# Purification of Specific Structured Lipids by Distillation: Effects on Acyl Migration

**Xuebing Xu*, Anja Skands[1], and Jens Adler-Nissen**

BioCentrum–DTU, Technical University of Denmark, 2800 Lyngby, Denmark

**ABSTRACT:** The cause and effects of acyl migration during the purification of specific structured lipids by distillation were studied in a conventional batch deodorizer with stripping steam. The mixture of specific structured lipids produced by lipase-catalyzed acidolysis between rapeseed oil and capric acid contained a large amount of free fatty acids and a small amount of partial acylglycerols besides triacylglycerols. Therefore, the effect of steam, free fatty acids, diacylglycerols, and monoacylglycerols on acyl migration was studied in a palm oil midfraction model. The results showed that all these factors influenced the rate of acyl migration, and their combinations made the effect more severe. However, diacylglycerols were found to be the main reason for acyl migration. In the distillation of the specific structured lipid product mixture, distillation temperature and time were the main factors to determine the degree of acyl migration and the extent of separation of free fatty acids. The results indicate that more efficient separation technology should be used to improve the quality of the purified structured lipids. In order to reduce the distillation temperature, vacuum should be made as low as possible with more effective pumps. To reduce the distillation time, thin-film principle in a packed column should be used, or other more efficient distillation techniques such as molecular distillation or short-path distillation should be exploited.

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**KEY WORDS:** Acyl migration, batch distillation, lipase-catalyzed acidolysis, specific structured lipids.

Production of structured lipids by lipase-catalyzed interesterification has recently attracted much attention from both academia and industry (1–4). The most exciting part of the technology is to exploit the regiospecificity of the lipases to produce tailor-made fats, which can be targeted for special nutritional requirements. The normal production method is to carry out an acidolysis reaction catalyzed by a lipase. The resulting product is a mixture of the expected structured lipids and free fatty acids (FFA), together with small amounts of partial acylglycerols. The purification of structured lipids is usually performed by distillation.

Acyl migration leads to nonspecific by-products. It occurs during lipase-catalyzed interesterification and is affected by factors such as water content, reaction time, reaction temperature, enzyme load, reactor type, and reaction system (5,6). To minimize acyl migration, it is necessary to choose the right reactor and to optimize the reaction system.

Acyl migration is also observed during the purification of specific structured lipids (SSL) by conventional distillation (7). It can be seen also that acyl migration occurs to a greater extent during the purification stage in the conventional batch deodorizer than in the reaction stage. No information so far has elucidated the phenomenon. This problem cannot be neglected with respect to the production of high-quality structured lipids.

In this study, two model distillations were conducted to study the causes of acyl migration and the parameters that influence acyl migration during the distillation of structured lipids produced in our pilot plant. The causes of acyl migration in palm oil midfraction (POMF) were elucidated by adding FFA or partial acylglycerols, or by pure steam stripping without adding anything. The effects of distillation parameters on the degree of acyl migration were studied with the reacted mixture of SSL produced in our pilot plant from rapeseed oil and capric acid by using a packed bed reactor and Lipozyme IM.

## MATERIALS AND METHODS

*Materials.* Refined, bleached, and deodorized (RBD) POMF and rapeseed oil were obtained from Aarhus Olie A/S (Aarhus, Denmark). Monoacylglycerols (MAG; Dimodan P), which contained a minimum of 90% monoesters, and diacylglycerols (DAG; HA 32-S3), which contained 30% monoesters and 70% diesters, were donated by Danisco Ingredients (Brabrand, Denmark). The fatty acid compositions of the products and oils are listed in Table 1. Capric acid (purity 99.6 mol%) was purchased from Henkel Kimianika (Selangor, Malaysia). Oleic acid (purity 78.5 mol%) was purchased from Riedel-de-Haen (Seelze, Germany). Lipozyme IM, in which *Rhizomucor miehei* lipase is immobilized on an anion exchange resin, was provided by Novo Nordisk A/S (Bagsvaerd, Denmark). All other chemicals and reagents for analysis were of analytical grade.

