit. When distillations were performed at the lowest temperatures (40–60 °C), a second distillate was recovered in the cold trap. The cold trap consisted of a mixture of acetone and dry ice that provides temperatures down to about −78 °C. This device was necessary to condense the most volatile FAEE that were not condensed as the main distillate. After each distillation under these conditions, the cold trap was melted by filling the cold trap with hot water. Then, it was removed and mixed with the main distillate for subsequent analysis.

Two different studies were carried out to investigate the fractionation of FAEE. The aim was to evaluate the recovery of short and medium FAEE present in the starting material. In Study 1, the product of chemical ethanolysis (300 g) was the starting material for distillations at the first temperature (40 °C) explored. The residue obtained was then distilled at a higher temperature, and distillates were removed again. This cycle of operation was repeated for as many temperature intervals as desired, and this procedure was also called cyclic short-path distillation. In this first study, three different feed rates of starting material: 100, 300 and 500 g/h were explored. The distillation temperatures ranged from 40–120, 40–140, and 40–160 °C, respectively, for each feed used. At the final temperature, almost the entire amount of FAEE present in the sample was evaporated. In Study 2, the starting material for all distillations at different temperatures was the product of chemical ethanolysis. Hence, in these processes residues were not used as starting material for subsequent distillations and they were just analyzed and discarded. In this second study, temperatures ranging from 50 to 110 °C and a feed rate of 500 g/h were investigated. 500 g of starting material was fed to the short-path for each distillation in this study.

Vacuum pressure was maintained at 0.14–0.2 mbar for all experiments. The rotor of the distillator (model RW 20, Ika-Works, Cincinnati, OH) was set at position 4, giving 250 rev/min for all distillations. Generally, the cooling water temperature was set at 0 °C. However, this temperature was increased to 10–20 °C when the distillates contained FAEE with a higher molecular weight and solid phases appeared at 0 °C at the condenser surface. The starting material was preheated by a double jacketed water heater at 55 °C for experiments performed at temperatures higher than 40 °C. For distillations carried out at 40 °C, the temperature of the water was also set at 40 °C. After the vacuum had been established, the starting material was passed over the evaporating surface at the different temperatures studied. After passing the entire sample over the evaporating surface, distillate and residue were collected and analyzed for fatty acid ethyl ester composition and acylglycerols content.

GC Analysis

FAEE (from the raw material produced by ethanolysis of the oil blend, residues and distillates from short-path distillation) were analyzed using an Agilent Technology (Santa Clara, CA) 6890 N gas chromatograph equipped with a flame ionization detector. Separation was achieved with an SP-2560 column, 100 m 0.25 mm i.d., and 0.20 μm film (Supelco Inc., Bellefonte, PA). Injection (1 μL) was performed at a split ratio of 20:1. Helium was used as the carrier gas at a constant flow rate of 1.1 mL/min. The injector temperature was 250 °C, and the FID set point was 260 °C. The temperature program was as follows: the sample was held at 140 °C for 5 min, and then increased up to 240 °C with ramping at 4 °C/min and held isothermally for 15 min. FAEE relative content was calculated by integration using a GC Chemstation software. Identification of the various FAEE was based on the retention times and relative area percentages of a Supelco

37 Component FAME mix (Supelco Inc., Bellefonte, PA). Quantification was effected via an external standard of stearic acid ethyl ester, palmitic acid ethyl ester, caprylic acid ethyl ester, caproic acid ethyl ester, and butyric acid ethyl ester. These standards were selected to quantify FAEE according to their chain length. The following formula was used for the FAEE quantification: mg injected = calibration factor × area. The calibration factors determined in the present study were as follows: 2.31×10^{-6} for butyric acid ethyl ester, 1.96×10^{-6} for caproic acid ethyl ester, 1.82×10^{-6} for caprylic acid ethyl ester, 1.59×10^{-6} for palmitic acid ethyl ester, and 1.60×10^{-6} for stearic acid ethyl ester. All samples were dissolved in hexane for GC analyses at 20–25 mg/mL. All analysis was performed in duplicate and average values reported. The standard deviation obtained for each compound in GC analysis is tabulated in Table 1.

HPLC Analysis

The products of the transesterification reaction, residues, and distillates were analyzed by HPLC to determine the purity of the FAEE and the amount of acylglycerols remaining. All samples were dissolved in chloroform for HPLC analyses at 20–25 mg/mL. Analyses were achieved with a Hewlett−Packard 1100 HPLC system (Avondale, PA) equipped with an autosampler, degasser, pump, and coupled to a Sedex 55 evaporative light-scattering detector (ELSD) (Richard Scientific, Novato, CA). The lipid species were separated with a Zorbax XL-Sil column 5 μm spherical 80 Å pore (250 mm × 4.6 mm). The ELSD was set to 40 °C, a nebulizer gas (N_2) pressure of 2.2 atm, and a gain of 6. The injection volume was 20 μL, and the column oven temperature was 40 °C. The mobile phase was: channel A, hexane; channel B, hexane/2-propanol/ethyl acetate/10% formic acid (80/10/10/1, vol/vol). The analysis procedure was based on a gradient method previously reported by Liu et al. [22]. A constant mobile phase flow of 2 mL/min was used during the analyses. The total run time was 19 min. When not in use, the column was rinsed with the solvent mixture of hexane/2-propanol/ethyl acetate (80/10/10, vol/vol) to remove formic acid. Blend of coconut oil and dairy fat 1:1 (vol/vol) was used as external standard for quantification of triacylglycerols. Di- and monoacylglycerols were isolated by TLC in order to perform an accurate quantification of these compounds. Residues recovered at highest temperatures in Study 1 were the starting material for TLC. Mobile phase used in TLC was composed of hexane/diethyl ether/acetic acid (50/50/1, vol/vol). Silica gel G (20 × 20 cm, 250 μm) (Analtech, Newark, DE) TLC plates were used as stationary phase. After TLC separation, bands containing di- and monoacylglycerols were scraped off from the TLC plate, extracted with 3 mL of diethyl ether, and evaporated under nitrogen. Fractions obtained were used as external standards to quantify di- and monoacylglycerols. All analysis was done in duplicate and averages reported. Standard deviation obtained for each compound in HPLC analysis is tabulated in Table 1.

