Fractionation of Chicken Fat Triacylglycerols: Synthesis of Structured Lipids with Immobilized Lipases

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ABSTRACT: Chicken fat was temperature-fractionated either without or with solvent, and by supercritical carbon dioxide extraction to produce triacylglycerol (TAG) fractions of varying monounsaturated fatty acid (MUFA) content. Solvent fractionation from acetone at low temperature (–38 and –18 °C) was the most effective process for enriching the MUFA-containing TAG of chicken fat in the liquid fractions. Caprylic acid was incorporated into the MUFA-enriched TAG fraction in lipase-catalyzed acidolysis reactions to produce structured lipids (SLs). Immobilization of the lipase within sol-gel phyllosilicate matrices allowed for the reusability of both lipases.

Key Words: fractionation, chicken fat, immobilization, phyllosilicates, structured lipids, enzymes

Introduction

MONOUNSATURATED FATTY ACIDS (MUFA), SUCH AS OLEIC acid (*cis*-9-octadecenoic acid), are known to reduce blood cholesterol levels in nonhypertriglyceridemic individuals (Mattson and Grundy 1985). Among vegetable oils, those of olive, peanut, rapeseed, and canola have been identified as being rich sources of MUFA, with the latter type fatty acids constituting 50% to 80% of their fatty acid composition. Because of the importance placed on dietary MUFA, it has been recommended that MUFA intake be as high as half of the of total recommended dietary intake of calories from fat (30%) as a means for reducing the risk of coronary artery disease (Nicolosi and others 1991; Bockisch 1998; Lee and Akoh 1998a).

In 1998, chicken was the most produced and consumed meat in the United States (USDA 1999). Despite its production and ready availability as a coproduct of chicken production, chicken fat, unlike beef tallow, is usually not used separately in other food or nonfood uses. Animal fats, in general, are of dietary concern because of their relatively high long-chain (C16 and C18 carbon atoms) saturated fatty acid (SFA) content. Chicken fat can be considered a source of MUFA since they constitute 45% to 50% of chicken fat fatty acids, while tallow contains only 30% to 40% MUFA (Brockerhoff and others 1966; Bockisch 1998).

Saturated medium-chain fatty acids (MCFA) contain C8 to C12 carbon atoms. Because of this, they are of lower caloric content than saturated long-chain fatty acids (LCFA). Since the adsorption of MCFA occurs by transport through the portal system, they are metabolized more rapidly than LCFA and thus are utilized in dietary energy supplements. Examples of the dietary use of MCFA include the treatment of fat malabsorption disorders, hyperlipidemia, obesity, and diabetes (Akoh and others 1998).

The lipase-catalyzed modification of natural fats and oils into structured lipids (SLs) has become an important topic in lipid chemistry. The general definition, application and nutritional properties of SLs have been described previously (Willis and others 1998; Lee and Akoh 1998a). When SLs are synthesized from animal fats for food uses, it often is desirable to reduce the saturated LCFA content of the starting fat and increase the MUFA and PUFA content as a means of improving the nutritional quality of the targeted structured lipid. This is done because LCFA, especially palmitic acid, are regarded as one risk factor in the development of coronary artery disease.

In this study, chicken fat was either temperature fractionated without solvent, fractionally crystallized from acetone, or extracted with supercritical carbon dioxide to produce MUFA-enriched triacylglycerol (TAG) fractions (MUFA-TAG). Selected liquid MUFA-TAG fractions were then subjected to enzyme-catalyzed acidolysis with caprylic acid. Lipases of *G. candidum* and *C. rugosa*, immobilized within phyllosilicate sol-gel matrices, were used as the biocatalysts in this process. This was done to produce SLs that would combine the beneficial dietary effects of the MUFA and MCFA classes of fatty acids.

Results and Discussion

TEMPERATURE FRACTIONATION OF A FAT OR OIL CAN BE REGARDED as a thermomechanical separation process wherein individual TAG species characteristic for a given fat or oil are selectively crystallized from the melt or liquid phase. During cooling of the liquid oil or melted fat, TAG species with the highest melting points preferentially crystallize, resulting in a solid phase within the liquid phase. Most natural fats and oils are complex mixtures of individual TAG that can contain from one to three different fatty acyl residues on their glycerol backbone. Because of this, there is a large variation in the melting points of the TAG species, which complicates the fractionation process.