*Production of specific structured lipids*. Structured lipids were produced by Lipozyme IM-catalyzed interesterification between rapeseed oil and capric acid in a packed bed reactor. The methodology was described in an earlier publication (6).

*To whom correspondence should be addressed at BioCentrum–DTU, Technical University of Denmark, Building 221, DK-2800 Lyngby, Denmark. E-mail: xuebing.xu@biocentrum.dtu.dk

[1]Present address: Biofac A/S, Ejbyvej 44, 4683 Lille Skendsved, Denmark.

**TABLE 1**
**Fatty Acid Composition (wt%) and Distribution of the Oils Used in the Studies[a]**

| | POMF | | MAG | DAG | SSL[b] | |
|---|---|---|---|---|---|---|
| *sn* | 1,2,3 | 2 | | | 1,2,3 | 2 |
| C8:0 | | | | | 1.9 | |
| C10:0 | | | | | 43.5 | 6.6 |
| C12:0 | | | 0.3 | | | |
| C14:0 | 0.6 | | 2.0 | 1.2 | | |
| C16:0 | 61.0 | 11.1 | 28.8 | 46.9 | 2.6 | |
| C17:0 | 0.2 | | 0.8 | 0.1 | | |
| C18:0 | 5.9 | | 66.0 | 50.9 | 0.8 | |
| C18:1n-9 | 28.9 | 80.7 | | | 29.0 | 42.3 |
| C18:2n-6 | 3.1 | 8.1 | | | 14.7 | 34.0 |
| C18:3n-3 | | | 2.2 | 0.9 | 7.5 | 17.1 |
| C20:0 | 0.3 | | | | | |

[a]POMF, palm oil midfraction; MAG, monoacylglycerols (Dimodan P); DAG, diacylglycerols (HA 32-S3); and SSL, specific structured lipids produced from rapeseed oil and capric acid by Lipozyme IM-catalyzed acidolysis. Dimodan P and HA 32-S3 from Aarhus Olie A/S (Aarhus, Denmark); Lipozyme IM from Novo Nordisk A/S (Bagsvaerd, Denmark).
[b]The results show the triacylglycerols in the reacted mixture. The reacted mixture contained 44% triacylglycerols, 5–6% DAG, 50% free fatty acids, and minor amounts of MAG.

The product mixture after reaction contained 44% triacylglycerols (SSL), 5–6% DAG, 50% FFA, and minor MAG. The compositional characteristics of the SSL are given in Table 1.

*Deodorization methods.* The distillation experiments were performed in a 10-L batch deodorizer. The process scheme is given in Figure 1. The equipment contained temperature control, steam/nitrogen sources, and a receiver flask connected to a vacuum sensor and a condenser. Vacuum was provided by a pump connected to the condenser. Six liters of mixture were fed into the flask, the vacuum was adjusted to 5 ± 0.5 mbar, and the total stripping steam consumption was 2 ± 0.5 wt% based on the mixture. For the POMF-based distillation, the temperature was first raised to 60°C in 10 min. After vacuum was applied, the temperature was raised to 140–150°C in 10 ± 3 min. This temperature was maintained for 90 ± 10 min. Samples were withdrawn by breaking the vacuum with nitrogen. Then the temperature was increased to 210–230°C in 10 ± 2 min and maintained at this temperature for 4 h. Samples were withdrawn after the temperature had been reduced to 50°C. For the SSL-based distillation, the temperature was first raised to 60°C in 10 min. After vacuum was applied, the temperature was raised to 150°C in 10 ± 3 min. This temperature was maintained for 70–80 ± 10 min to remove medium-chain fatty acids. Then the temperature was increased to 200, 220, or 240°C in 10 ± 2 min according to the plan and maintained at this temperature for 0, 2, or 4 h. Samples were withdrawn each time during the distillation by breaking the vacuum with nitrogen.