Results and Discussion

Two studies were performed to obtain fractions with high purity and yield of short and medium chain FAEE. As described in Methods section of Study 1 (cyclic short-path distillation), the residue obtained from the previous distillation was used as feed material for distillation at subsequent temperatures. The same starting material was used in Study 2 for all temperatures, and there were no recycling of residues. It was a one time distillation of the same ethanolysis products.

Throughout this work, two main responses were evaluated:

a. Purity or composition, defined as % (weight of x-compound in a fraction/weight of entire fraction) × 100.
b. Yield, defined as % (weight of x-compound in a fraction/weight of x-compound present in feed material) × 100.

Study 1 (Cyclic Short-Path Distillation)

Conditions of Study 1 were selected in order to fractionate FAEE with similar molecular weights in successive steps (temperatures) at three different feed rates studied. Performing a cyclic short-path distillation and understanding the behavior of these distillations, it was possible to recover the FAEE present in the starting material in several fractions with different compositions and yields. Tables 1 and 2 report the purity and yield, respectively, of each FAEE obtained in distillates under different distillation conditions explored. Results of purity and yield in residues are shown in Table 3. Only the yields of major FAEE were reported, since yields of minor FAEE present in the starting material could give a false sense of accuracy in this response.

Figure 1 shows the variation of FAEE compositions versus distillation temperature (a), and variation of FAEE yield versus distillation temperature (b), when a feed rate of 500 g/h was employed. Curves represented are called elimination curves and the temperature at the maximum is known as the elimination temperature [19]. As shown in Fig. 1, the elimination temperatures of FAEE, ranging from 40 to 160 °C, increased with the carbon number chain length of the FAEE. As Embree [23] reported, we have to consider the maximum yield to define these elimination curves. According to this definition, using a feed rate of 500 g/h, it

Table 1 Purity or composition (%) of distillates and raw material in Study 1 (cyclic short-path distillation), standard deviation, and fatty acid composition of anhydrous milk fat and coconut oil

Feed (g/h)	100					300					
Temperature (°C)	40	60	80	100	120	40	60	80	100	120	140
Purity of distillates (%)											
C4:0	11.4	0.4	0.0	0.0	0.0	19.8	0.1	0.0	0.0	0.0	0.0
C6:0	8.7	0.7	0.0	0.0	0.0	15.0	2.3	0.1	0.0	0.0	0.0
C8:0	30.2	3.1	0.1	0.0	0.0	36.2	19.2	0.8	0.1	0.0	0.0
C10:0	20.1	7.1	0.3	0.0	0.0	11.6	22.4	6.3	0.4	0.0	0.0
C11:0	0.7	0.3	0.0	0.0	0.0	0.4	0.8	0.3	0.0	0.0	0.0
C12:0	26.0	62.9	17.8	0.9	0.5	12.8	47.6	65.2	23.3	0.8	0.3
C14:0	2.1	17.7	33.7	4.9	0.9	2.1	5.6	18.5	32.3	6.7	1.3
C14:1 n9	0.1	0.6	0.9	0.2	0.0	0.1	0.2	0.6	0.9	0.2	0.0
C15:0	0.0	0.3	1.1	0.5	0.1	0.1	0.0	0.3	1.0	0.5	0.1
C16:0	0.4	5.0	29.5	32.2	10.6	1.1	1.3	5.6	26.4	32.6	11.4
C16:1 n7	0.0	0.2	1.2	0.9	0.2	0.0	0.1	0.2	1.0	1.0	0.3
C18:0	0.0	0.4	3.6	15.6	25.6	0.3	0.1	0.5	3.6	16.7	25.0
C18:1 n9	0.1	1.1	9.6	32.4	40.4	0.5	0.3	1.3	9.1	34.1	39.4
C18:2 n6	0.0	0.2	1.7	5.0	5.5	0.1	0.0	0.2	1.5	5.1	5.6
C18:3 n6	0.0	0.0	0.2	0.5	0.9	0.0	0.0	0.0	0.2	0.6	0.6
C18:3 n3	0.0	0.0	0.1	0.6	1.3	0.0	0.0	0.0	0.1	0.6	0.9
TAG	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.7
DAG	0.0	0.0	0.0	1.7	0.9	0.0	0.0	0.0	0.0	1.4	1.0
MAG	0.0	0.0	0.0	0.7	2.8	0.0	0.0	0.0	0.0	0.9	3.0
C4–10	71.2	11.6	0.5	0.1	0.1	83.0	44.8	7.5	0.5	0.1	0.0
C4–12	97.2	74.6	18.3	0.9	0.5	95.8	92.4	72.7	23.8	0.9	0.3