In this study, temperature-fractionation of chicken fat over the range of 14 to 30 °C, as exemplified at 21 °C in Fig. 1, without solvent, resulted in a poor enrichment of MUFA-containing TAG in the isolated TAG fractions. This was concluded since there were little or no statistical differences in the FAME compositions of the isolated fractions (Table 1). This is because chicken fat is a complex mixture of TAG species having a wide range of molecular weights and degrees of unsaturation (Brokerhoff and others 1966). Figure 1 illustrates the temperature fractionation of chicken fat into liquid and solid fractions at 21 °C. The solid fraction was then subfractionated at 30 °C, after which the liquid fraction (21S30L) was subfractionated at 13 °C. The liquid fraction from the 21 °C fractionation was subfractionated at 6 °C and the solid fraction obtained therefrom (21L6S) was further subfractionated at 9 °C. Even though the MUFA content of several fractions (for example, fractions 21L6L and 21L6S9L, Table 1) increased significantly ($p \leq 0.05$) from that of neat chicken fat, the recovery (wt %

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Table 1 — Fatty acid composition (wt%) of chicken fat fractionated without solvent at various temperatures

FA	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:3	MUFA[a]	PUFA[a]	SFA[a]
Chicken fat	0.7	0.2	25.2	7.8[hi]	5.9	40.5	18.4	0.7	0.1	0.5	0.1	49.0[fg]	19.2[g]	31.9[ef]
21L [b]	0.7	0.3	23.6	8.1[ghi]	5.2	41.5	19.1	0.8	0.1	0.5	0.1	50.4[ef]	20.0[fg]	29.6[fg]
21S [b]	0.8	0.2	27.9	7.5[hi]	6.5	38.2	17.4	0.7	0.1	0.5	0.2	46.4[fg]	18.3[hi]	35.3[e]
21S30S[c]	1.0	0.3	32.6	7.8[hi]	6.8	33.3	16.6	0.7	0.1	0.6	0.2	42.0[h]	17.5[i]	40.5[d]
21S30L	0.8	0.3	25.4	8.9[efg]	5.0	39.2	18.9	0.8	0.1	0.5	0.2	48.9[fg]	19.9[fg]	31.3[f]
21L6S	0.8	0.4	24.1	9.1[d]	4.7	39.8	19.4	0.9	0.2	0.5	0.2	49.8[fg]	20.5[f]	29.8[fg]
21L6L	0.8	0.4	18.7	10.0[de]	3.5	43.2	21.6	1.0	0.1	0.6	0.2	54.2[d]	22.8[d]	23.1[i]
21S30L13S	0.7	0.2	28.2	7.3[i]	6.7	38.5	17.1	0.7	0.1	0.4	0.1	46.4[fg]	17.9[hi]	35.7[e]
21S30L13L	0.7	0.3	21.6	8.4[fgh]	4.8	42.6	20.0	0.8	0.1	0.5	0.2	51.8[def]	21.0[ef]	27.2[gh]
21L6S9S	0.7	0.3	25.5	7.7[hi]	5.7	40.2	18.2	0.8	0.4	0.5	0.1	48.7[fg]	19.1[gh]	32.3[ef]
21L6S9L	0.7	0.3	19.1	9.0[def]	4.0	44.1	21.1	0.9	0.1	0.5	0.2	53.9[de]	22.2[de]	23.9

[hia]MUFA is sum of monounsaturated fatty acids; PUFA is sum of polyunsaturated fatty acids; SFA is sum of saturated fatty acids.
[b]21L and 21S are recovered liquid and solid fraction, respectively, after fractional crystallization at 21°C for 24h.
[c]21S30S is recovered solid fraction after fractionation at 21°C for 24 h followed by fractionation at 30°C, respectively (Figure 1).
[d-i] Mean values within the same column having a similar subscript do not differ significantly ($p \leq 0.05$).

of fraction, Fig. 1) was poor. Moreover, for these fractions only the palmitoleic acid content was statistically different from that of neat chicken fat for the fractionation temperatures studied (Table 1). The concentration of the MUFA-containing TAG in the liquid fractions also was accompanied by an increase in PUFA-containing TAG ($p \leq 0.05$) and, as expected, a concomitant decrease in SFA-containing TAG ($p \leq 0.05$). The fractionation of chicken fat also was studied at 14 and 30 °C including the subfractionation temperatures shown in Fig.1. These fractionation temperatures, however, yielded fractions whose fatty acid compositions were not significantly different from chicken fat. Because of the low enrichment of MUFA in TAG fractions from temperature-fractionation, we turned our attention to the solvent fraction of chicken fat.

It is known that TAG at low temperature generally form more stable crystals from a solvent than without solvent. Among solvents, acetone is regarded as more suitable for promoting TAG crystal formation than any other solvents (Yokochi and others 1990). However, compared to solvent-free fractional crystallization, solvent-fractionation processes are more complicated in that they typically require lower temperatures for crystallization to occur, there is a need to recover the solvent after fractionation, and regulatory solvent residue levels must be met. Currently, the maximum permissible residue level for acetone is 30 ppm (Code of Federal Regulations 21CFR173.210). These added steps result in a more expensive process, especially when dealing with large amount of materials.

