Solubility of Simple and Mixed Triacylglycerols in Supercritical CO_2

W.B. Nilsson* and **J.K. Hudson**
Utilization Research Division, Northwest Fisheries Science Center, NMFS, National Oceanic & Atmospheric Administration, Department of Commerce, Seattle, Washington 98112

A simple mixture of triacylglycerols (TAGs) was synthesized enzymatically from palmitic acid and oleic acid. The mixture contained tripalmitoylglycerol (PPP), trioleoylglycerol (OOO), and various isomers of palmitoyl-dioleoylglycerol (POO) and oleoyl-dipalmitoylglycerol (PPO). The behavior of this TAG mixture in equilibrium with supercritical carbon dioxide (SF-CO_2) was studied at 40 and 60°C between 172 and 310 bar. Generally, the partition coefficients varied inversely with species molecular weight. The solubilities of PPP and OOO in SF-CO_2 were determined at the same conditions. PPP was markedly more soluble in SF-CO_2 when present in the TAG mixture than as a pure compound.

KEY WORDS: Oleic acid, oleoyl-dipalmitoylglycerol, palmitic acid, palmitoyl-dioleoylglycerol, partition coefficients, solubilities, supercritical carbon dioxide, triacylglycerols, trioleoylglycerol, tripalmitoylglycerol.

Review of the literature on edible oils points to strong interest in purification and fractionation processes involving near-critical and supercritical fluids (SFs) (1). Potential applications range from extraction of vegetable oils from various crops such as soybeans (2) and canola (3) to fractionation of alkyl esters derived from fish oils, resulting in concentration of individual ω-3 fatty acids (4–7). There have also been a number of attempts to fractionate complex triacylglycerol (TAG) mixtures with either near-critical or SF propane (8–10), supercritical fluid carbon dioxide (SF-CO_2) (11–12) or mixtures of these two solvents (11). Generally, some fractionation of TAG mixtures has been observed, but no significant concentration of any single fatty acid moiety in a given fraction has been demonstrated.

Difficulties associated with efforts to concentrate individual fatty acids by fractionation of TAGs have generally been attributed to the complex manner in which specific fatty acid acyl groups are distributed in natural TAG mixtures (12), rather than to an absence of significant selectivity of SF-CO_2 for individual TAG species. This assertion has been based more upon speculation than any wealth of data describing the vapor-liquid behavior of mixtures of TAGs in equilibrium with SF-CO_2. To date, the only reported data describing the phase behavior of such systems is that of Bamberger *et al.* (13). In their work, solubilities of 99% trilauroyl- (LLL), trimyristoyl- (MMM) and tripalmitoylglycerol (PPP) in SF-CO_2 were determined at 40°C between *ca.* 100 and 300 bar. These values were then compared to partition coefficients (PCs) obtained over the same range of conditions for a quaternary system of LLL/MMM/PPP/CO_2. It was noted that while the PC of the most soluble component in the model mixture (LLL) was numerically equal to its solubility in the binary LLL/CO_2 system, the PCs of the two less soluble components (MMM and PPP) were up to one order of magnitude greater than the numerical values of their solubilities in the corresponding simple binary systems. This behavior was attributed to unspecified intermolecular interactions between unlike TAGs in both phases. Whatever the origin of this phenomenon, the data indicate that simple solubility data may be of limited value in predicting the vapor-liquid equilibrium behavior of multicomponent systems composed of SF-CO_2 and TAGs.

The simple (or monoacid) saturated TAGs studied in the work of Bamberger *et al.* (13) are not usually major components in most natural oils, which predominantly consist of mixed TAGs (*i.e.*, TAGs containing more than one structurally distinguishable acyl group). For example, a study of the structure of extra virgin olive oil has been published recently (14). A fatty acid profile indicated that it contained 75.4% oleic acid (OA). By use of silver ion high-performance chromatography and stereospecific analysis, it was determined that the simple TAG trioleoylglycerol (OOO) accounted for only about 45 mol% of all species. Nearly all other TAG species contained at least one other acyl group other than the OA moiety, *e.g.*, various isomers of palmitoyldioleoylglycerol (POO) and oleoyl-dipalmitoylglycerol (PPO). As has been previously pointed out (15,16), a better understanding of the phase behavior of real systems will require vapor-liquid equilibrium data for systems containing such mixed TAGs. In this study, we report PCs for a model TAG/CO_2 system. The simple, enzymatically synthesized TAG mixture contained significant quantities of all isomers of POO and PPO along with the corresponding simple TAGs, OOO and PPP.