Feed (g/h)	500							RM	StD	AMF	CO
Temperature (°C)	40	60	80	100	120	140	160				
C4:0	26.6	0.6	0.0	0.0	0.0	0.0	0.0	1.5	1.5 E-4	1.8	0.0
C6:0	19.5	3.3	0.2	0.0	0.0	0.0	0.0	1.3	1.2 E-5	1.7	0.6
C8:0	35.4	26.6	2.4	0.2	0.0	0.0	0.0	4.5	1.0 E-5	1.3	7.6
C10:0	8.4	22.7	9.0	0.7	0.0	0.0	0.1	4.5	4.0 E-5	3.0	6.1
C11:0	0.3	0.8	0.4	0.0	0.0	0.0	0.0	0.2	6.9 E-6	0.0	0.0
C12:0	7.9	39.3	64.4	30.3	3.8	0.4	0.4	25.2	1.2 E-3	3.5	47.0
C14:0	1.2	4.7	16.3	32.7	17.8	3.4	1.1	14.4	3.7 E-4	11.3	18.1
C14:1 n9	0.0	0.2	0.6	1.0	0.5	0.1	0.0	0.4	9.0 E-5	0.9	0.0
C15:0	0.0	0.1	0.3	0.9	0.9	0.3	0.1	0.5	1.2 E-4	1.2	0.0
C16:0	0.5	1.2	4.7	21.9	37.0	24.0	9.0	16.8	3.7 E-2	30.5	9.1
C16:1 n7	0.0	0.1	0.2	0.9	1.2	0.7	0.2	0.6	9.7 E-4	1.8	0.0
C18:0	0.1	0.1	0.4	2.8	10.2	19.9	21.4	6.6	0.18	12.3	3.1
C18:1 n9	0.2	0.3	1.0	7.1	22.4	36.8	30.5	12.7	0.13	26.5	6.4
C18:2 n6	0.0	0.1	0.2	1.2	3.6	5.3	4.5	2.0	7.4 E-3	3.1	1.9
C18:3 n6	0.0	0.0	0.0	0.1	0.4	0.6	0.9	0.2	1.7 E-4	0.5	0.1
C18:3 n3	0.0	0.0	0.0	0.1	0.4	0.8	0.9	0.3	8.2 E-4	0.6	0.0
TAG	0.0	0.0	0.0	0.0	0.0	0.0	2.0	2.4	0.09	–	–
DAG	0.0	0.0	0.0	0.0	0.0	1.9	5.2	5.9	0.01	–	–
MAG	0.0	0.0	0.0	0.0	0.0	1.6	8.5	1.1	0.04	–	–
C4–10	90.1	54.0	12.0	0.9	0.1	0.1	0.1	12.0	7.4 E-5	7.8	14.2
C4–12	98.0	93.3	76.4	31.3	3.9	0.5	0.4	37.2	1.2 E-3	11.3	61.2

RM Raw Material; *StD* Standard Deviation; *AMF* Anhydrous Milk Fat; *CO* Coconut Oil

Table 2 Yield (%) of distillates in Study 1 (cyclic short-path distillation)

Feed (g/h)	100					300						500						
Temp. (°C)	40	60	80	100	120	40	60	80	100	120	140	40	60	80	100	120	140	160
Yield of distillates (%)[a]																		
C4:0	89.6	7.4	0.2	0.0	0.0	90.9	0.6	0.2	0.0	0.0	0.0	55.2	26.9	0.6	0.0	0.0	0.0	0.0
C6:0	79.8	13.0	0.6	0.1	0.0	80.6	16.3	1.2	0.0	0.0	0.0	47.3	32.2	3.0	0.0	0.0	0.0	0.0
C8:0	81.2	17.1	0.7	0.2	0.0	56.9	40.2	3.3	0.3	0.1	0.0	34.7	41.6	11.8	0.5	0.0	0.0	0.0
C10:0	55.6	39.9	1.6	0.2	0.0	18.8	48.2	27.7	1.9	0.2	0.0	10.7	31.7	45.7	2.5	0.0	0.0	0.0
C12:0	12.6	62.7	16.4	0.8	0.1	3.6	17.9	50.2	19.5	0.7	0.1	1.5	9.0	58.0	19.2	2.8	0.2	0.0
C14:0	1.8	30.8	54.1	7.7	0.3	1.0	3.7	25.1	47.6	10.1	0.4	0.2	1.6	25.7	36.1	22.8	3.0	0.2
C16:0	0.3	7.4	40.7	43.7	3.3	0.5	0.7	6.5	33.6	42.0	3.2	0.0	0.3	6.3	20.9	40.7	18.8	1.3
C18:0	0.1	1.4	12.9	54.1	20.3	0.3	0.1	1.5	11.9	54.9	17.8	0.0	0.1	1.3	6.9	28.8	39.8	8.0
C18:1 n9	0.1	2.1	17.5	58.2	16.6	0.3	0.2	2.1	15.3	58.1	14.5	0.0	0.1	1.8	9.0	32.7	39.0	5.9
C18:2 n6	0.1	2.5	19.4	56.9	14.3	0.3	0.2	2.2	16.3	55.3	13.1	0.0	0.1	2.0	9.1	31.8	35.0	5.2
C4–10	75.7	23.2	0.9	0.1	0.0	50.8	36.1	12.2	0.8	0.1	0.0	40.9	35.8	21.9	1.2	0.1	0.1	0.0
C4–12	34.6	49.7	11.1	0.6	0.1	19.9	25.3	40.2	14.1	0.5	0.0	14.6	20.3	45.6	13.7	2.0	0.2	0.0
Weight (g)	39.7	74.5	68.0	66.2	15.0	23.2	30.6	61.7	66.4	62.1	13.3	16.6	24.3	66.7	48.8	58.8	41.4	6.3

[a] FAEE lower than 1% in the composition of the starting material are not included in the yield. Only major FAEE are reported

was observed that the rate of evaporation is in the order C4-6:0 > C8:0 > C10:0 > C12:0 > C14:0 > C16:0 > C18, with elimination temperatures at 40, −55, −75, 80, 100, 120 and 140 °C, respectively (see Table 4). This pattern can be explained because fractionation by short-path distillation is based on the different volatility of compounds and FAEE with a longer chain length will have a decreased volatility. For that reason, higher temperatures were required to fractionate FAEE with higher molecular weights.

Elimination curves give a characteristic but relative temperature for each kind of FAEE, but this also depends on the apparatus and the operating conditions [19]. It should also be noted that complete elimination of each FAEE occurs at a broader range of temperatures.

