Integrated Synthesis and Extraction of Short-Chain Fatty Acid Esters by Supercritical Carbon Dioxide

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We developed an efficient, integrated reaction-extraction process for the production of short-chain fatty acid ethyl esters (FAEE) from milk fat, using carbon dioxide as the only processing solvent. FAEE were synthesized using a short-chain fatty acid selective lipase. The expansion of the liquid mixture of reactants by dense carbon dioxide enhanced the apparent lipase selectivity. In situ extraction of FAEE by a continuous flow of supercritical carbon dioxide proved to increase the lipase production rate. When the integrated process was operated with alternated periods of synthesis and product removal, the overall selectivity for short-chain FAEE increased as well, as a result of the combination of the selectivities of lipase and extraction solvent. A two-fold increase of the lipase productivity was achieved at these conditions, compared to a single batch reaction. The developed process enables the synthesis and isolation of high-value fatty acid derivatives from a natural source such as milk fat. © 2009 American Institute of Chemical Engineers *AIChE J*, 56: 1080–1089, 2010

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Introduction

Milk fat contains a particularly high amount of short and medium chain fatty acids compared to other natural fats and oils.[1] Fatty acids with a chain length of 4–12 carbon atoms represent about 25% of the total fatty acids in milk fat triglycerides, on a molar basis.[2] Short-chain fatty acids and their esters find many industrial applications as flavoring agents, emollients, or emulsifiers. Currently, these compounds are mostly obtained via chemical synthesis, fermentation, or extraction from plants.[3,4] Disadvantages of these processes are that synthesis products cannot be labeled as natural,[3] while the low concentrations of the desired compounds make fermentation and extraction processes costly.[4] An alternative, cost-effective biotechnological process for the production of short-chain fatty acid esters is therefore interesting from an industrial perspective.

Fatty acids can be released from triglycerides by lipase-catalyzed transesterification reactions, for example alcoholysis.[5,6] By using ethanol as the alcoholysis acyl acceptor, fatty acid ethyl esters (FAEE) are formed in this reaction. The selectivity of lipases can be used to enrich one of the reaction products in a certain type of fatty acids. In the case of milk fat ethanolysis, lipase selectivity for short-chain fatty acids can be used to produce a FAEE product mixture that is enriched in the valuable short-chain fatty acid fraction.[7]

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Lipase-catalyzed reactions can be carried out in supercritical fluids (SCF).[8] Supercritical carbon dioxide (scCO_2) is one of the favorite SCF due to its lack of toxicity, mild critical point, and wide availability.[8] SCF are interesting reaction solvents featuring a solvent power tunable by pressure and temperature and excellent transport properties.[8–11] The main drawback of SCF is that, due to their relatively low solvent power, high amounts of fluid at elevated pressure are necessary to dissolve the reactants. A novel approach in the field of supercritical catalysis involves the dissolution of a gas in the liquid mixture of reactants at pressures on the order of tenths of bars, for creating a gas-expanded liquid phase (GXL) where the dissolved gas acts as a co-solvent.[12] The GXL has lower viscosity than the original mixture, resulting in higher diffusion coefficients. The increase in diffusivity can in turn enhance the equilibrium conversion, reaction rate, and/or selectivity in GXL as compared to solvent-free or conventional organic media.[7,12,13] For systems consisting of edible fats and carbon dioxide, several researchers have shown that considerable amounts of the gas are dissolved in the molten fat at moderate pressures,[14,15] which is accompanied by a decrease of the mixture viscosity.[15]

The advantages of CO_2 as reaction solvent or co-solvent can be maximized by making additional use of it for the separation of reaction products. Separation of products after a supercritical reaction is often accomplished in a succession of separators operated at decreasing pressures. The less soluble compounds condense in the first separator, at the highest pressure, and the most soluble in the last one, at the lowest pressure.[16,17] Marty et al.[16] reported good separations of product (ethyl oleate) and reactants (oleic acid and ethanol) using a cascade of four separators. Weber et al.[17] separated FAEE from partial glycerides using a similar configuration, taking advantage of the high solubility of FAEE in carbon dioxide as compared to that of glycerides.

In situ removal of reaction products provides supplementary benefits to the reaction: it prevents the products from reacting back or undergoing side-reactions; may enhance reaction selectivity and, in the case of equilibrium reactions, increases the production rate due to the equilibrium shift imposed by the removal of products, as demonstrated by Adschiri et al.[18] and Shishikura et al.[19] in lipase-catalyzed transesterification reactions.

Our objective is developing a simple and efficient process for the synthesis and separation of short-chain fatty acid esters from milk fat, a natural source of these components. To this end, we explore the advantages of using carbon dioxide as reaction co-solvent and the adequate process conditions for the selective extraction of short-chain FAEE from the reaction mixture. Finally, we investigate the additional advantages of integrating synthesis and extraction by the use of carbon dioxide, which allows taking advantage of the synergic selectivities for short-chain FAEE of the lipase and the extraction solvent.

Materials and Methods

Materials

A low melting fraction (O-fraction) of concentrated milk fat was obtained from Friesland-Campina (Lochem, Netherlands) and stored below 4°C before use. The fatty acid composition of this milk fat, normalized to the nine major fatty acids, was experimentally determined by gas chromatography, and is shown in Table 1. In this work, butyric, hexanoic, octanoic, decanoic, and lauric acids are considered short-chain fatty acids (s-FA). The rest are considered long-chain fatty acids (l-FA). Carbon dioxide was obtained from Linde Gas Benelux (Schiedam, Netherlands). The immobilized enzyme Novozym 435 was kindly donated by Novozymes A/S (Bagsvaerd, Denmark). Absolute ethanol (99.9%) was purchased from Sigma-Aldrich (Zwijndrecht, Netherlands). Ethyl butyrate (99.5%), hexanoate (99.8%), octanoate (99.8%), decanoate (99.5%), myristate (98.5%), laurate (99.8%), palmitate (99.8%), oleate (99.5%), and stearate (99.5%), used as analytical standards, were also purchased from Sigma-Aldrich.