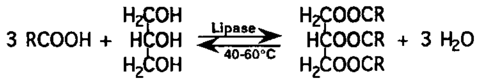
EXPERIMENTAL PROCEDURES

Synthetic reagents and standards. The OA and palmitic acid (PA), and 1,2-dioleoyl-3-palmitoyl-*rac*-glycerol (all of 99% purity) were purchased from Sigma Chemical Company (St. Louis, MO). Anhydrous glycerol was purchased from J.T. Baker (Phillipsburg, PA). A custom chromatographic standard, containing 1,2-dioleoyl-3-palmitoyl-*rac*-glycerol (POO, 39.18 wt%), 1-oleoyl-2,3-palmitoyl-*rac*-glycerol (PPO, 16.97 wt%), PPP, 11.10 wt% and OOO, 32.76 wt%, was prepared by Matreya, Inc. (Pleasant Gap, PA). Synthesis of the TAG test material was catalyzed by Lipozyme™ IM-20 (described subsequently), obtained from Novo Nordisk Bioindustrials, Inc. (Danbury, CT).

TAG synthesis. TAG test materials were synthesized according to the procedure of Ergan *et al.* (17). In this method, the reaction is catalyzed at moderate temperatues in a solvent-free system with a lipase. The enzyme, formally classified as a TAG acylhydrolase (EC 3.1.1.3), is isolated from a selected strain of the fungus *Mucor miehei* and immobilized on a weak anion exchange resin (Lipozyme™ IM-20). A study of the mechanism and kinetics of this reaction has been published recently (18). For purposes of this report, the overall reaction can be written simply, as in Scheme 1:

---

*To whom correspondence should be addressed at Utilization Research Division, Northwest Fisheries Science Center, NMFS, National Oceanic & Atmospheric Administration, Department of Commerce, 2725 Montlake Boulevard East, Seattle, WA 98112.



$$3\ RCOOH + \begin{matrix}H_2COH\\ HCOH\\ H_2COH\end{matrix} \underset{40\text{-}60^\circ C}{\overset{Lipase}{\rightleftharpoons}} \begin{matrix}H_2COOCR\\ HCOOCR\\ H_2COOCR\end{matrix} + 3\ H_2O$$

where RCOOH is any fatty acid. Equilibrium is shifted toward the right by removal of water from the stirred reactor in some manner. After 48 h, yields of up to 90% were reported for the synthesis of OOO at 60°C from a 3:1 (*i.e.*, stochiometric) mole ratio of OA to glycerol.

In choosing the fatty acid substrate for the TAG synthesis in this work, it was assumed that the lipase would incorporate PA and OA onto the glycerol backbone in a completely random manner. Furthermore, we wished to maximize the content of the product with respect to one of the mixed TAG components. With the obvious exception of palm oil, OA is generally more predominant in natural oils than PA. Therefore, an attempt was made to maximize the level of POO in the final product. (Structural and stereoisomers are not distinguished in this work. Therefore, "POO" includes 1,2-dioleoyl-3-palmitoylglycerol, 1-palmitoyl-2,3-dioleoylglycerol and 1,3-dioleoyl-2-palmitoylglycerol.) Random statistics predict that an OA/PA mole ratio of 2:1 will maximize the content of POO in the TAG product. In addition, previous work (17) suggested that a mole ratio of fatty acid to glycerol of 3:1 gave optimum yields. Therefore, all TAG syntheses were conducted with an OA/PA/glycerol mole ratio of 2:1:1.

Batch syntheses were carried out on either a 5- or 10-g scale with similar results. As an example, 6.78 g OA (0.024 mole), 3.08 g PA (0.012 mole) and 1.12 g glycerol (0.012 mole) were combined in a 50-mL round-bottom flask containing 1.12 g lipase. The flask was partially immersed in an oil bath at 60°C and magnetically stirred. With a water aspirator, a vacuum of *ca.* 0.035 bar was then applied for 50 h, at which time the reaction was terminated. The reaction mixture was then filtered, and the enzyme was rinsed with diethyl ether. After removal of solvent, a total of 10.0 g of product was recovered. High-performance liquid chromatography (HPLC) of this material (discussed below) indicated that the product contained *ca.* 84% TAG. Nearly all of the by-products were diacylglycerols. Several batch syntheses were performed to obtain *ca.* 95 g of the crude product.