Using a feed rate of 500 g/h, almost a complete elimination of C4:0 and C6:0 was achieved at 40 °C, without significant differences between both types of FAEE. In addition, no significant differences in elimination temperature were observed between C18:0, C18:1, and C18:2. The explanation can be that their structural differences are based on the number and position of double bonds. These FAEE (C18) present identical chain length and hence, similar molecular weight and similar volatility. However, it may be possible to establish differences by exploring a closer range of temperatures [24].

A similar pattern was defined with the other two feed rates investigated, 100 and 300 g/h. However, different values of elimination temperatures were observed at these conditions. Examination of Fig. 2a indicates elimination temperatures when a feed rate of 100 g/h was used. These elimination temperatures are given in Table 4. Some authors reported similar results for C16:0 and C18:1, obtaining elimination temperatures of 80 and 100 °C, respectively, using a higher vacuum $(0.67–4) \times 10^{-3}$ mbar. However, their study was focused on the fractionation of squid visceral oil ethyl esters and recovery of EPA and DHA, and no data were reported for short and medium chain fatty acids [19]. Furthermore, Fig. 2b indicates intermediate values when the feed rate was 300 g/h. Table 4 shows the elimination temperatures using a feed rate of 300 g/h.

These results indicate that it is possible to remove the same amount and type of FAEE with either low feed rates and low temperatures or high feed rates and high temperatures. The reason could be that when a low feed rate was employed, the sample remains in contact with the distillator surface for a longer residence time. This time can play an important role in the evaporation of the desired compounds. On the contrary, higher feed rates involve a shorter residence time for FAEE in the distillator and, in these cases, higher temperature values were required to remove similar amounts and types of FAEE. Moreover, other possible factors can also explain these results. For instance, when a high feed rate is used, the film thickness could increase, and thus retard the evaporation of FAEE into the distillator. Figure 3 shows how different types of FAEE were removed from residues at successive temperatures studied when a feed rate of 500 g/h was used.

Throughout this first study, fractions with a high purity of specific FAEE can be obtained, since each group of FAEE was recovered in a different fraction. In Study 1, we found that the same amount of raw material was fractionated better than in Study 2. For example, the fraction recovered at 80 °C using feed rates of 300 and 500 g/h,

Table 3 Purity or composition (%) and yield (%) of residues in Study 1 (cyclic short-path distillation)

Feed (g/h)	100					300						500						
Temp. (°C)	40	60	80	100	120	40	60	80	100	120	140	40	60	80	100	120	140	160
Purity of residues (%)																		
C4:0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
C6:0	0.2	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.3	0.1	0.0	0.0	0.0	0.0	0.0
C8:0	0.9	0.0	0.0	0.0	0.0	1.7	0.1	0.0	0.0	0.0	0.0	2.6	0.6	0.0	0.0	0.0	0.0	0.0
C10:0	2.2	0.1	0.0	0.0	0.0	3.7	1.0	0.0	0.0	0.0	0.0	4.1	2.7	0.1	0.0	0.0	0.0	0.0
C11:0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0
C12:0	24.6	6.8	0.2	0.0	0.0	26.3	20.6	4.9	0.2	0.0	0.0	26.2	25.5	9.3	1.6	0.1	0.0	0.0
C14:0	16.1	15.0	2.8	0.2	0.0	15.6	16.8	13.3	3.7	0.3	0.1	15.2	16.2	15.3	8.8	1.7	0.2	0.0
C14:1 n9	0.5	0.4	0.1	0.0	0.0	0.5	0.5	0.4	0.1	0.0	0.0	0.4	0.5	0.4	0.2	0.0	0.0	0.0
C15:0	0.5	0.6	0.3	0.0	0.0	0.5	0.6	0.6	0.3	0.0	0.0	0.5	0.5	0.6	0.5	0.2	0.0	0.0
C16:0	19.0	25.2	21.0	3.7	0.4	18.3	20.9	25.0	20.4	3.9	0.7	17.8	19.2	23.8	24.6	14.7	2.0	0.0
C16:1 n7	0.7	0.8	0.6	0.1	0.0	0.6	0.7	0.8	0.6	0.1	0.0	0.6	0.7	0.8	0.8	0.4	0.1	0.0
C18:0	7.5	10.7	14.4	12.0	1.2	7.2	8.3	10.9	14.3	11.1	1.7	7.0	7.5	9.9	12.7	14.6	6.5	0.6
C18:1 n9	14.4	20.3	26.5	16.4	1.6	13.8	16.0	21.6	27.0	15.9	2.2	13.4	14.5	19.1	23.8	24.8	8.9	0.7
C18:2 n6	2.3	3.3	4.2	2.4	0.2	2.2	2.5	3.4	4.1	2.4	0.3	2.2	2.3	3.1	3.9	4.0	1.3	0.1
C18:3 n6	0.3	0.4	0.5	0.5	0.1	0.3	0.3	0.4	0.5	0.4	0.1	0.3	0.3	0.4	0.5	0.6	0.3	0.0
C18:3 n3	0.3	0.4	0.5	0.5	0.0	0.3	0.4	0.4	0.5	0.4	0.1	0.3	0.3	0.4	0.5	0.6	0.3	0.0
TAG	3.2	3.8	6.3	14.4	25.5	2.9	3.0	4.2	6.7	14.1	23.4	1.9	2.1	3.8	5.3	8.5	17.7	26.6
DAG	7.2	10.0	16.4	37.1	63.2	6.6	8.0	11.1	16.5	43.8	69.8	5.0	5.3	10.0	13.8	21.8	57.7	63.0
MAG	2.0	2.7	4.0	7.9	5.2	1.8	2.1	2.6	3.4	4.2	5.1	1.1	1.0	2.5	2.9	3.8	6.0	5.1
Yield of residues (%)[a]																		
C4:0	8.0	1.3	0.0	0.0	0.0	5.3	1.2	0.0	0.0	0.0	0.0	6.0	2.4	0.0	0.0	0.0	0.0	0.0
C6:0	15.6	1.2	0.0	0.0	0.0	16.2	1.5	0.0	0.0	0.0	0.0	22.5	4.0	0.0	0.0	0.0	0.0	0.0
C8:0	17.7	0.6	0.1	0.0	0.0	35.1	2.0	0.1	0.0	0.0	0.0	53.0	12.3	0.2	0.0	0.0	0.0	0.0
C10:0	43.9	0.9	0.0	0.0	0.0	75.8	17.3	0.4	0.0	0.0	0.0	86.1	52.4	1.9	0.1	0.0	0.0	0.0
C12:0	85.4	16.7	0.3	0.0	0.0	96.7	64.5	11.1	0.4	0.0	0.0	97.5	88.7	23.8	2.9	0.1	0.0	0.0
C14:0	97.5	64.6	7.4	0.2	0.0	100	91.6	53.4	9.3	0.3	0.0	99.1	98.5	68.7	28.8	3.1	0.1	0.0
C16:0	99.4	93.1	48.0	3.2	0.2	100	98.2	86.0	43.9	3.2	0.3	99.2	100	91.5	69.2	22.4	1.2	0.1
C18:0	100	100	3.9	26.3	1.3	100	99.4	96.2	78.5	23.4	1.9	99.4	100	97.6	91.0	57.2	10.1	0.5
C18:1 n9	99.6	99.3	79.9	18.6	0.9	100	99.2	98.3	76.8	17.3	1.3	99.1	100	97.4	88.3	50.2	7.1	0.3
C18:2 n6	100	100	80.7	17.6	0.8	100	98.7	99.2	74.7	16.5	1.2	100	100	99.9	91.6	51.3	6.6	0.3
Weight (g)	262.5	184.1	112.3	41.7	20.3	277.2	233.7	169.4	104.8	39.6	20.5	281.1	259.6	189.6	136.8	73.8	28.9	17.2