Table 1. Fatty Acid Composition of Milk Fat Fraction Used in the Experiments

Fatty Acid (Type/Name)		mol %
Short-chain	Butyric acid (C4)	9.7 ± 0.1
	Caproic acid (C6)	4.8 ± 0.1
	Caprylic acid (C8)	2.0 ± 0.1
	Capric acid (C10)	4.1 ± 0.1
	Lauric acid (C12)	4.5 ± 0.1
Long-chain	Myristic acid (C14)	11.9 ± 0.1
	Palmitic acid (C16)	28.1 ± 0.2
	Stearic acid (C18)	9.2 ± 0.2
	Oleic acid (C18:1)	25.7 ± 0.1

Analytical methods

Analyses of the FAEE and partial glyceride mixtures were carried out in a gas chromatograph Chrompack CP9002 (Varian, Middelburg, Netherlands) equipped with flame ionization detector, using a VF5-ht GC-column (Varian, Middelburg, Netherlands). Injection and detection temperatures were 350°C. N_2 was the carrier gas. Oven temperature was kept at 50°C for 2 min, then raised at 20°C/min to 350°C and kept for 2 min at the final temperature. This temperature profile allowed elution of all components and quantification of FAEE.

GXL reaction and reaction-extraction experiments

The experimental set-up is depicted in Figure 1. A jacketed stirred autoclave (Autoclave Engineers) was used for ethanolysis under CO_2 pressure. The autoclave has a volume of 385 mL and is equipped with two sapphire windows, a bottom mesh filter and several inlet/outlet ports. In regular reaction-extraction experiments, 120 g of molten fat and corresponding amounts of ethanol and immobilized lipase were introduced in the autoclave, which was then closed and pressurized by the addition of carbon dioxide. In our previous work, we studied the ethanolysis reaction of milk fat in diverse reaction media at 315 K,[7] consequently this temperature was fixed and used in the present study for reaction, extraction, and integrated reaction-extraction experiments. Temperature was kept at the set point by a thermostatic bath and the mixture was stirred using a 6-blade Rushton turbine impeller at 150 rpm. Samples from the reaction mixture, free of enzyme particles, were taken regularly by opening the bottom valve of the

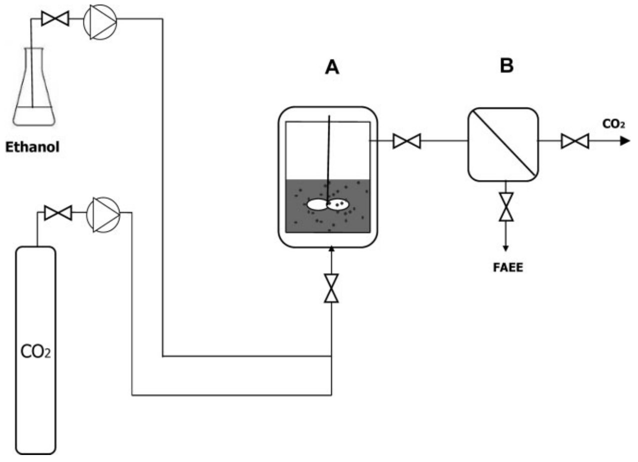

Figure 1. Reaction-extraction set-up.

The jacketed high-pressure reactor (A) is initially charged with lipase and reactants, and has a feed inlet for CO_2 and ethanol make-up. The outlet valve connects the reactor with the separator (B), where the extracted material is condensed.

autoclave. The size of the samples was ~2 g. In the reaction-extraction experiments, extraction of the reaction products was started by initiating a continuous flow of $scCO_2$ of ~100 g/min, after allowing a certain initial time for reaction. The stirring speed was then reduced to 120 rpm for reducing the chance of liquid droplet entrainment by the gas flow. The extracted material was condensed from the gas stream at 2.8 MPa and 281 K and was collected in the separation vessel, maintained at low temperature by a thermostatic bath. During the extraction period, ethanol was fed to the reactor using an ISCO pump, to compensate its loss due to extraction in the carbon dioxide stream.

Results and Discussion

Our objective was synthesizing and extracting short-chain FAEE from milk fat using carbon dioxide as the only process solvent. The effect of carbon dioxide was first investigated separately on reaction and extraction, to elucidate the impact of the dissolved gas on the reaction medium; and to determine the optimal pressure for selective extraction of short-chain FAEE at 315 K. The integration of both tasks was then carried out in two different ways: simultaneous and cyclic reaction-extraction.

Effect of carbon dioxide on the enzymatic reaction

Lipase-catalyzed ethanolysis of milk fat was carried out in CO_2-expanded milk fat (GXL) at 11 MPa and 315 K. Ethanolysis of fat is an equilibrium reaction, in which up to three FAEE are formed from each triglyceride (TAG):

$$\mathrm{TAG} + \mathrm{E} \leftrightarrow \mathrm{DAG} + \mathrm{FAEE}$$

$$\mathrm{DAG} + \mathrm{E} \leftrightarrow \mathrm{MAG} + \mathrm{FAEE}$$

$$\mathrm{MAG} + \mathrm{E} \leftrightarrow \mathrm{G} + \mathrm{FAEE}$$

Mono- and di- acylglycerols (MAG, DAG) and glycerol (G) are also produced. The presence of glycerol in reactions catalyzed by immobilized lipases is sometimes problematic, because glycerol may adsorb onto the lipase carrier and inactivate the enzyme. However, only small concentrations of glycerol were expected to be produced in our reaction system, as we did not intend to attain full conversion. It has been shown that the lipase used (Novozym 435) produces glycerol from MAG only when the concentration of TAG is very low.[20] In addition, the hydrophobic support of Novozym 435 is most likely surrounded by a layer of apolar substrates and products that prevent it from glycerol adsorption. Several authors have reported very good stability of Novozym 435 in alcoholysis reactions.[21]