When low temperature (−38 and −18 °C) acetone crystallization was used for fractionation of chicken fat, there was an increase ($p \leq 0.05$) in the MUFA and PUFA content of the liquid fractions (Table 2). In general, the MUFA content of the liquid fractions increased from that of neat chicken fat to between 14.3% to 17.8% at −38 °C and 16.1% to 22.0% at −18 °C, respectively. In addition, lowering the temperature from −18 to −38 °C resulted in a significant increase in the PUFA content of the liquid fractions (Table 2). Depending on the solvent ratio used, the liquid fractions at −38 °C increased in PUFA content in the range of 64.6% to 91.7%. On the other hand, as expected, the content of SFA decreased in these fractions as the temperature of crystallization was lowered. Solid fractions at 0 °C were composed of 49.9% to 62.3% SFA, whereas the liquid fractions at −38 °C contained only 7.2% to 10.8% SFA. Other studies have shown that higher solvent ratios and lower crystallization temperatures were necessary for the best separation of SFA from soybean oil (Bull and Wheeler 1943). In this study, the SFA content of the fractions varied with the solute-to-solvent ratio used for fractionation. However, varying the solvent ratios at the fractionation temperatures used in this study did not result in increased enrichment of

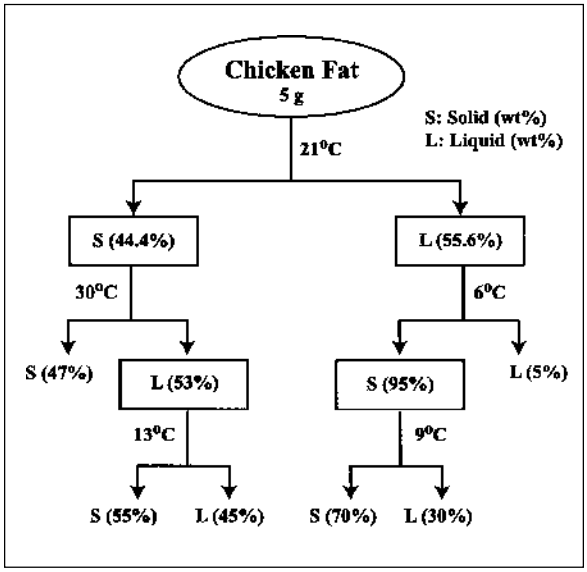

Fig. 1—Protocol for temperature fractionation of chicken fat without solvent

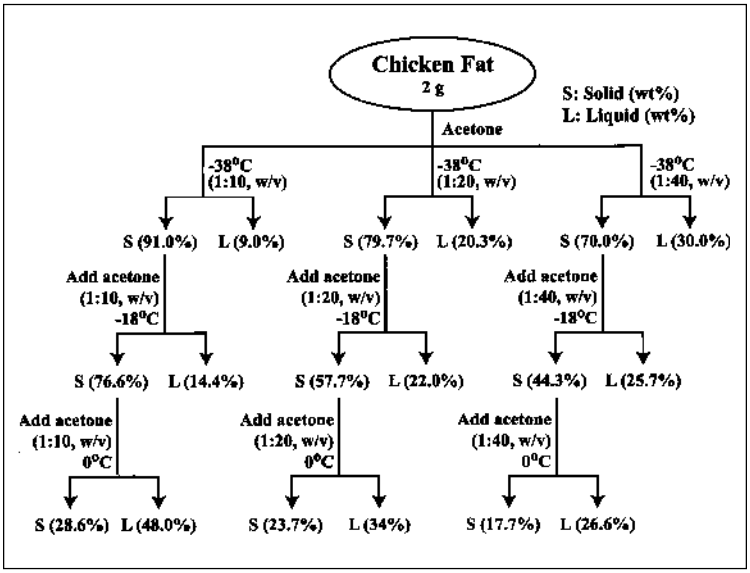

Fig. 2—Protocol for fractional crystallization of chicken fat from acetone

Table 2—Fatty acid composition (area %) of chicken fat fractions after solvent fractionation at low temperature from acetone[a]