[a] FAEE lower than 1% in the composition of the starting material are not included in the yield. Only major FAEE are reported

gave a purity of C12:0 approximately 65%. It is also remarkable that distillates obtained at 40 °C with feed rates of 300 and 500 g/h, were composed of 20–26% C4:0 and approximately 35% C8:0. However, yields achieved at these conditions must also be evaluated in order to optimize the process of fractionation. Detailed summaries of these results are given in Tables 1 and 2.

In addition, after the distillation processes were performed and several fractions were recovered, some of the fractions were pooled to obtain products with the desired composition of short and medium chain FAEE. Figure 4 shows the results when each distillate was pooled with distillates recovered at lower temperatures. Figure 4a, b indicate purity and yield, respectively, of short and medium chain FAEE, excluding lauric acid (C12:0). Figure 5a, b show results of purity and yield, respectively, of short and medium chain FAEE, including lauric acid (C12:0). It can be observed in Figs. 4 and 5 that when a feed rate and a temperature were selected, similar results of yield and purity can be obtained by selecting higher feed rates at higher temperatures. Nevertheless, no appreciable differences were observed between feeds of 300 and 500 g/h at some ranges of temperatures.

Figures 4 and 5 provide conclusive information for the production of desired fractions with specific composition and yields of different FAEE. Hence, for example, carrying

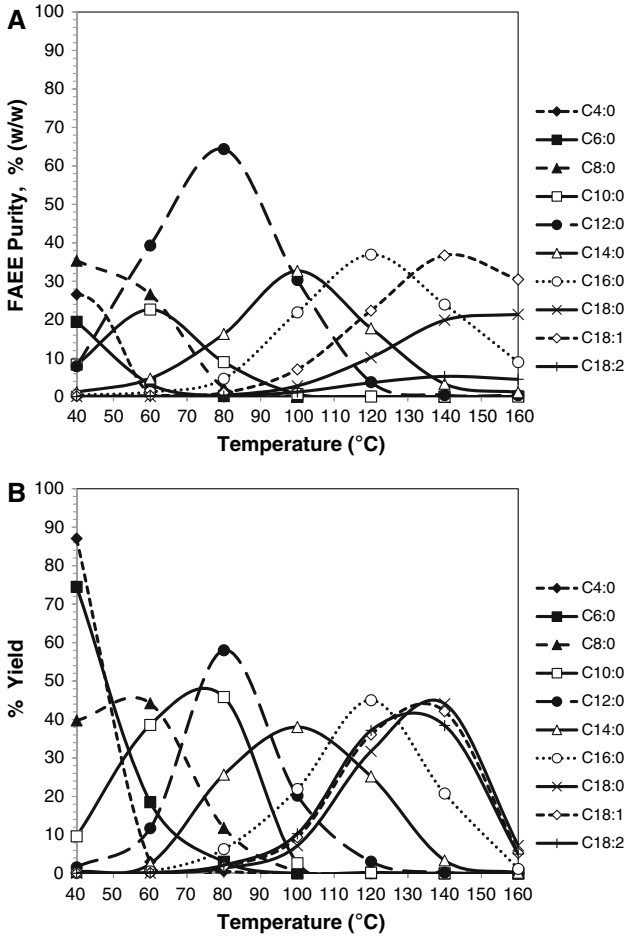

Fig. 1 Purity (**a**) and yield (**b**) of distillates at 500 g/h in Study 1 (cyclic short-path distillation)