Milk fat triglycerides typically contain both short and long fatty acids (FA) in the same molecule. Novozym 435 is selective for substrates of small volume (i.e., glycerides containing short-chain FA) due to the limited space of its active site.[22] In our previous study on lipase-catalyzed milk fat ethanolysis, Novozym 435 produced FAEE mixtures richer in short-chain FA than the original milk fat, particularly during the first hours of reaction. As the reaction proceeded in time, the percentage of short chains in the FAEE product decreased, approaching that of the original milk fat. We concluded that the lipase catalyzes the transesterification of all fatty acid chain lengths, but that of long-chain FA is catalyzed at a lower rate. In addition, we observed that lipase activity reached its maximum for equimolar concentration of reactants (ethanol to fatty acid ratio $E/FA = 1$).[7]

For milk fat ethanolysis experiments catalyzed by Novozym 435, Figure 2 shows the evolution of the lipase selectivity for short-chain fatty acids (S_L) with conversion (c). The graph includes one solvent-free experiment and two experiments in GXL at different E/FA ratios (0.5 and 1.0). Reaction conversion (c) is defined as moles of FAEE produced (m_{FAEE}) over total moles of FA in the original fat ($m_{\mathrm{FA},0}$):

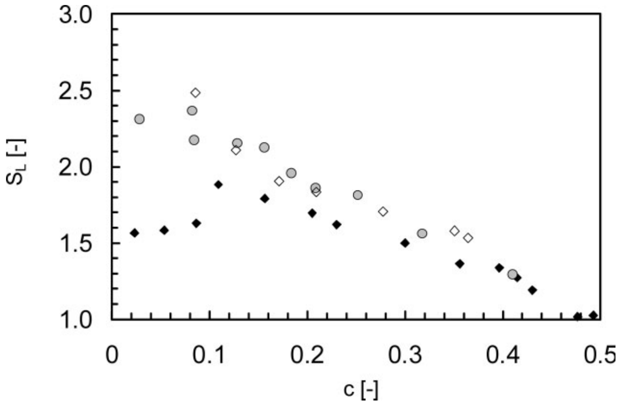

Figure 2. Lipase selectivity (S_L) vs. conversion (c), in ethanolysis experiments catalyzed by Novozym 435.

Black diamonds: solvent-free, $E/FA = 0.5$; gray circles: GXL, $E/FA = 0.5$; empty diamonds: GXL, $E/FA = 1.0$. Reaction temperature was 315 K. S_L decreased with conversion in all experiments, indicating that short-chain fatty acids were transesterified at a faster rate. S_L was in overall higher in the experiments carried out in GXL than in the solvent-free, reference experiment.

$$c = \frac{m_{\mathrm{FAEE}}}{m_{\mathrm{FA},0}}$$

The lipase selectivity (S_L) is defined as the ratio of short-chain fatty acid conversion (c_s) to long-chain fatty acid conversion (c_l):

$$S_L = \frac{c_s}{c_l} = \frac{m_{\mathrm{s-FAEE}}/m_{\mathrm{s-FA},0}}{m_{\mathrm{l-FAEE}}/m_{\mathrm{l-FA},0}}$$

The lipase selectivities S_L were, at low conversions, well above 1 in all experiments, meaning that the percentage of s-FA converted to s-FAEE was larger than the percentage of converted l-FA. This reflects the lipase preference for short- over long-chain fatty acids. An enhancement of S_L was observed in the carbon-dioxide expanded liquid (GXL), in relation to the solvent-free reactions. In the GXL, a maximum S_L of ~2.5 was reached, vs. a maximum of 1.9 in the absence of solvent.

This apparent selectivity increase can be attributed to the enhanced internal diffusion in the GXL medium, as the medium viscosity is considerably reduced by dissolution of carbon dioxide. Venter et al.,[15] for example, reported at 313 K a viscosity of ~43 mPa s for cocoa butter and only 6 mPa s for CO_2-saturated cocoa butter at 9 MPa. Given that the lipase is immobilized onto a porous support, diffusion is the prevalent mechanism for the transport of substrates towards the catalytic sites. By enhancing diffusion and thus reducing mass transfer limitations, the reaction becomes more kinetically controlled. As a consequence, the difference in the intrinsic reaction rates of short-chain and long-chain FA appears more evident.

In addition, the lipase selectivity S_L vs. conversion c profile differed in each reaction medium: in GXL, S_L decreased steadily with c, while it passed through a maximum in solvent-free medium. This maximum in the S_L curve could be related to the time required for the substrates to diffuse into the support pores and reach a stationary concentration at which the lipase selectivity was maximal.

In our previous work, we noticed that the lipase selectivity in GXL decreased at high E/FA ratios (E/FA > 1.5).[7] This effect was explained by a possible change in the lipase structure induced by the interactions between ethanol and carbon dioxide at high ethanol concentrations. Therefore, both considering activity and selectivity, the equimolar ratio of reactants (E/FA = 1.0) was chosen for further use.

Extraction of s-FAEE from partially converted milk fat

FAEE are fairly soluble in carbon dioxide at pressures and temperatures above the critical point.[23–25] For a homologous series of a component type (e.g., FAEE of varying chain length), the solubility in carbon dioxide decreases with increasing molecular weight.[24] Lipid molecules other than FAEE (MAG, DAG, TAG) are present in the reaction mixture. In general, these compounds are less soluble in CO_2 than FAEE, due to their higher polarity and molecular weight.[24] The solubility of glycerol, which could be present in the reactor in small concentrations, is very low.[26]

Generally, the solubility of all compounds increases with carbon dioxide density. Therefore, the separation selectivity decreases at increasing pressures. As our components of interest are the short-chain FAEE (s-FAEE), it is desirable to operate the extraction at the minimum possible pressure that allows their extraction in considerable amount, while minimizing the extraction of other components—primarily long-chain FAEE (l-FAEE).