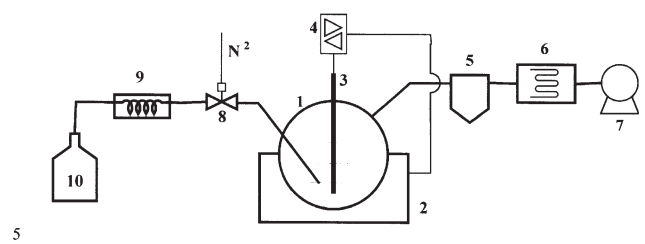


**FIG. 1.** Process scheme and configuration of the batch deodorizer for distillation studies. 1 Evaporation vessel and oil mixture, 2 Heater, 3 Temperature monitor, 4 Temperature/heater controller, 5 Distillate receiver, 6 Condenser, 7 Vacuum pump, 8 Three-way valve, 9 Steam heater, 10 Steam generator.

*Grignard degradation.* The same method with allyl magnesium bromide was used as described previously (5). The 2-MAG fractions were separated by thin-layer chromatography (TLC) after degradation.

*Fatty acid composition analysis.* The methods and procedures were described in a previous publication (5). The triacylglycerols in the samples were isolated by TLC. Sample mixtures, partial acylglycerols, oils, or the isolated triacylglycerols were methylated by the acidic method. The *sn*-2 MAG were methylated with potassium hydroxide. The fatty acid methyl esters were analyzed by gas chromatography (GC). The area percentages were recalculated into molar percentages based on the measured response factors and fatty acid molecular weights.

*FFA contents.* FFA content in rapeseed oil (wt%) and rapeseed oil content in FFA (wt%) were determined with standard alkali titration using phenolphthalein as indicator (8).

## RESULTS AND DISCUSSION

There has been no information so far regarding the acyl migration occurring during oil and fat refining and deodorization. Generally, little acyl migration is observed during the refining and deodorization of normal vegetable oils and fats (Xu, X., unpublished results). However, strong acyl migration was observed during the purification of SSL in a batch deodorizer (7). The mixture of SSL for purification is different from normal vegetable oils that contain small quantities of partial that acylglycerols and FFA.

To elucidate the causes of the acyl migration during the distillation of specific structured lipids, we used the POMF as the model for triacylglycerols. Effects of steam, FFA, MAG, DAG, or their combinations were studied. Five different oil mixtures were utilized in the experiment, as illustrated in Table 2, in order to elucidate the cause of acyl migration. The results, given in Table 3, show that steam alone did not induce much acyl migration of the POMF; however, when FFA were

**TABLE 2**
**Initial Experimental Mixtures of Oils Used for the Elucidation of Acyl Migration During Steam Distillation in the Batch Deodorizer[a] (wt%)**

| Experiment | POMF | DAG | MAG | FFA[b] | Total |
|---|---|---|---|---|---|
| Exp 1 | 100 | | | | 100 |
| Exp 2 | 43 | | | 57 | 100 |
| Exp 3 | 96 | | 4 | | 100 |
| Exp 4 | 86 | 10 | 4 | | 100 |
| Exp 5 | 37 | 4 | 2 | 57 | 100 |

[a]For abbreviations see Table 1.
[b]Free fatty acids, a mixture of capric and oleic acids (2:3, mol/mol).

**TABLE 3**
**The Change of the Major Fatty Acids Contained in POMF Triacylglycerols and Its *sn*-2 Position During Steam Distillation Under Different Batch Deodorizer Conditions[a]**