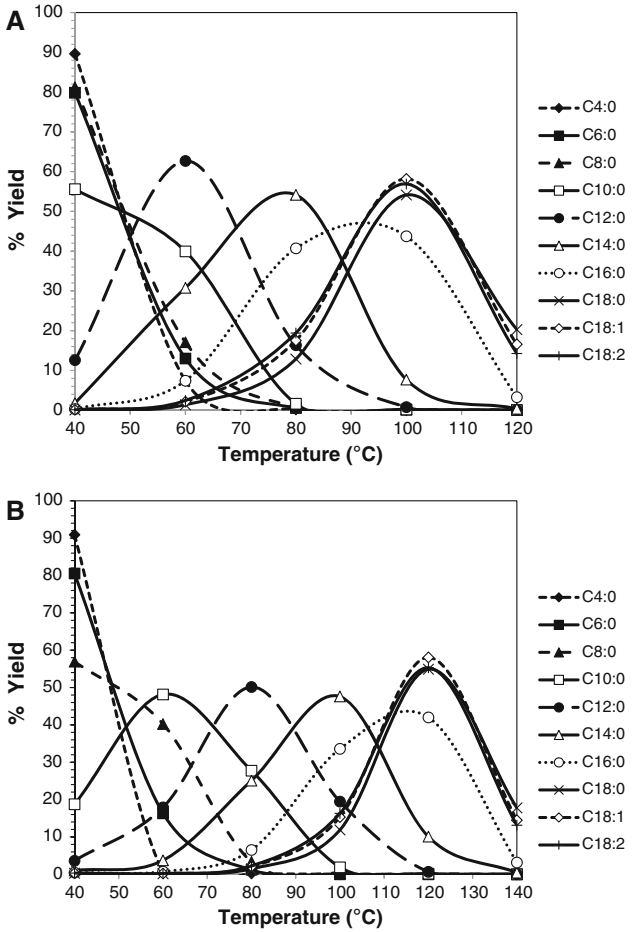

Fig. 2 Yield of distillates at 100 g/h (**a**) and 300 g/h (**b**) in Study 1 (cyclic short-path distillation)

Table 4 Elimination temperatures (°C) of FAEE at the different feeds studied

FAEE	Elimination temperature (°C)		
	100 (g/h)	300 (g/h)	500 (g/h)
C4:0	40	40	40
C6:0	40	40	40
C8:0	40	40	~55
C10:0	40	60	~75
C12:0	60	80	80
C14:0	80	100	100
C16:0	~90	~115	120
C18:0	100	120	140
C18:1	100	120	140
C18:2	100	120	140

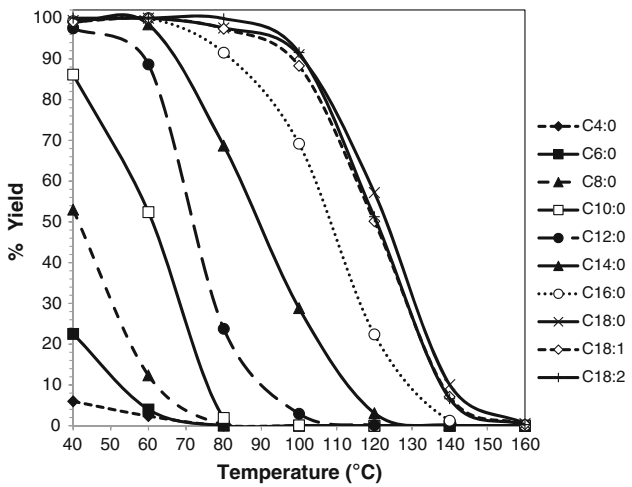

Fig. 3 Yield of residues at 500 g/h in Study 1 (cyclic short-path distillation)

out the process by pooling distillates up to 60 °C at 300 g/h and up to 65 °C at 500 g/h, a good balance between composition (94%) of short and medium chain FAEE and yield (45%) could be achieved. Thus, a fraction highly enriched in short and medium chain FAEE (C4–12) with acceptable yields was recovered. In order to obtain higher

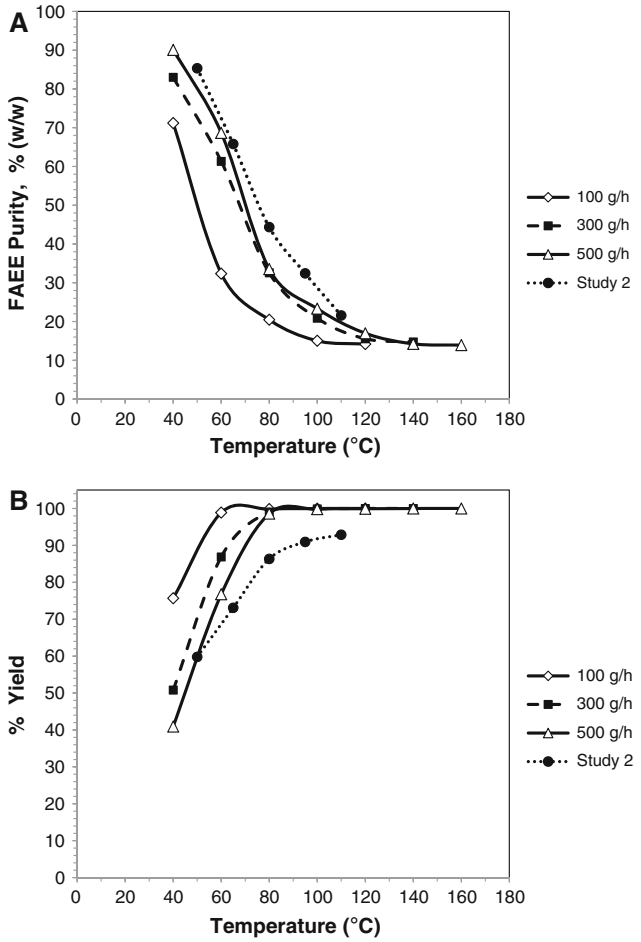

Fig. 4 **a** Comparison of purity of C4–10 between Study 1 (pooled fractions) and Study 2 distillates. **b** Comparison of yield of C4–10 between Study 1 (pooled fractions) and Study 2 distillates