Purification of reaction mixtures. By-products (mostly diacylglycerols) were partially removed from the reaction mixtures with SF-CO_2. The apparatus and general aspects of this method are thoroughly described elsewhere (19). In this work, all purifications were carried out at 60°C in a two-step process. Table 1 describes the first step of the overall process, along with estimates (by HPLC) of the composition of each fraction collected. The final fractions from several batches were then combined to give a new mixture containing *ca.* 6% by-product. In a final purification step, this material was then "stripped" of a substantial portion of the remaining by-product, at 60°C and 172 bar, to yield a mixture that was *ca.* 98% TAG.

HPLC methodology. TAG materials were analyzed with a Shimadzu LC-6A Series HPLC with ultraviolet-visible detection at 212 nm (Kyoto, Japan). Species were resolved on two 3-μ reverse-phase C18 Zorbax™ ODS columns (6.2 mm i.d. × 8 cm o.d.) connected in series (Mac Mod, Chaddes Ford, PA). All analyses were performed isocratically with a mobile phase of ethanol/acetonitrile (60:40, vol/vol) at a flow of 1 mL/min with an oven temperature of 35°C.

TABLE 1

Typical Results for Supercritical CO_2 Fractionation of the Crude Reaction Mixture from Enzymatic Syntheses of Triacylglycerols (TAGs)[a]

Fraction	Fraction wt (g)	Pressure (bar)	Liters[b] CO_2	TAG (wt%)	By-product (wt%)
1	1.63	172	850	23.1	76.9
2	1.77	190	800	48.7	51.3
3	1.69	207	400	66.6	33.4
4	14.00	345	1000	95.6	4.4
Charge	20.38	—	—	87.8	12.2

[a] The estimated wt% of TAGs and by-products are given. The fractionation was performed at 60°C with methodology described previously (Ref. 19).

[b] At 1 bar and 25°C.

This method led to the resolution of four distinct peaks in the trace that were identified by means of standards as OOO, POO (all isomers), PPO (all isomers) and PPP. Preliminary analyses of synthetic products indicated that the detector was not as sensitive to the presence of those species containing palmitoyl groups. Detector response correction factors were established with the custom standard described in the materials section. Peak areas were multiplied by the following factors to correct for detector response: OOO, 0.78; POO, 1.05; PPO, 1.20; PPP, 1.70. It will be shown below that the correction factor for a given species appears in both the numerator and denominator of the expression for the PC and thus cancels. Application of these correction factors is therefore not required in computing PCs.

Apparatus and data acquisition procedures. Solubility and PC data were obtained in a flow-through apparatus that has been described elsewhere (20,21). Briefly, the system consists of a double-ended diaphragm-type compressor (Newport Scientific, Inc., Jessup, MD) with a maximum working pressure of 10,000 psi. System pressure is maintained by use of a back pressure regulator (Tescom Corp., Elk River, MN) and monitored by a pressure transducer (Validyne Engineering, Northridge, CA). Pressure fluctuations were damped to *ca.* 1 bar by use of surge vessels. The extraction vessel is a 1′ length (30 cm) of pipe nipple (9/16″ i.d. × 1″ o.d.) purchased from Autoclave Engineers, Inc. (Erie, PA). The extractor, a preheater and, in some cases, connecting tubing were heated by use of heating tapes. Current to the tapes was supplied by digital proportional rate derivative temperature controllers (Syscon Int., Inc., Elkhart, IN), which in turn were connected to thermocouple probes. The probes monitored internal temperatures of the extractor and preheater, while probes for connecting tubing measured surface temperature. Extracted material was expanded through a heated metering valve (High Pressure Equipment Co., Erie, PA) and collected in glass U-tubes immersed in an ice bath. Gas flow was monitored with a rotameter (Fischer & Porter Co., Warminister, PA), and the total gas volume was measured with a dry test meter (American Meter Co., Philadelphia, PA). The apparatus also included a Jerguson gauge (Clark-Reliance Corp., Strongsville, OH) for visual observation of lipid/CO_2 phase behavior.

The procedure for acquisition of both solubility and PC data was essentially identical. The test material (5–10 g) was suspended on glass wool inside the extractor. The remaining volume was packed with 0.16″ Propak, a stainless-steel packing material (Scientific Development Co., State College, PA). After the system was pressurized and the extraction vessel brought to thermal equilibrium, CO_2 flow was begun. Gas flow rate was generally held to less than 3 L/min (measured at 1 bar and 25°C). Previous work indicated that this flow rate is low enough to at least closely approach saturation of the fluid with test material.