FA	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:3	MUFA[b]	PUFA[b]	SFA[b]	Yield[c]
CF[d]	0.7	0.2^{fg}	25.2^{no}	7.8^{hijk}	5.9^{jklm}	40.5^{hij}	18.4^{jk}	0.7^{fgh}	0.1	0.5	0.1	49.0^{hi}	19.2^{jk}	31.9^{mn}	—
–38L10[e]	0.6	0.6^{f}	5.4^{r}	13.3^{f}	1.1^{p}	41.6^{hi}	35.0^{f}	1.6^{f}	0.1	0.5	0.2	56.0^{fg}	36.8^{f}	7.2^{r}	9.0
–38L20	0.6	0.5^{fg}	6.3^{r}	12.6^{f}	1.4^{p}	44.2^{fgh}	32.4^{g}	1.4^{fg}	—	0.4	0.2	57.7^{f}	34.0^{fg}	8.3^{r}	20.3
–38L40	0.7	0.5^{fg}	8.3^{qr}	12.0^{f}	1.7^{op}	44.7^{fgh}	30.1^{g}	1.3^{fgh}	0.1	0.5	0.2	57.7^{f}	31.6^{g}	10.8^{qr}	30.0
–38S10	0.7	0.2^{fg}	29.2^{l}	6.7^{jklm}	6.8^{hijkl}	39.6^{hij}	15.3^{lm}	0.6^{fgh}	0.1	0.5	0.2	47.0^{hi}	16.1^{lm}	36.8^{lm}	91.0
–38S20	0.6	0.2^{fg}	27.7^{lmn}	7.2^{ijkl}	6.9^{hijklm}	37.2^{ijk}	18.9^{jk}	0.5^{gh}	0.1	0.4	0.2	45.0^{ijkl}	19.6^{jk}	35.3^{lm}	79.7
–38S40	0.7	0.2^{fg}	25.9^{mno}	7.3^{ijkl}	6.2^{jklm}	37.8^{ijk}	20.5^{ij}	0.6^{fgh}	0.1	0.4	0.3	45.7^{ijk}	21.4^{ij}	32.9^{mn}	70.0
–18L10	0.7	0.3^{fg}	10.7^{q}	9.9^{g}	2.8^{nop}	49.0^{f}	24.6^{h}	1.0^{fgh}	0.1	0.6	0.2	59.8^{f}	25.8^{h}	14.3^{pq}	15.8
–18L20	0.7	0.3^{fg}	14.7^{p}	9.3^{gh}	3.7^{mnop}	47.0^{fg}	22.5^{hi}	0.9^{fgh}	0.1	0.6	0.2	57.2^{f}	23.6^{hi}	19.2^{op}	27.6
–18L40	0.6	0.3^{fg}	16.7^{p}	8.6^{ghi}	4.3^{lmno}	47.4^{fg}	20.4^{ij}	0.8^{fgh}	0.1	0.6	0.2	56.9^{f}	21.4^{ij}	21.7^{o}	36.8
–18S10	0.7	0.2^{fg}	40.2^{h}	5.9^{lmn}	9.5^{gh}	33.6^{klm}	8.8^{q}	0.4^{gh}	0.1	0.4	0.2	39.8^{lm}	9.4^{pq}	50.8^{gh}	84.2
–18S20	0.7	0.2^{fg}	36.6^{ij}	5.7^{lmn}	8.3^{ghij}	35.0^{jkl}	12.3^{o}	0.4^{gh}	0.1	0.4	0.2	41.3^{klm}	12.9^{no}	45.7^{ij}	72.4
–18S40	0.6	0.3^{fg}	34.6^{jk}	4.9^{no}	7.9^{hijk}	35.7^{jkl}	14.7^{mn}	0.5^{gh}	0.1	0.4	0.3	41.3^{klm}	15.5^{lmn}	43.2^{jk}	63.2
0L10	0.7	0.2^{fg}	23.9^{o}	7.8^{hij}	5.0^{klmn}	43.3^{gh}	17.6^{kl}	0.7^{fgh}	0.1	0.5	0.1	51.8^{gh}	18.4^{kl}	29.7^{n}	62.2
0L20	0.7	0.2^{fg}	28.5^{lm}	6.9^{jklmn}	5.8^{jklm}	41.7^{hi}	14.9^{mn}	0.5^{gh}	0.1	0.4	0.1	49.2^{hi}	15.5^{lmn}	35.1^{lm}	58.9
0L40	0.8	0.2^{fg}	32.4^{k}	6.1^{klmn}	6.4^{ijklm}	39.9^{hij}	13.0^{no}	0.5^{gh}	0.1	0.4	0.2	46.6^{hij}	13.7^{mno}	39.7^{kl}	59.3
0S10	0.8	0.2^{fg}	39.2^{hi}	5.4^{mno}	9.8^{ghi}	31.8^{lm}	11.9^{op}	0.4^{gh}	0.1	0.3	0.1	37.7^{mn}	12.4^{no}	49.9^{hi}	37.8
0S20	0.7	0.1^{g}	43.4^{g}	4.6^{no}	11.2^{fg}	29.3^{mn}	9.6^{pq}	0.3^{h}	0.2	0.4	0.1	34.4^{no}	10.0^{pq}	55.5^{g}	41.1
0S40	0.8	0.1^{g}	48.0^{f}	3.7^{o}	13.1^{f}	25.5^{n}	7.7^{q}	0.3^{h}	0.1	0.3	0.1	29.6^{o}	8.1^{q}	62.3^{f}	40.7

[a]Fractionation temperatures were 0, −18, and –38 °C; solvent ratios used were 1:10; 1:20; and 1:40 (w:v).
[b]MUFA is sum of monounsaturated fatty acids; PUFA is sum of polyunsaturated fatty acids; SFA is sum of saturated fatty acids.
[c]Weight % recovery of liquid and solid fractions at same temperature and solvent ratio (fo example −38L10 + −38S10 is equal to 100%).
[d]CF is chicken fat.
[e]-38L10 is liquid fraction recovered by solvent crystallization of chicken fat from acetone (1:10, w/v) at –38 °C. Other recovered fractions similarly designated.
[f-r] Mean values within the same column having a similar superscript do not differ significantly ($p \leq 0.05$).

the MUFA-content of the fractions (Table 2).