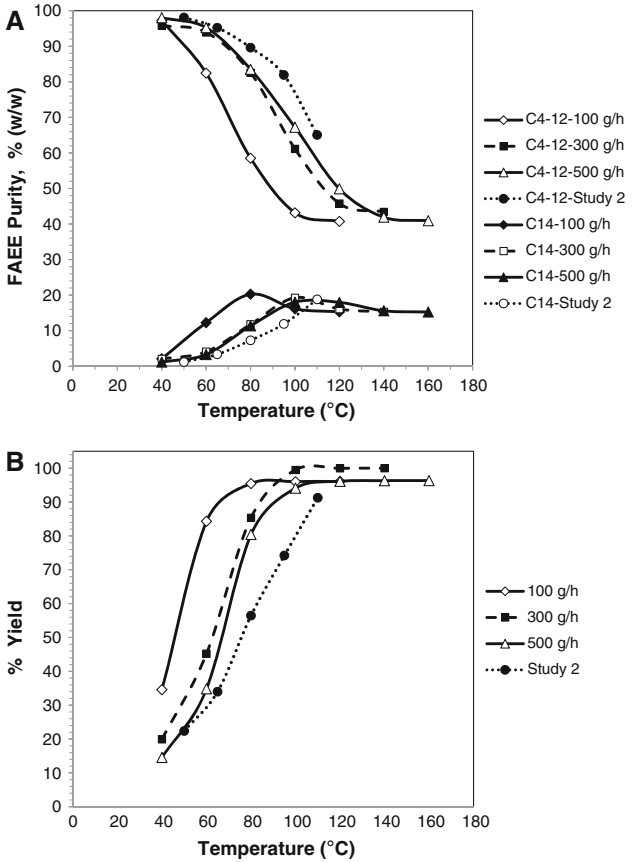

Fig. 5 **a** Comparison of purity of C4–12/C14 between Study 1 (pooled fractions) and Study 2 distillates. **b** Comparison of yield of C4–12 between Study 1 (pooled fractions) and Study 2 distillates

yields of short and medium chain FAEE (C4–12), other conditions should be explored. For example, pooling distillates up to 60 °C at 100 g/h and up to 80 °C at 300 or 500 g/h, yields of 80–85% were obtained, although in these cases the purity of C4–C12 decreased to 82–84%. This purity drop was caused by the presence of FAEE with a higher molecular weight, since at these conditions, distillation of FAEE with longer chains started to become feasible. Table 5 summarizes these results and the different possibilities to operate.

Study 2 (Single Non-Cyclic Short-Path Distillations)

Conditions of Study 2 were established in order to determine the yield and composition of fractions obtained at 5 different temperatures: 50, 65, 80, 95 and 110 °C, by carrying out a direct distillation from final product of ethanolysis. The raw material was fractionated into two samples and only one distillate and one residue was recovered from the same amount of starting material. An advantage associated to this procedure was that the raw material can be processed directly and the desired fractions can be recovered using one single step. This characteristic can make this process faster and suitable for industrial applications. A feed rate of 500 g/h was used for the second study since a larger amount of sample can be processed by this procedure and it may provide an important advantage for scaled-up industrial processes.

From the results presented in Table 6 the following conclusions can be derived. As observed from previous study, as the temperature was increased, the purity of short and medium FAEE decreased, giving the highest values at the lowest temperatures. However, large amount of these compounds still remained in the residues and thus, low yields were achieved under these conditions. In other words, as we observed in Study 1, processes with high purity of MCFAEE result in low yields of these compounds. On the contrary, temperatures that provide high yields were associated with low values of purity. Under these conditions almost all MCFAEE present in the starting material were removed, although less volatile compounds

Table 5 Summary of results at different distillations conditions in Study 1 (pooled fractions)

Temperature (°C)	Feed (g/h)	Purity C4–10 (%)	Yield C4–10 (%)	Purity C4–12 (%)	Yield C4–12 (%)	Comments
60	100	32.4	98.9	82.5	84.3	Low purity
80	300	32.6	99.1	82.6	85.4	High yield
80	500	33.5	98.6	83.5	80.4	
55	100	~40.0[a]	~94.5	~87.0	~75.0	
75	300	~40.0	~97.0	~85.0	~75.0	
75	500	~42.5	~94.0	~87.0	~68.0	<purity
70	300	~47.5	~94.5	~88.0	~64.0	>yield
50	100	~50.0	~88.0	~90.0	~62.0	
70	500	~52.0	~88.0	~90.0	~56.0	
65	300	~55.0	~91.0	~92.0	~54.0	
45	100	~61.0	~82.5	~94.0	~47.0	
65	500	~61.0	~84.0	~93.0	~45.0	Purity/yield
60	300	61.3	86.9	93.9	45.2	
55	300	~67.5	~79.0	~95.0	~38.0	
60	500	68.7	76.8	95.2	34.8	>purity
40	100	71.2	75.7	97.2	34.6	<yield
50	300	~72.5	~70.0	~95.0	~32.0	
40	300	83.0	50.8	95.8	19.9	High purity
50	500	~80.0	~60.0	~97.0	~23.0	Low yield

[a]Temperatures preceded by ~ estimations from Figs. 1 and 2. These are not in multiples of 20 °C

were also distilled, mainly C14 and C16, decreasing the purity of MCFAEE.

According to these results, processes carried out at 50–65 °C could provide good results in terms of purity (65–85% of C4–10) and yield (60–73% of C4–10) if a fraction more enriched in short chain FAEE is desired. On the other hand, processes at 80 °C could offer a good compromise solution between purity (89%) and yield (56%) when C12 is desired to be recovered. In addition, an improved yield of C4–12 could be obtained (74%) when a temperature of 95 °C was selected, even though at these conditions a lower purity of C4–C12 was attained (82%). Other researchers reported similar yields of SCFAEE and MCFAEE using supercritical fluid extraction in a single step from a similar raw material. However, in their study, no purities higher than 70% of SCFAEE and MCFAEE were attained at these conditions and the mass balance of SCFAEE (mainly ethyl butyrate) was significantly lower (50–70%) [18]. As described in Table 6, improved yields of ethyl butyrate and other SCFAEE were obtained in this study (greater than 90%).