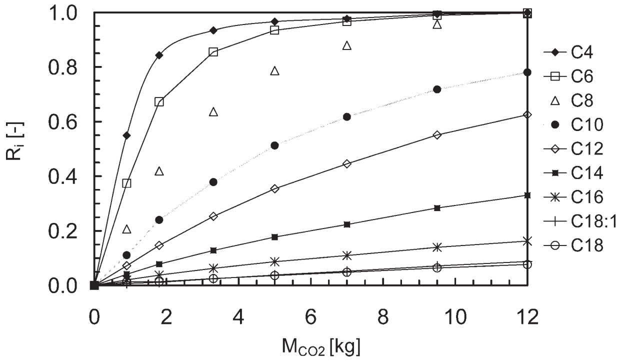

Figure 3. FAEE recovery (R_i) vs. amount of CO_2 used (M_{CO2}), for an extraction experiment at 9.1 MPa and 315 K.

The rate of recovery R_i was highest at the start of the extraction and decreased later as FAEE were depleted in the liquid phase. The rate of recovery was higher the lighter the FAEE.

Extraction of FAEE was applied batch-wise to milk fat partially converted to FAEE and glycerides in ethanolysis experiments at E/FA = 1.0, the optimal reactants ratio. All extraction experiments were done at 315 K and used an average carbon dioxide flow rate of 100 g/min.

Figure 3 shows the evolution of a typical extraction experiment, carried out at 315 K and 9.1 MPa. The recovery R_i of each individual FAEE is plotted vs. amount of CO_2 passed through the vessel (M_{CO2}). The recovery (R_i), a measure of the extraction degree, is defined as the amount of compound i extracted divided by the initial amount of compound i in the feed (mol/mol):

$$R_i = \frac{m_{\mathrm{Ex},i}}{m_{0,i}}$$

The first part of the recovery curves were nearly straight lines for all FAEE, indicating a constant extraction rate. The slope of the recovery curves is proportional to the gas-phase loading (VPL_i), which is the amount of FAEE extracted over the amount of CO_2 used:

$$\mathrm{VPL}_i = \frac{\Delta m_{\mathrm{Ex},i}}{\Delta m_{\mathrm{CO_2}}}$$

The vapor phase loadings (VPL_i) were constant at the start of the extraction. Comparison of VPLs to FAEE solubility data from literature[26] indicates that gas-liquid equilibrium was not achieved during extraction. Therefore, constant VPLs were probably caused by constant mass transfer resistance from the liquid to the gas phase.[27]

After the constant VPL period, the slope of the recovery curves started to decrease for the lighter (shorter chain) FAEE. This was caused by the decline of gas-liquid mass

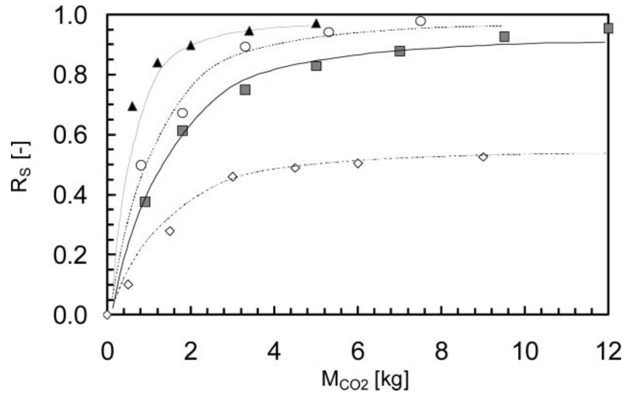

Figure 4. s-FAEE recovery (R_S) vs. amount of CO_2 used (M_{CO2}).

White rhombus: 8.1 MPa; gray squares: 9.1 MPa; white circles: 9.6 MPa; black triangles: 10.1 MPa. At a given pressure, the increase of R_S with M_{CO2} was highest at the start of the extraction and leveled off as the concentration of s-FAEE in the liquid phase decreased. The rate of recovery increased with the extraction pressure.

transfer rates (J_i) due to the concentration decrease of s-FAEE in the liquid phase (x_i). The liquid phase concentration x_i determines the equilibrium concentration in the gas, y_i*, as described by the following equations:

$$y_i^* = K_i \cdot x_i$$

$$J_i = k_{GL,i}\ c_G\left(y_i^* - y_i\right) = k_{GL,i} c_G (K_i x_i - y_i)$$

Short- and long-chain FAEE recoveries were lumped into s-FAEE recovery (R_S) and l-FAEE recovery (R_L), respectively, as described by the equations below. Short-chain FAEE recovery R_S and extraction selectivity S_E, defined as the ratio of R_S to R_L, were the parameters used for comparing the effect of the extraction pressure.

$$R_S = \frac{\sum_{i=C4-C12} m_{Ex,i}}{\sum_{i=C4-C12} m_{0,i}}$$

$$R_L = \frac{\sum_{i=C14-C18} m_{Ex,i}}{\sum_{i=C14-C18} m_{0,i}}$$

$$S_E = \frac{R_S}{R_L}$$

Figure 4 shows the evolution of the short-chain FAEE recovery (R_S) with the amount of carbon dioxide used for extraction (M_{CO2}). For a given extraction pressure, the recovery R_S increased rapidly at the start of the extraction and leveled off as the concentration of s-FAEE in the raffinate decreased, as observed in Figure 3 for the individual components.

The rate of recovery increased with pressure, due to the increased solvent power of CO_2. At the highest pressure (10.1 MPa), practically complete recovery of s-FAEE was achieved in relatively short time, using 5 kg CO_2. The amount of carbon dioxide needed for complete recovery increased at lower pressures, due to the lower solubility of the components in those conditions. At the lowest pressure, 8.1 MPa, a relatively low rate of recovery was observed. The extraction was stopped at a s-FAEE recovery R_S of 53%, after using 9 kg CO_2.

In Figure 5, the extraction selectivity (S_E) is plotted vs. s-FAEE recovery (R_S), for the same set of experimental data. The selectivity S_E decreased with pressure, due to the enhancement of the solvent power of CO_2 for both short- and long-chain FAEE. For example, for a $R_S = 0.7$, the extraction selectivity S_E was 8.7 at 9.1 MPa, 4.8 at 9.6 MPa, and 3.0 at 10.1 MPa.