The solubilities of PPP and OOO were determined in a straightforward manner from the fraction weights and gas volumetric data. The PC of any component A of a mixture is defined as:

$$PC(A) = y_A/x_A \qquad [1]$$

where y_A is the concentration of A in the CO_2-rich vapor phase and x_A is the concentration of A in the lipid-rich phase. All concentrations in this work were determined on a w/w basis. The definition implies the coexistence of only two phases at all experimental conditions used in this work. This assumption was confirmed visually with the Jerguson gauge. The quantity y_A was determined from the fraction weight, HPLC analysis of the content of the fraction with respect to component A and gas volumetric data. On the other hand, x_A was inferred from mass balance considerations with knowledge of the composition of the original charge as well as the content of any previous fractions. Strictly speaking, these data are not true PCs becaue this procedure gives no information regarding the CO_2 content of the bottom phase. The values reported here are on a CO_2-free basis and are thus systematically low. Nonetheless, the data do provide information regarding the effect of TAG structure upon the solubility of various components of the model mixture.

RESULTS AND DISCUSSION

Solubilities of OOO in SF-CO_2. Solubilities of OOO in SF-CO_2 at 60 and 40°C for a range of pressures are given in Table 2. Data for OOO at 60°C were reported previously (21). Figure 1 compares these data with those of King *et al.* (22) and Gonçalves *et al.* (23). Although these two sets of previously published data do not cover pressures above 220 bar, and solubilities were obtained at 35°C rather than 40°C, they still provide some basis for evaluating the data of Table 2.

Agreement between values given in Table 2 at 60°C and those of King *et al.* (22) is only fair. While these two sets of data nearly agree at *ca.* 200 bar, King *et al.* (22) report a significantly higher solubility around 170 bar. This discrepancy may be explained by noting that although King *et al.* (22) also used a flow technique, their apparatus allowed for recirculation of the extracted material through the lipid-rich phase. In principle, equilibrium is more closely approached than in the single-pass method used in this work. On the other hand, solubilities at 40°C are only slightly lower than those found at 35°C in the two previous studies. Solubilities at 40°C are expected to be slightly less than those at 35°C by virtue of the fact that at relatively low pressures, OOO solubility decreases with increasing temperature. This phenomenon, known as retrograde condensation, generally vanishes at higher pressures as indicated by the values at 40 and 60°C at 310 bar.

TABLE 2

Solubilities (w/w × 10^3) of Trioleoylglycerol (OOO) and Tripalmitoylglycerol (PPP) in Supercritical Fluid CO_2 at 40 and 60°C

Pressure (bar)	OOO 40°C	OOO 60°C[a]	PPP 40°C	PPP 60°C
172	2.1	0.6	0.28	0.9
207	3.3	1.4	0.32	2.3
241	4.9	3.1	—	4.4
276	6.2	5.3	0.38	7.9
310	7.0	7.6	0.38	10.8

[a] From Reference 21.

Solubilities of PPP in SF-CO_2. Comparison of the 40 and 60°C data in Table 2 reveals that the behavior of PPP differs from that of OOO in that the former does not exhibit retrograde behavior at any pressure investigated. As a practical matter, this presents certain difficulties that required the tubing between the extractor and expansion valve to be heat-traced. Otherwise, PPP dissolved in the extractor at an elevated temperature tended to fall out of solution when entering cooler connecting tubing. At best, this can result in erroneously low solubility data. At worst, if solvent loadings are high enough, this can result in completely clogged tubing.

Figure 2 provides a comparison of the data of Table 2 with solubilities reported by Bamberger *et al.* (13) and Pearce (16). Data obtained at 40°C in this work compare fairly well with those of the previous reports. In all cases, solubilities above *ca.* 200 bar display weak dependence on pressure. In fact, the data of this work display essentially little pressure dependence between 170 and 300 bar.