Figures 4 and 5 represent a comparison between Study 1 and Study 2 distillations. As we explained previously for these figures, each temperature from Study 1 represents analysis of pooled fractions distilled off up to the current temperature. This characteristic permits one to compare purities and yields achieved between Study 1 and Study 2. It should be noted that when the same temperatures are compared at the same feed rate (500 g/h), higher yields of short and medium chain FAEE were reached in Study 1 than in Study 2. On the other hand, under these conditions, the purity obtained in Study 1 was lower than in Study 2. These differences could be explained by the different raw materials employed throughout the distillations for the two studies. Whereas doing a cyclic short-path distillation, FAEE present in starting material were partially removed during subsequent distillations, in Study 2 the same amount of FAEE should be removed in a single step to reach similar results of purity and yield at the same temperature. Hence, under these conditions, it is possible that the residence time at the distillator surface was not long enough to remove this higher amount of FAEE. Figures 4 and 5 show that an increased temperature was required in Study 2 to obtain results of purity and yield similar to those in Study 1. In our opinion, a decreased feed rate and hence, a longer residence time of sample in the distillator, can also provide similar results.

From this work, short-path distillation proved to be capable of effectively fractionating short and medium chain FAEE. The studies we performed provide various options to carry out the fractionation processes with regard to different responses, such as the number of steps involved, FAEE desired, purities and yields obtained, or amount of material processed. This work permits us to

Table 6 Purity or composition (%) and yield (%) of distillates and residues in Study 2

Temperature (°C)	Distillates					Raw material	Residues				
	50	65	80	95	110		50	65	80	95	110
Purity (%)											
C4:0	18.2	8.4	5.8	4.2	2.7	1.7	0.1	0.1	0.0	0.0	0.0
C6:0	14.6	8.9	5.1	3.7	2.4	1.4	0.1	0.1	0.0	0.0	0.0
C8:0	39.0	29.9	17.6	12.5	8.2	4.9	1.5	0.4	0.1	0.0	0.0
C10:0	13.1	18.1	15.3	11.7	8.0	4.8	4.0	2.5	0.8	0.3	0.1
C11:0	0.4	0.6	0.6	0.4	0.3	0.2	0.2	0.1	0.0	0.0	0.0
C12:0	12.7	29.4	45.3	49.5	43.5	27.3	28.9	26.8	19.9	12.7	2.9
C14:0	1.2	3.4	7.3	11.9	18.8	15.5	17.0	17.7	18.0	17.6	10.6
C14:1 n9	0.0	0.1	0.3	0.4	0.6	0.5	0.5	0.5	0.5	0.5	0.3
C15:0	0.0	0.1	0.1	0.2	0.4	0.5	0.5	0.6	0.6	0.7	0.6
C16:0	0.4	0.9	1.9	3.9	9.9	17.9	19.7	21.1	23.3	26.6	30.0
C16:1 n7	0.0	0.0	0.1	0.2	0.4	0.6	0.7	0.7	0.8	0.9	1.0
C18:0	0.1	0.1	0.2	0.4	1.2	7.0	7.7	8.3	9.3	11.1	15.8
C18:1 n9	0.2	0.2	0.5	1.0	3.0	13.5	14.8	15.9	17.9	22.1	29.6
C18:2 n6	0.0	0.1	0.1	0.2	0.5	2.2	2.3	2.6	2.8	3.2	4.6
C18:3 n6	0.0	0.0	0.0	0.0	0.1	0.3	0.3	0.4	0.4	0.4	0.6
C18:3 n3	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.3	0.3	0.5	0.6
TAG	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.2	0.2	0.3	0.4
DAG	0.0	0.0	0.0	0.0	0.0	0.9	0.8	1.0	1.3	1.4	2.0
MAG	0.0	0.0	0.0	0.0	0.0	0.6	1.0	1.1	1.3	1.3	2.0
C4–10	85.3	65.8	44.4	32.4	21.6	13.0	5.9	3.1	0.9	0.3	0.2
C4–12	98.1	95.2	89.6	81.9	62.1	40.4	34.7	30.0	20.8	13.0	3.1
Yield (%)[a]											
C4:0	95.0	74.0	88.1	90.6	86.5		5.1	3.4	1.4	0.0	1.1
C6:0	92.0	88.6	89.6	92.4	91.7		8.5	3.3	1.4	0.0	0.7
C8:0	72.6	87.5	90.5	92.5	94.3		27.6	7.7	1.2	0.0	0.3
C10:0	25.2	54.2	80.8	89.1	94.1		74.3	43.6	12.3	3.6	0.5
C12:0	4.3	15.5	42.2	66.2	90.5		95.1	83.2	55.1	29.3	4.6
C14:0	0.7	3.1	12.0	28.1	69.0		98.8	96.0	87.9	71.4	29.3
C16:0	0.2	0.7	2.7	7.9	31.5		99.5	98.5	98.2	93.5	72.2
C18:0	0.1	0.2	0.7	2.0	9.8		99.6	98.9	100	99.9	97.1
C18:1 n9	0.1	0.2	0.9	2.6	12.7		99.6	98.7	99.9	100	94.7
C18:2 n6	0.1	0.3	1.0	2.8	13.9		100	100	100	94.0	95.4
C4–10	59.8	73.1	86.3	90.9	92.9		40.2	20.5	5.5	1.3	0.5
C4–12	22.4	34.0	56.5	74.2	91.3		77.2	63.1	39.1	20.3	3.2
Weight (g)	46.3	72.2	124.4	182.8	284.6		450.9	425.4	370.9	314.1	214.9

[a] FAEE lower than 1% in the composition of the starting material are not included in the yield. Only major FAEE are reported

select the most suitable conditions for producing fractions enriched in short and medium FAEE with a desired composition and yield. In further steps, these fractions can be used as starting materials for the production of structured lipids, nutraceuticals or functional lipids, antimicrobial lipids, and emulsifiers.

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