For a given extraction pressure, the extraction selectivity S_E generally decreased with the recovery of s-FAEE, R_S, and thus with the amount of CO_2 used. As observed in Figure 3, the period of constant VPL was shorter the lighter the FAEE, and increased with molecular weight regardless of the initial concentration of each individual FAEE in the liquid mixture. As a consequence, the mass fraction of l-FAEE in the extract increased with the amount of CO_2 used, resulting in the observed decrease of the extraction selectivity S_E with the extraction degree R_S observed in Figure 5.

In the extraction done at 8.1 MPa, the selectivity seems to pass through a maximum value during the extraction process, instead of decreasing with the amount of carbon dioxide used (M_{CO2}). A possible explanation could be the difficulty of accurately measuring the very small amount of l-FAEE present in the first extracts obtained in this extraction, as small errors in the measured amounts of l-FAEE lead to relatively large errors in the calculated values of S_E.

Regarding the separation of individual FAEE, the selectivity was higher the larger the difference in chain length of the FAEE pair under consideration. This is illustrated in Figure 6, where the extraction selectivity S_{ij} of the pairs C12/C14 and C4/C18:0 is plotted vs. the recovery R_i of the lighter component of each pair, together with the S_E vs. R_S curve of the lumped s-FAEE and l-FAEE. The data shown corresponds to the extraction done at 9.1 MPa. The selectivity S_{ij} ranged from 63 to 13 for the pair formed by the lightest and the heaviest FAEE (C4 and C18:0), with complete

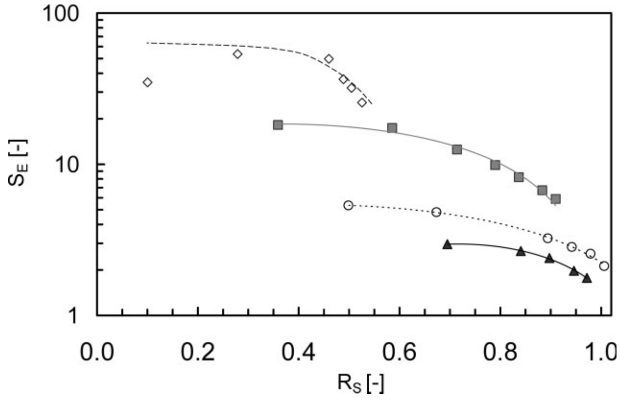

Figure 5. Extraction selectivity (S_E) vs. recovery of s-FAEE (R_S).

White rhombus: 8.1 MPa; gray squares: 9.1 MPa; white circles: 9.6 MPa; black triangles: 10.1 MPa. At a given pressure, the selectivity S_E generally decreased with the recovery R_S. The selectivity S_E decreased at increasing pressures.

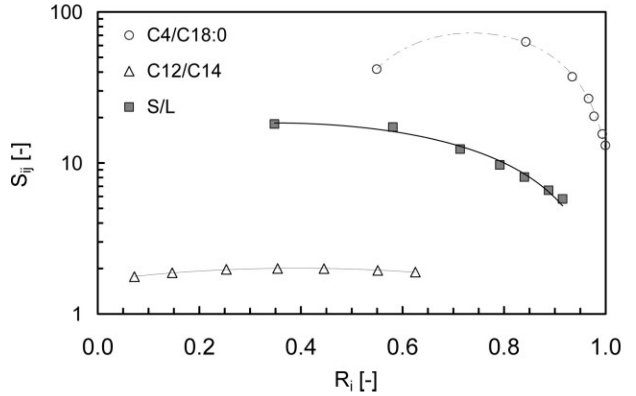

Figure 6. Extraction selectivity (S_{ij}) for selected FAEE pairs, vs. recovery R_i of the lighter FAEE of each pair.

White circles: C4/C18:0; gray squares: lumped s-FAEE/l-FAEE; white triangles: C12/C14. Extraction was performed at 9.1 MPa and 315 K. The extraction selectivity was higher the larger the difference in chain length of the FAEE pairs under consideration.

end recovery of C4. For the pair C12/C14, however, S_{ij} varied between 2.0 and 1.8 with a final C12 recovery R_{12} = 0.63. For achieving sharp separations between FAEE of similar chain length, multistage countercurrent fractionation would be required. Nevertheless, the semi-batch, single stage $scCO_2$ extraction operation proved to be a good method for the separation of short-chain FAEE from long-chain FAEE.

Effect of in situ product removal on reaction rate and selectivity

The hypothesis under investigation was that the integration of reaction and extraction could lead to positive effects on the reaction rate and short-chain fatty acid selectivity, derived from the in situ removal of reaction products and the combination of reaction and extraction selectivity. The reactants ratio was set to E/FA = 1 and the temperature at 315 K. We chose 9.1 MPa for the operation pressure as a trade-off between the recovery R_S and the extraction selectivity S_E. At this pressure, considerably high recoveries were obtained with an acceptable extraction selectivity ($S_E = 5.9$ for a s-FAEE recovery $R_S = 0.91$). Two possible process configurations were tested: simultaneous reaction-extraction and cyclic reaction-extraction.

Simultaneous Reaction-Extraction. In a typical simultaneous reaction-extraction experiment, the reaction was first let to proceed in the gas-expanded mixture (without CO_2 circulation) until a significant amount of FAEE was produced. Thereafter, the extraction process was initiated.

Figure 7 shows the cumulative productivity of the lipase *P* vs. time for two integrated reaction-extraction experiments. The cumulative lipase productivity *P* is defined as total mol of FAEE produced per gram of lipase.

The productivity curve of a regular ethanolysis experiment with no simultaneous extraction is plotted in Figure 7 as reference (solid curve). For the simultaneous extraction-reaction experiments, the moment in which the carbon dioxide flow was started is indicated by the dashed vertical line. In the first reaction-extraction tests, the conversion was observed to stop as soon as the carbon dioxide flow was started. This is represented by the gray circles series. The dotted horizontal line indicates the final productivity attained. The fact that the reaction stopped was due to the complete extraction of the reactant ethanol from the liquid mixture.