In contrast to temperature-fractionation of chicken fat, the weight percent recovery of TAG from the liquid fractions in solvent-fractionation (Fig. 2) was higher depending on the solute-to-solvent ratio and temperature used for fractionation. More importantly, the saturated fatty acid content for several of the liquid fractions produced by solvent-fractionation was significantly reduced ($p \leq 0.05$), compared to chicken fat. For the chicken fat used in this study, the sum of unsaturated (mono- and polyunsaturated) fatty acids (UFA) to SFA ratio was approximately 68:32 (Table 1). Temperature-fractionation of the chicken fat gave liquid fractions (Table 1) that had unsaturated-to-saturated fatty acid ratios as low as 77:23, an SFA reduction of about 25%. For solvent-fractionation (Table 2), liquid fractions were obtained that had UFA:SFA ratios as low as 93:7, a SFA reduction of about 75%. Because of their lower SFA content, the latter fractions were chosen in this study for the production of structured lipids.

Supercritical fluid extraction (SFE) technology has received increased attention because of its advantages over conventional solvent-extraction processes. Because the critical temperature of carbon dioxide is around 31 °C, extractions can be performed at moderate temperature without chemical solvent. In this study, SFE fractions of 0.7, 0.4, and 6.8 g were recovered from 11 g of chicken fat at three different pressures (4.7, 11, and 24 MPa, at 40 °C) of supercritical carbon dioxide. Previously, other studies successfully fractionated anhydrous milk fat and tallow under supercritical conditions (Bhaskar and others 1993; Merkle and Larick 1995). With the extraction conditions used in this study, however, only minor fractionation of chicken fat was obtained since the fatty acid profiles for the extracted fractions were similar to that of the starting material.

From the results of various fractionation schemes explored in this study, chicken fat and enriched MUFA-TAG fractions (for example, fraction -38L20, abbreviation in Table 2) obtained therefrom were used for the production of SLs. This was done by lipase-catalyzed acidolysis of the TAG with caprylic acid. After acidolysis, 5.8% to 8.1% of caprylic acid was incorporated into the TAG molecules of the synthesized SLs (Table 3). Incorporation of caprylic acid into the chicken fat and chicken fat fractions was intentionally kept low (>10%), since the goal was to prepare a SL wherein the long-chain SFA acyl groups at the 1,3 positions of the original TAG species were replaced preferentially with capryloyl residues. The low efficiency of incorporation of caprylic acid (approx. 8%) was not of concern since it can be readily recovered for reuse (Akoh and others 1998). The data in Table 3 show that the content of several fatty acids in the SL products differed significantly ($p \leq 0.05$) when compared to the starting fats. When acidolysis was catalyzed with the immobilized lipase from *G. candidum*, the products from the fractionated chicken fat contained 25.3% more MUFA and 65.7% less palmitic acid, the major SFA in chicken fat, than that of unfractionated chicken fat. Of the 18% to 24% total SFA in the product from the fractionated chicken fat, caprylic acid constituted 24% to 42% of the SFA. This is to be compared to the products prepared from unfractionated chicken fat where total SFA content ranged between 38% to 40% with caprylic acid constituting only 20% to 21% of total SFA (Table 3).

The acidolysis of chicken fat with caprylic acid catalyzed by

Table 3—Fatty acid composition (area %) of products obtained by the lipase-catalyzed acidolysis of neat or fractionated chicken fat with caprylic acid

			C. rugosa[a]		*G. candidum*[b]	
Fatty acid	Chicken fat	Fractionated chicken fat	Product from chicken fat	Product from fractionated chicken fat	Product from chicken fat	Product from fractionated chicken fat
	nd[d]	nd	7.6	5.8	8.1	7.8
C14:0	0.7	0.6	0.5	0.5	1.0	0.6
C14:1	0.2	0.5	0.4	0.5	0.3	0.3
C16:0	25.2^{f}	6.3^{g}	23.5^{f}	14.2^{g}	23.9^{f}	8.2^{g}
C16:1	7.8^{g}	12.6^{f}	7.1	7.9	6.1^{g}	9.6^{f}
C18:0	5.9^{f}	1.4^{g}	6.8^{f}	3.6^{g}	6.2^{f}	2.1^{g}
C18:1	40.5	44.2	36.3^{g}	42.2^{f}	37.1^{g}	44.4^{f}
C18:2	18.4^{g}	32.4^{f}	16.1^{g}	23.8^{f}	15.9^{g}	25.4^{f}
C18:3	0.7	1.4	0.9	0.8	1.0	1.0
C20:0	0.1	—	0.03	nd	nd	nd
C20:1	0.5	0.4	0.7	0.6	0.3	0.5
C20:3	0.1	0.2	0.05	0.1	0.05	0.1
Σ MUFA[e]	49.0^{g}	57.7^{f}	44.5^{g}	51.2^{f}	43.8^{g}	54.9
Σ PUFA[e]	19.2^{g}	34.0^{f}	17.1^{g}	24.7^{f}	17.0^{g}	26.5^{f}
Σ SFA[e]	31.9^{f}	8.3^{g}	38.4^{f}	24.1^{g}	39.2^{f}	18.7^{g}
%C8 in Σ SFA	nd	nd	19.8^{g}	24.1^{f}	20.7^{g}	41.7^{f}