The pressure dependence of the solubility of PPP at 60°C in the same regime (Fig. 2) is more pronounced. These data are somewhat lower than those obtained by Pearce (16) at 55°C. Since PPP displays nonretrograde behavior under the conditions of this work, values at 60°C

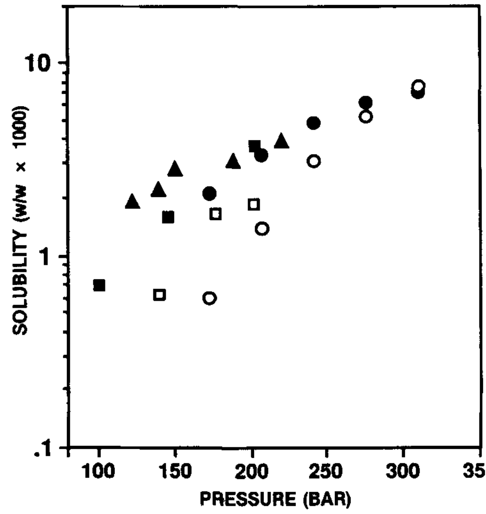


FIG. 1. Solubility of trioleoylglycerol in supercritical CO_2 as a function of pressure. Key: King *et al.* (Ref. 22), 60°C (□) and 35°C (■); Gonçalves *et al.* (Ref. 23), 35°C (▲); Nilsson *et al.* (Ref. 21), 60°C (○); this work, 40°C (●).

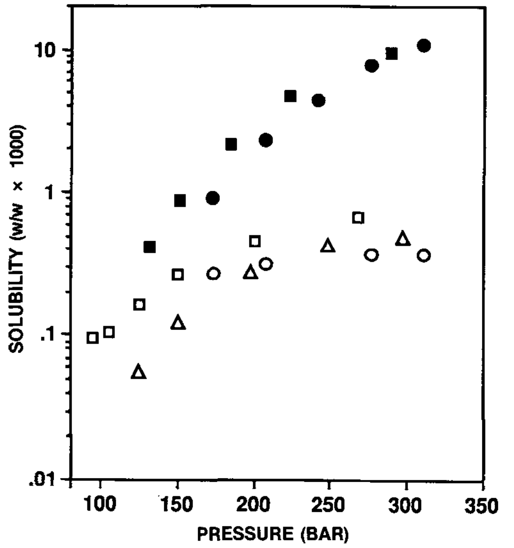


FIG. 2. Solubility of tripalmitoylglycerol in supercritical CO_2 as a function of pressure. Key: Bamberger *et al.* (Ref. 13), 40°C (△); Pearce (Ref. 16), 40°C (■) and 55°C (□); this work, 40°C (●) and 60°C (○).

would be expected to be somewhat greater than those at 55°C. This discrepancy could be attributed to two factors. In the method used by Pearce (16), the fluid was sampled directly from an equilibrium cell into a calibrated sample loop and analyzed by HPLC. Again, in the single-pass flow-through method used in this work, it is possible that the system was not at equilibrium, and therefore, the fluid was incompletely saturated. Alternatively, ineffective heat tracing of tubing downstream from the vessel could result in the presence of cool regions leading to measured solubilities that are erroneously low. Obviously, this potential problem would be eliminated by enclosing the extractor and connecting line in a well-controlled oven.

The strikingly different pressure dependence of the solubility of PPP at the two temperatures may be due to a phase change. Visual observation of the PPP/CO_2 system at 172 bar through the Jerguson gauge indicated that the melting point of PPP is depressed under pressure to *ca.* 57°C. (The normal melting point of PPP is 65.5°C.) The melting point of PPP was not checked at the higher pressures, but it seems unlikely that the melting point depression even at 310 bar would be great enough to result in a liquid phase at 40°C. Therefore, for all 60°C data, PPP is present as a liquid while for all data collected at 40°C, we believe PPP is present as a solid.

PPP is less soluble than OOO at 40°C, but not at 60°C. This is a consequence of the fact that in this pressure regime OOO displays retrograde behavior while PPP does not. On the other hand, as was demonstrated by Bamberger *et al.* (13), solubility data for a pure substance may be of little value in predicting the behavior of that substance in multicomponent mixtures.

PCs for components of the synthetic TAG mixture. Application of HPLC detector correction factors (discussed above) resulted in a synthetic TAG mixture described in the first column of Table 3. The second column gives the composition of the mixture with respect to only the TAGs as a class. Comparison of these values with those predicted based upon purely random statistics (column 3) gives good evidence that the enzymatic synthesis is primarily a random process. Column 4 gives the composition of the TAG mixture from which most by-products have been removed by the two-step SF-CO_2 process (Table 1). The level of PPP in this purified product was deemed to be too low to yield accurate PC data. The mixture was therefore supplemented with PPP to increase the level of this component to *ca.* 10%. The composition of the final test material is given in the last column of Table 3.