To overcome the extraction of ethanol, a continuous ethanol make-up stream was incorporated with the objective of maintaining a constant ethanol concentration in the liquid mixture during the reaction-extraction experiments. As the partition coefficient of ethanol between the liquid fat mixture and the gas phase was unknown, a range of ethanol/CO_2 feed ratios between 0.015 and 0.050 g ethanol/g CO_2 were tested. Using 0.024 g ethanol/g CO_2, the concentration of ethanol in the liquid phase during the reaction-extraction period was ~15 wt %, corresponding to the desired E/FA = 1.0 (considering the amount of milk fat-bonded FA present at the start of the experiment). This feed ratio of ethanol to CO_2 was subsequently used in the reaction-extraction experiments.

As shown by the white triangles series in Figure 7, FAEE productivity (*P*) increased from the moment that simultaneous FAEE extraction and ethanol make-up were started (*t* = 3.3 h). The cumulative productivity *P* increased by 45% after 3 h of simultaneous reaction-extraction, compared to the reference single reaction. This increase in productivity was presumably a consequence of the disappearance of one of the reaction products (FAEE) from the reaction mixture, shifting the reaction equilibrium towards the products side according to le Chatelier principle. A similar effect was observed by Adschiri et al.[18] for the transesterification of methyl oleate and tricaprylin with simultaneous extraction of the product methyl caprylate. They reported an increase of 45% in the conversion after 14 h of extractive reaction.

These results confirm the hypothesis that in situ product removal increases the production rate of FAEE by the lipase.

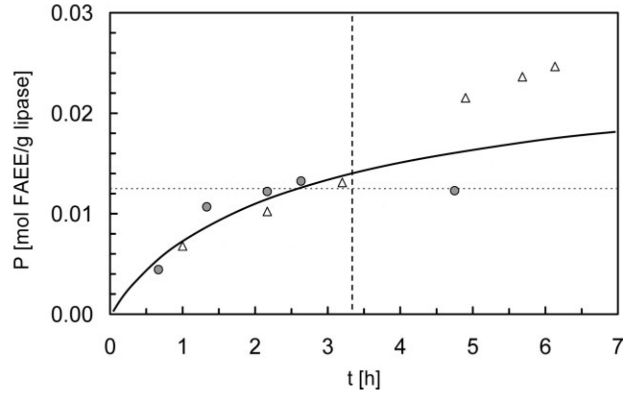

Figure 7. Cumulative lipase productivity (*P*) of FAEE vs. time (*t*).

All experiments were carried out at 315 K, with E/FA = 1.0 and 4 wt % of Novozym 435. Solid line: reference reaction experiment without extraction. Gray circles: experiment without ethanol make-up. The start of extraction is indicated by the dashed vertical line. White triangles: experiment with ethanol make-up. In the experiment without ethanol make-up, the reaction stopped upon start of extraction due to the removal of ethanol from the liquid mixture. When an ethanol make-up stream was added, the lipase productivity increased in relation to the reference synthesis experiment, due to the in situ removal of reaction products.

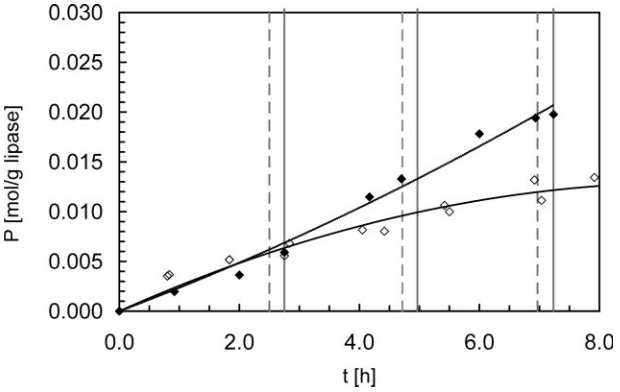

Figure 8. Cumulative productivity of FAEE (*P*) vs. time, for a cyclic reaction-extraction of three cycles (black rhombus).

Reaction conditions were 315 K, E/FA = 1.0 and 10 wt % of Novozym 435. The start and the end of the extraction periods are indicated by the dashed and solid vertical lines, respectively. The cumulative productivity *P* increased by 62% after 7.25 h, in relation to the reference single reaction (empty rhombus and solid line).

Extraction of FAEE from the reaction mixture pulls the reaction towards synthesis of FAEE by lowering the concentration of FAEE available for reverse reaction. Since larger amounts of s-FAEE than l-FAEE were extracted, the production rate of s-FAEE could be expected to increase more in comparison to the production rate of l-FAEE. However, this was not the case: the relative amounts of s-FAEE and l-FAEE produced were approximately the same as in the reference, reaction-only experiment. This effect can be explained based on the supposition that the ratio of short- to long-chain FAEE produced by the lipase depends directly on the concentration of short- and long-chain FA still bonded to glycerides, rather than on the concentration of short- and long-chain FAEE present in the reactants mixture.

Cyclic Reaction-Extraction Operation. In situ extraction of reaction products resulted in an advantageous increase of lipase productivity. To avoid the continuous addition of ethanol required in the simultaneous reaction-extraction mode, the integrated reaction-extraction was operated in a cyclic manner. On the basis of the previous results on reaction and extraction rates, the duration of the reaction periods was set to 2–2.5 h and followed by 15 min of extraction. The experiments consisted of 3 or 4 cycles. After each extraction period, the amount of extracted ethanol was added to the reactor for restoring the E/FA ratio to 1.