[a]Reaction with lipase from *C. rugosa* immobilized within phyllosylicates.
[b]Reaction with lipase from *G. candidum* immobilized within phyllosylicates.
[c]Fractionated chicken fat used for reaction was –38L20 (abbreviation in Table 2).
[d]nd, not detected.
[e]MUFA, PUFA, and SFA, see abbreviations in Table 2.
[f-g]Mean values having different superscripts within a row for chicken fat:fractionated chicken fat and each enzyme-catalyzed reaction for each differ significantly ($p \leq 0.05$).

the immobilized lipases in general was favored at higher temperature (Fig. 3). The *G. candidum* lipase gave the highest incorporation of caprylic acid at 55 °C (7.8%), while for the *C. rugosa* lipase 65 °C (5.9% incorporation) was the optimal temperature—but the difference between the reactions was not significant ($p \leq 0.05$). At 35 °C, the incorporation of caprylic acid decreased by 52.1% and 38.3%, compared to the reaction at 55 °C for the *G. candidum* and 65 °C for the *C. rugosa* lipases, respectively.

With this immobilization procedure, lipases are intercalated within dispersed phyllosilicate layers, which are subsequently crosslinked with silicate polymers formed by the controlled hydrolysis of tetramethyl orthosilicate, forming the sol-gel matrix (Hsu and others 1998). Immobilization within this matrix stabilizes the lipase, which allows for reuse in subsequent reactions in contrast to other physical adsorption methods (Lee and Akoh 1998b). In this study, both immobilized lipases were reused for the acidolysis of chicken fat fractions with caprylic acid (Fig. 4). Only a 4.5% (*G. candidum lipase*) decrease in incorporation of caprylic acid was noted after 3 sequential uses of this immobilized lipase ($p \leq 0.05$). After the 4th reaction, however, the incorporation of caprylic acid into the MUFA-TAG fractions by the immobilized *G. candidum* and *C. rugosa* lipases decreased by 19.2% and 25.6%, respectively.

Conclusion

THROUGH THE FRACTIONATION OF CHICKEN FAT, SEVERAL MUFA-TAG-enriched fractions were obtained that had desirable fatty acid compositions for the production of structured lipids (SLs). Acetone fractionation at low-temperature (−38 °C and −18 °C) was most effective for enriching of the MUFA and PUFA containing TAG of chicken fat. SLs were synthesized by lipase-catalyzed acidolysis of chicken fat and MUFA-TAG enriched fractions with caprylic acid. Immobilization of lipases within a sol-gel phyllosilicate matrix gave a stable immobilized lipase that could be reused for the production of the SLs.

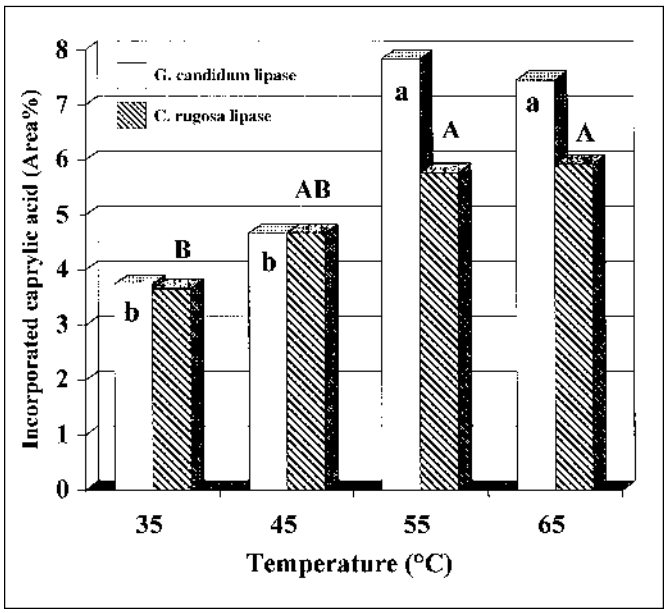

Fig. 3 — Effect of temperature on the lipase-catalyzed incorporation of caprylic acid into fractionated chicken fat (fraction-38L20, Table 2). Percent caprylic acid incorporated with immobilized *G. candidum* lipase; immobilized *C. rugosa* lipase. Values with different letter designations (*G. candidum* lipase: a, b; or *C. rugosa* lipase: A, B) are significantly different ($p \leq 0.05$).