| | | Triacylglycerols | | | | *sn*-2 position | | | |
|---|---|---|---|---|---|---|---|---|---|
| Oils[b] | Conditions | C16:0 | C18:0 | C18:1 | C18:2 | C16:0 | C18:0 | C18:1 | C18:2 |
| POMF | | 61.0 | 5.9 | 28.9 | 3.1 | 11.1 | 0.0 | 80.7 | 8.1 |
| Exp 1 | 215°C (4 h) | 62.2 | 5.2 | 28.5 | 3.0 | 11.9 | 0.2 | 79.5 | 8.3 |
| Exp 2 | 152°C (1.5 h) | 60.8 | 5.7 | 29.2 | 3.2 | 11.5 | 0.0 | 80.1 | 8.4 |
| | 210°C (4 h) | 60.8 | 5.6 | 29.2 | 3.2 | 12.7 | 0.9 | 77.7 | 8.2 |
| Exp 3 | 140°C (1.5 h) | 62.6 | 6.8 | 26.5 | 3.0 | 11.5 | 2.9 | 77.3 | 8.0 |
| | 210°C (4 h) | 61.1 | 6.5 | 28.2 | 3.1 | 13.7 | 4.7 | 73.6 | 7.4 |
| Exp 4 | 140°C (1.5 h) | 60.7 | 7.7 | 27.5 | 3.0 | 17.1 | 7.9 | 67.5 | 6.9 |
| | 210°C (4 h) | 58.1 | 12.3 | 25.7 | 2.8 | 54.1 | 12.1 | 29.7 | 3.1 |
| Exp 5 | 150°C (1.5 h)[c] | 60.0 | 10.2 | 25.3 | 2.9 | 17.3 | 8.2 | 66.4 | 6.8 |
| | 230°C (4 h)[d] | 54.0 | 11.1 | 28.5 | 2.8 | 23.8 | 8.2 | 58.8 | 5.9 |

[a]For experimental procedure see the deodorization method for POMF-based distillation in the Materials and Methods section. For abbreviation see Table 1.
[b]See Table 2 for starting reactant mixtures used in Exp 1–5.
[c]C10:0 in the triacylglycerols (0.4%) and at the *sn*-2 position (1.2%).
[d]C10:0 in the triacylglycerols (2.5%) and at the *sn*-2 position (2.8%).

added, slightly higher levels of acyl migration occurred. The addition of 4% MAG into the mixture (Experiment 3) caused a 7–8% increase in palmitic and stearic acids in the *sn*-2 position, and the addition of a mixture containing 10% DAG + 4% MAG (Experiment 4) increased the two acids at the *sn*-2 position up to 56%. It can be concluded that DAG had a significant influence on the fatty acid distribution in the triacylglycerols during distillation compared to MAG alone. Of course, it could be also interpreted as the synergistic interaction between DAG and MAG, but it is more likely that DAG are more important for this phenomenon. One of the reasons is that DAG could be transformed more quickly into triacylglycerols than MAG with respect to the reaction steps, if reactions occur between partial acylglycerols and FFA during the distillation under high temperatures. Therefore, most of the acyl migration could come from the synthesized triacylglycerols from DAG during distillation because DAG mainly contain palmitic and stearic acids. Experiment 5, with the combination of POMF, DAG (4%), MAG (2%), and FFA (57%), was also found to produce a large increase of palmitic and stearic acids at the *sn*-2 position, from 11 to 35%, but not as high as that by adding 10% DAG. This again shows that DAG play a more important role leading to the higher acyl migration after distillation regardless of the reasons.

**TABLE 4**
**Acyl Migration and FFA Amounts Observed After the Steam Distillation of SSL Mixture in the Batch Deodorizer at Various Temperatures and Times[a]**

| Entry | Temperature (°C) | Time (h) | Acyl migration[b] (mol%) | FFA[c] (%) |
|---|---|---|---|---|
| 1 | 200 | 0 | 7.9 | 18.8 |
| 2 | 200 | 2 | 9.9 | 17.2 |
| 3 | 200 | 4 | 10.9 | 16.3 |
| 4 | 220 | 0 | 8.5 | 16.2 |
| 5 | 220 | 2 | 10.2 | 11.6 |
| 6 | 220 | 4 | 10.2 | 6.5 |
| 7 | 240 | 0 | 10.7 | 8.4 |
| 8 | 240 | 2 | 12.6 | 0.4 |
| 9 | 240 | 4 | 12.8 | 0.1 |

[a]For the experimental procedure see the deodorization method for specific-structured-lipids (SSL)-based distillation in the Materials and Methods section.
[b]The acyl migration was defined as the increase of capric acid at the *sn*-2 position of the triacylglycerols beyond the original capric acid content (6.6 mol%).
[c]Remaining FFA content after distillation. For abbreviation see Table 1.