Figures 8 and 9 show the cumulative FAEE productivity curves (*P*) vs. time for a three-cycle and a four-cycle reaction-extraction experiment, respectively. The reference, reaction-only productivity is also shown in both graphs. The productivity *P* was considerably higher in the cyclic operation as compared to the reaction-only experiment, similar to the increase in the simultaneous synthesis and extraction. After 7.25 h of operation, the cumulative productivity *P* reached 0.020 mol/g lipase in the three-cycle experiment (Figure 8) and 0.024 mol/g lipase after in the four-cycle experiment (Figure 9). This is respectively an increase of 62% and 94% in relation to productivity *P* obtained in the reference ethanolysis reaction (solid curve) after the same operation time.

The increase in productivity was due to the fact that the intermediate extraction periods lowered substantially the concentration of FAEE in the liquid mixture, minimizing the reverse reaction (synthesis of glycerides from FAEE). The cyclic removal of reaction products had thus a comparable effect on the lipase productivity to that of simultaneous product removal.

The percentage of short chains in the FAEE product at the end of the cyclic reaction-extraction (adding up the FAEE recovered in the extract and in the raffinate) was between 32 and 35%, similar to that obtained in the reaction-only experiments. This observation confirms that no clear increase on the apparent lipase selectivity for short-chain fatty acids, S_L, derived from in situ product removal.

Effect of in situ product removal on extraction selectivity

Figure 10 shows the extraction selectivity S_E vs. the extraction degree of s-FAEE (R_S) for the three operation modes described. The extraction selectivity S_E was lower in the integrated reaction-extraction operations (curves B and C), than in a single reaction followed by extraction (sequential mode, curve A). Particularly low values of S_E were obtained in the simultaneous reaction-extraction mode (B). This decrease in the extraction selectivity can be explained by two arguments. During simultaneous synthesis-extraction, the concentration of FAEE in the liquid mixture was very low due to their continuous removal by the carbon dioxide flow. The rate of extraction of s-FAEE was probably higher than its production rate, resulting in concentration-limited VPL values. As this limitation was not critical in the case of l-FAEE due to their lower inherent solubility in CO_2, the result was poor separation selectivity. For improving the separation selectivity, it would be necessary to decrease further the flow rate of CO_2 so that the production rate of s-FAEE does not exceed its extraction rate.

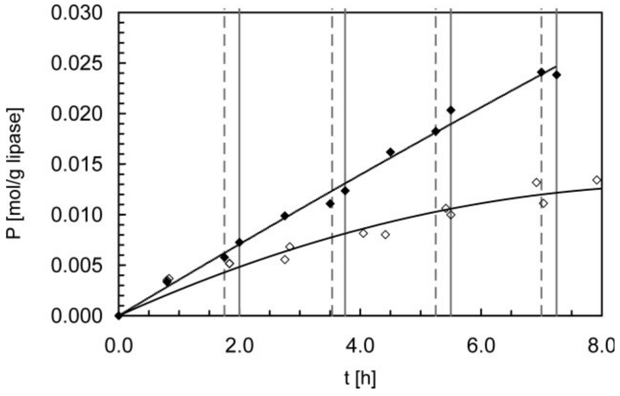

Figure 9. Cumulative productivity of FAEE (*P*) vs. time, for a cyclic reaction-extraction of four cycles (black rhombus).

Reaction conditions were 315 K, E/FA = 1.0 and 10 wt % of Novozym 435. The start and the end of the extraction periods are indicated by the dashed and solid vertical lines, respectively. The cumulative productivity *P* increased by 94% after 7.25 h, in relation to the reference single reaction (empty rhombus and solid line).

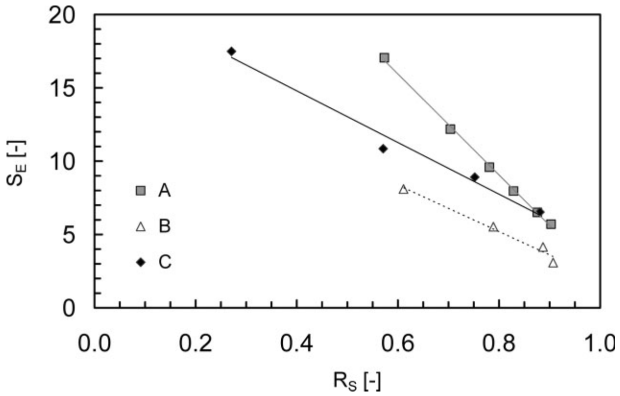

Figure 10. Extraction selectivity (S_E) vs. short-chain recovery (R_S) for three operation modes.

A: sequential reaction and extraction, B: simultaneous reaction-extraction, and C: four-cycle reaction and extraction. Extraction conditions were 9.1 MPa and 315 K.

Similarly, the decrease of extraction selectivity in the case of cyclic operation seems to be a consequence of the overall lower concentration of FAEE in the liquid mixture during extraction. The concentration of ethanol during extraction was considerably higher in the simultaneous (B) than in the sequential (A) and the cyclic operation mode (C), as ethanol was continuously fed to the reactor. Ethanol has a co-solvent effect that increases the solubility of all FAEE in the gas phase, and this solubility enhancement results in a decrease of the CO_2 selectivity for different FAEE.[28]

Towards an industrial process: comparison of operation modes

We compared the overall performance of the three operation modes (sequential, simultaneous, and cyclic reaction-extraction) to highlight the advantages of the integration and assess its potential for industrial implementation.

The comparison was based on processes taking the same total time (7.25 h) and using the same initial amount of reactants and reactants ratio (E/FA = 1.0), temperature of 315 K and pressure of 9.1 MPa. The results are summarized in Table 2. The factors used for the comparison were: total ethanol used, total CO_2 used, final conversion of milk fat, total recovery of s-FAEE, and overall process selectivity for s-FAEE. The total recovery of s-FAEE, $R_{S'}$ is defined as the amount of s-FAEE extracted over the initial amount of s-FA in milk fat. Analogously, $R_{S'}$ is the total recovery of l-FAEE. The overall process selectivity S', defined as $R_{S'}$ over $R_{L'}$, reflects the effect of combining the selectivity of the lipase and the selectivity of the extraction for s-FAEE. The percentage of s-FAEE in the FAEE extract, directly related to S', is also shown in Table 2.