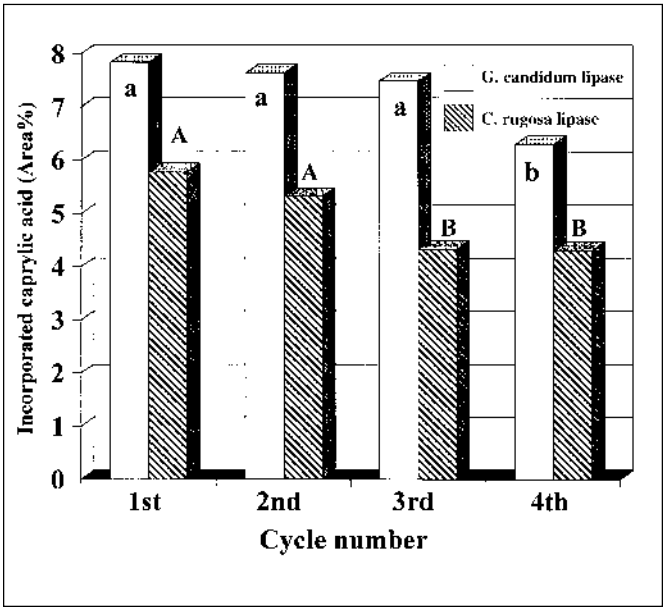

Fig. 4 — Reusability of immobilized lipases of *G. candidum* and *C. rugosa* in lipase-catalyzed acidolysis of fractionated chicken fat (fraction -38L20, Table 2) with caprylic acid at 55 °C. Percent caprylic acid incorporated with immobilized *G. candidum* lipase; immobilized *C. rugosa* lipase. Values with different letter designations (*G. candidum* lipase: a, b: or *C. rugosa* lipase: A, B) are significantly different ($p \leq 0.05$).

Materials and Methods

Materials

Chicken fat was a gift to the authors from Tyson Foods Inc. (Springdale, Ark., U.S.A.). Lipases used in this study were from *G. candidum* (Amano Enzyme Inc., Troy, Va., U.S.A.) and *C. rugosa* (Enzeco, New York, N.Y., U.S.A.). Cetyl trimethylammonium chloride (CTAC) and tetramethyl orthosilicate (TMOS) were purchased from Aldrich Chemical (Milwaukee, Wis., U.S.A.). The phyllosilicate clay was obtained from Source Clay Minerals Depository, Columbia, Mo. Sodium fluoride (NaF) and caprylic acid were obtained from Sigma (St. Louis, Mo., U.S.A.) Acetone, HPLC analytical grade, was obtained from Baxter Health Corp. (Muskegon, Mich., U.S.A.).

Fractionation of chicken fat triacylglycerols

Temperature fractionation. Chicken fat (5 g) was placed into a 50-mL polypropylene centrifuge tube and incubated at various temperatures (14 to 30 °C) for 24 h. The crystals were pelleted in a centrifuge (7650 × g, 10 min) adjusted to the fractionation temperature and the liquid phase decanted from the crystallized TAG. The fractionation process at 21 °C, as well as the subfractionation of the initially obtained solid and liquid TAG fractions, is shown in Fig.1. In all examples, the chicken fat or isolated TAG fractions were held at 80 °C for 10 min before fractionation to remove memory effects of polymorphic

TAG forms.

Acetone fractionation. Chicken fat (2 g) was placed into a 50-mL polypropylene centrifuge tube and fractionally crystallized from acetone solvent. Three solute-to-solvent ratios (1:10, 1:20, and 1:40; chicken fat: acetone; wt/v) were employed, each at three temperatures (−38, −18, and 0 °C) (Table 2). For all fractionations, each centrifuge tube was placed in an insulated 250-mL wide-mouth centrifuge bottle to minimize temperature changes during centrifugation. All fractions were incubated for 24 h. After centrifugation (2300 × g, 15 min) in the prechilled centrifuge, the liquid and solid phases were separated by decantation of the liquid phase from the crystal pellet. Acetone was evaporated from the fractions at 60 °C under nitrogen. Each recovered solid fraction at −38 °C was subsequently subfractionated as outlined in Fig. 2.

Supercritical fractionation. A Supercritical Fluid Extractor, Model 680 BAR (Applied Separations Inc., Allentown, Pa., U.S.A.) was used for the supercritical extraction experiments. Chicken fat (11 g) was loaded into a [15 cm x 1.5 cm (i.d.)] stainless steel extraction vessel packed with hydromatrix (Varian Inc., Harbor City, Calif., U.S.A.). Supercritical carbon dioxide was used as the extraction solvent at a flow of 1L/min. The extraction was performed stepwise over the range of 4.7, 11, and 24 MPa at 40 °C. Extraction time was 60 min at each pressure condition. The extracted fractions were collected separately.