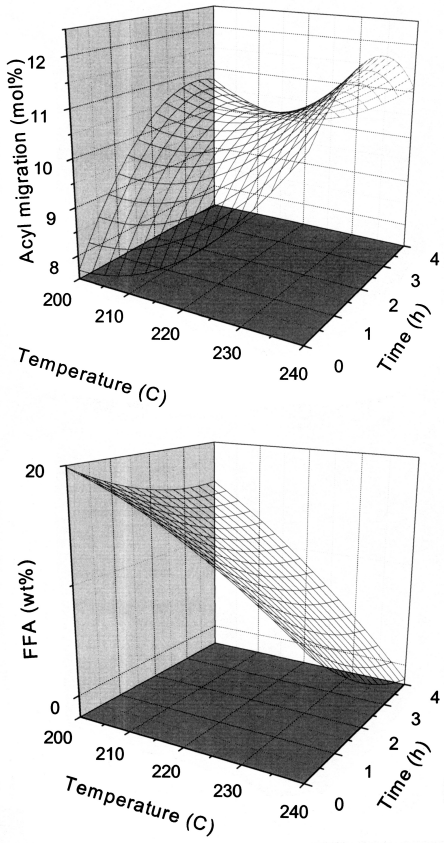


**FIG. 2.** Response surfaces of the acyl migration of capric acid into the *sn*-2 position (upper) and residual free fatty acid content (FFA) in the distilled products (lower) between temperature (°C) and time (h) for the steam distillation of specific structured lipids in a batch deodorizer.

DAG was previously identified as the main cause of acyl migration during lipase-catalyzed interesterification (5). The mechanism of acyl migration *via* DAG was described early, especially in an acidic environment (9). If DAG is the main component causing acyl migration, esterification or interesterification between DAG and FFA or between DAG and triacylglycerols must occur during the distillation, because the analysis of acyl migration was only on triacylglycerols. From Table 3, the fatty acid composition of the triacylglycerols after distillation was also changed; notably, the content of stearic acid increased from 6 to 11%, and the content of capric acid increased from 0.0 to 2.5% in the last experiment. This result supports the above assumption. It can therefore be speculated that reactions occurred between the partial acylglycerols, FFA, and POMF, probably *via* hydrolysis and esterification together with acyl migration. It is not clear, however, if interesterification occurs between each component in the mixture during the high-temperature distillation.

For the mixture of SSL produced in a pilot packed-bed reactor by the Lipozyme IM-catalyzed acidolysis between rapeseed oil and capric acid, the distillation conditions of the FFA removal in the same batch deodorizer were studied. The reacted mixture, containing 44% SSL, 5–6% DAG, 50% FFA, and minor MAG, was directly used for the experiment. The content of capric acid in the triacylglycerols of the product before distillation was 6.6 mol% (Table 1). Therefore, the increase of capric acid at the *sn*-2 position of triacylglycerols beyond 6.6 mol% was defined as acyl migration in this experiment. The remaining FFA after distillation were also monitored. The conditional setup for the experiments and the results of acyl migration and remaining FFA observed are given in Table 4. It appears that both temperature and distillation time influenced the acyl migration of capric acid; on the other hand, these two parameters had opposite influences on the residual FFA content in the distilled products. This can be further illustrated by two surface plots generated from the setup and results in Table 4 (Fig. 2). The surface plots were generated as a full design without star points (two factors and three levels) assisted by Modde 4.0 (Umetri AB, Umeå, Sweden). Higher temperature and longer time increased the extent of acyl migration, whereas they decreased the content of FFA in the distilled products. It is clear that acyl migration cannot be totally avoided and a compromise has to be made in such a deodorizer in terms of the extent of acyl migration and FFA content.

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