In the sequential reaction-extraction (A) no ethanol make-up was required, resulting in the lowest overall ethanol usage. The highest percentage of short-chains in the FAEE extract was obtained: 79.7%, corresponding to an overall selectivity $S' = 11.9$. However, the conversion of milk fat was the lowest, as there was no possibility of increasing the productivity of FAEE by in situ product removal. Low conversion resulted in a low overall recovery of short-chain FAEE ($R_{S'} = 0.30$). From an industrial prospective, the sequential operation would be relatively easy to implement, either batchwise or in a continuous process. However, as the lipase cost is likely to represent an important contribution to the overall operating costs,[29] the low lipase productivity obtained here is a drawback of this operating mode.

In the simultaneous reaction-extraction mode (B), the largest amount of ethanol make-up was required as this reactant was continuously extracted from the reactor by the carbon dioxide flow. This would not necessarily be a disadvantage in a production scale, as a recycle stream of the extracted ethanol back into the reaction vessel could be implemented. A final conversion of 41% was achieved. This was higher than in mode A because the productivity increased due to in situ product removal. The total recovery of s-FAEE was consequently higher than in case A. On the other hand, the extraction selectivity was relatively low, resulting in the lowest concentration of s-FAEE in the extract (61.3%) from all the compared processes.

Applying intermediate cycles of reaction products extraction (C-3 and C-4) caused the maximum increase of lipase productivity (up to 94%), which at its turn resulted in the highest final conversion of milk fat, as high as 55% in the four-cycle process. Considerably higher extraction selectivity was attained in comparison to mode B, and lower amounts of ethanol were used. A fairly high concentration of s-FAEE in the extract at the end of the process was therefore obtained, nearly as high as in the sequential mode (76.3% in C-4 vs. 79.7% in mode A). In addition, the total s-FAEE recovery $R_{S'}$ was doubled in the cyclic process as compared to the sequential operation.

Comparing the evolution of the overall process selectivity with the total recovery of s-FAEE gives an interesting insight on the different operation modes. In Figure 11, the process selectivity S' is plotted vs. the total recovery of s-FAEE, $R_{S'}$.

The graph shows that the absolute value of the slope of S' with $R_{S'}$ was lower in the integrated than in the sequential process, meaning that higher separation selectivities could be

Table 2. Comparison of Operation Modes

Exp.	Mode	Ethanol Used (g)	CO_2 Used (kg)	Final Conversion (mol %)	R_S'	S'	% SC in Extract (mol %)
A	R+E, sequential	18	7.0	26	0.30	11.9	79.7
B	In situ product removal, simultaneous	186	6.7	41	0.69	4.8	61.3
C-3	In situ product removal (3 cycles)	50	5.0	46	0.60	9.4	75.7
C-4	In situ product removal (4 cycles)	66	6.0	55	0.77	9.7	76.3

Final overall recovery (R_S') refers to the total amount of s-FAEE obtained in the extract over the total amount of s-FA available in the original milk fat. Final overall selectivity S' is R_S' over R_L'.

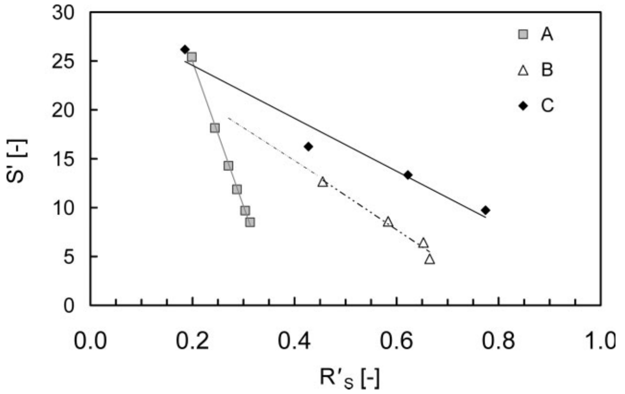

Figure 11. Overall process selectivity (S') vs. total short-chain recovery (R'_S) for three operation modes.

A: sequential reaction and extraction, B: simultaneous reaction-extraction, and C: four-cycle reaction-extraction. Extraction conditions were 9.1 MPa and 315 K.

obtained at high recoveries. This increase, particularly high for the cyclic operation, is the result of exploiting the combination of lipase selectivity and extraction selectivity. For a certain targeted value of process selectivity S', higher short-chain recoveries $R_{S'}$ are obtained when applying in situ product removal. For example, for an overall selectivity $S' = 10$, the short-chain recovery $R_{S'}$ was $\sim$0.30 in the sequential operation (A), 0.55 in the simultaneous reaction-extraction (B), and 0.78 in the cyclic operation (C).

In summary, integration of reaction-extraction proved to provide significant advantages in the synthesis and extraction of short-chain FAEE from milk fat. In particular, the cyclic operation resulted in higher lipase productivity and higher overall selectivity S' at high recoveries. The results presented here correspond to batch experiments with three and four extraction cycles. It seems possible to enhance further the lipase productivity and the separation selectivity by optimizing the number and length of the extraction cycles.

Conclusions

The use of dense carbon dioxide as reaction co-solvent during milk fat ethanolysis enhanced the lipase selectivity for short-chain fatty acids. Supercritical carbon dioxide extraction experiments on partially converted fat showed that a good balance between short-chain FAEE recovery and selectivity was obtained at 9.1 MPa and 315 K. The potential advantages of integrating reaction and extraction by the use of carbon dioxide were investigated. It was concluded that in situ removal of FAEE from the reaction mixture clearly increased the lipase productivity. The integrated reaction-extraction operated in cycles resulted in an increase of the overall process selectivity at high recoveries, as a result of coupling lipase selectivity and extraction selectivity. Using four reaction-extraction cycles, the lipase productivity was nearly doubled in comparison to the reference single reaction. The total recovery of short-chain FAEE was 2.6 times higher than in a sequential reaction-extraction, for a comparable concentration of short chains in the FAEE extract (76%).

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