Fatty acid analysis

Fatty acid composition of chicken fat and triacylglycerol (TAG) fractions obtained from it were determined by gas chromatography (GC) after conversion to fatty acid methyl esters (FAME) with 14% boron trifluoride (BF_3) in methanol (Foglia and others 1993). FAME compositions were obtained with a Hewlett-Packard Model 5890 Series II GC equipped with a split automatic injector, a flame ionization detector (FID), and an HP-INNOWAX column (30 m x 0.25 mm, 53 m film thickness) (Hewlett-Packard, Wilmington, Del., U.S.A.). The column was held at 120 °C for 2 min, then programmed to 230 °C at a rate of 5 °C/min and held at a final temperature for 22 min. The injector and detector temperatures were 260 °C and the carrier gas was helium at a flow of 5.5 mL/min. A Hewlett Packard Model 5890 Series II GC with an HP Mass Selective Detector (MSD) Model 5972 Series was used for identification of FAME. The MSD was scanned from m/z 10 to m/z 600 at 1.2 scans per sec. An HP-5 capillary column (30 m x 0.25 mm i.d., 25 μm film thickness) was used to separate FAME. The column was held at 80 °C for 2 min and programmed to 230 °C at a rate of 10 °C/min. The carrier gas was helium at a flow of 1 mL/min at a split ratio of 50:1. The injector and detector temperatures were 230 °C and 280 °C, respectively.

Lipase immobilization

Lipases were immobilized within phyllosilicates by the method of Hsu and others (1998). Enzyme solutions were prepared by mixing crude lipase powder (1.0 g) in 10 mL of 0.1 M Tris-HCl buffer (pH 7.5). CTMA (600 μL) was mixed with 4.4 mL of 3.3% by wt sodium-ion saturated clay suspension. Then 3.1 mL enzyme solution, 246 μL deionized water, 1046 μL TMOS, and 1 mL 10% NaF solution were added, and the mixture was vortexed for 2 min and placed onto ice for 2 h. The crosslinked lipase-phyllosilicate complex was kept at room temperature (24 ± 1 °C) overnight. Ten mL deionized water was added, and the mixture vortexed for 2 min. After filtration, the supernatant was used for protein analysis and the filtered immobilized lipase was dried under vacuum for 48 h at 24 ± 1 °C.

Table 4—Protein content and water activity of lipases immobilized within phyllosilicate sol-gel matrix

	C. rugosa	*G. candidum*
Lipolytic activity of crude lipase[b]	89.9	119.2
Total protein before immobilization (mg)	29.1	36.1
Amount of protein immobilized (mg)	26.2	33.2
Lipolytic activity of immobilized lipase[c]	27.2	34.2
Water activity of dried immobilized lipase	0.1	0.1

[a]mg protein/ mL crude lipase solution.
[b]One unit of lipolytic activity is defined as mmole of fatty acid released from olive oil per min per mg of crude lipase.
[c]One unit of lipolytic activity is defined as μmole of fatty acid released from olive oil per min per 100 mg of immobilized lipase.

Protein and water determination. A modified Lowry assay was used to measure the protein concentration before and after immobilization (Bensadoun and Weinstein 1976). Protein content was measured at 660 nm using a standard curve for bovine serum albumin. Water activity (a_w) of the immobilized lipases was determined with an Aqualab CX-2 (Decagon, Devices Inc., Pullman, Wash., U.S.A.).

Lipase activity. Lipolytic activity of the immobilized lipases was measured by titration of the fatty acids released from olive oil with 0.05 N NaOH using a VIT 90 Video Titrator (Radiometer, Copenhagen, Denmark). The prepared olive oil emulsion was composed of 20 mL of deionized water, 9 mL of olive oil, and 21 mL of 10% gum arabic solution. Lipolytic activity was determined by vigorously mixing 110 ± 2 mg of immobilized lipase with the emulsion for 30 min at 30 °C. One unit of lipase activity is defined as mole of fatty acids liberated per minute per mg of immobilized lipase. The lipolytic activity of the immobilized *C. rugosa* and *G. candidum* lipases were 27.2 and 34.2 U, respectively (Table 4).

Synthesis of structured lipids

Acidolysis reactions were conducted by reacting 1.0 g of a chicken fat fraction with 1.2 g of caprylic acid (approx. 1:2 molar ratio) in the presence of 700 mg of immobilized lipase. Chicken fat before and after fractionation (−38L20, abbreviations listed in Table 2) were transesterified. Reactions were performed in a screw cap vial with magnetic stirring at 200 rpm for 30 h. To assess the effect of temperature on caprylic acid incorporation, a series of acidolysis reactions were run at 35, 45, 55, and 65 °C. To determine enzyme stability, the 55 °C acidolysis sample was centrifuged, the immobilized lipase recovered, and fresh substrates added for subsequent reactions at 55 °C. Reaction products were analyzed by spotting 100 μL of reactants onto silica gel G thin layer chromatography plates (500 m thickness). The plates were developed with toluene: ethyl acetate: diethyl ether: acetic acid (80:10:10:1, v/v). After spraying with 0.2% 2,7-dichlorofluorescein in methanol, plates were visualized under UV light and the band corresponding to triacylglycerols (TAG) scraped from the plate and the TAG extracted with ether. The solvent was removed under nitrogen and the TAG converted to FAME for determination of fatty acid composition.

Statistical analysis

Statistical analysis was performed by Statistical Analysis System (1996; SAS, Cary, N.C., U.S.A.). Student t test and Bonferroni (Dunn) t test were performed on the means of values. The tested significance level was $p \leq 0.05$.

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