PCs for each component of the TAG mixture collected between 172 and 310 bar at 40 and 60°C are given in Tables 4 and 5, respectively. Each PC represents an average of from three to ten separate determinations. Perhaps the most striking feature of the 40°C data (Table 4) is the magnitude of the PC for PPP when compared to the solubility of PPP in the binary system (Table 2). At higher pressures, PPP is from 20 to 30 times more soluble when present in the TAG mixture than as a pure component. Furthermore, even though in the binary systems PPP was found to be less soluble than OOO at 40°C at all pressures investigated, when present as part of the TAG mixture PPP actually had a slightly larger PC than OOO. The PC of OOO, unlike that of PPP, was numerically equal to its binary solubility (Table 2). These data indicate that the PC of OOO is essentially independent of the level of this species present in the mixture. In contrast, it seems reasonable to suppose that as the level of PPP is increased, the PC for this TAG should decrease, eventually approaching a value numerically equal to its solubility in the binary sysem. Due to the expense of sufficient quantities of the two mixed TAGs (POO and PPO), no binary solubility data for these species could be obtained for direct

TABLE 3

Estimated Composition of Various Materials Described in This Work (in units of wt%)[a]

Component	Crude	Normalized	Predicted	Purified	Supplemented
OOO	25.3	29.1	29.6	33.1	29.8
POO	41.0	47.2	44.4	45.7	43.4
PPO	17.6	20.3	22.2	17.0	16.2
PPP	2.9	3.3	3.7	2.3	9.1
By-products	13.2	—	—	1.9	1.5

[a] Crude: Enzymatically synthesized triacylglycerol (TAG) mixture prior to purification with supercritical CO_2. Normalized: composition of crude TAG mixture after normalization to exclude by-products. Predicted: TAG composition predicted by random statistics. Purified: Composition of TAG mixture following purification with SF-CO_2. Supplemented: Composition of final test material after supplementation with PPP. Abbreviations: See Table 2 and POO, palmitoyl-dioleoylglycerol; PPO, oleoyl-dipalmitoylglycerol.

TABLE 4

Partition Coefficients for Triacylglycerol Components in Supercritical CO_2 at 40°C[a]

Pressure (bar)	Partition coefficient × 10^3 OOO	POO	PPO	PPP
172	2.0	2.4	2.7	2.2
207	3.0	3.4	3.8	3.6
241	4.6	5.1	5.6	4.8
276	6.2	6.8	7.9	6.8
310	7.7	9.0	10.6	10.9

[a]See Tables 2 and 3 for abbreviations.

TABLE 5

Partition Coefficients for Triacylglycerol Components in Supercritical CO_2 at 60°C[a]

Pressure (bar)	Partition coefficient × 10^3 OOO	POO	PPO	PPP
172	1.1	1.3	1.6	1.7
207	1.8	2.1	2.6	3.1
241	3.2	4.1	5.0	5.5
276	5.3	6.5	7.7	9.1
310	7.7	8.9	10.2	10.8

[a]See Tables 2 and 3 for abbreviations.

comparison with mixture PC data. However, both of these mixed TAG species are seen to have somewhat higher PCs than OOO. Of these two TAGs, PPO consistently has the highest PC.

The behavior of the TAG test material is somewhat different at 60°C (Table 5). The PC for PPP is greater than those of the other three components, with OOO having the smallest value. Careful comparison of PC values from Tables 4 and 5 and indicates that, although all species of the mixture undergo retrograde condensation at lower pressures, the PC for PPP is more weakly affected by the 20°C rise in temperature. Furthermore, PPP crosses over from retrograde to nonretrograde behavior between 240 and 270 bar, while OOO remains retrograde (as do POO and PPO) up to around 310 bar. At 60°C, the selectivity of CO_2 for PPP relative to OOO ranges from 1.5 to 1.7. The selectivities of CO_2 for the two mixed TAGs are somewhat less, as might be predicted based purely on molecular weight considerations. These findings contradict recent speculation that POO would have a lower solubility in SF-CO_2 than OOO (23). The argument given was based upon the data of Chrastil (24), which indicated that PPP is less soluble in CO_2 than OOO, which in turn led to the supposition that substitution of a palmitoyl group for an oleoyl group would result in a decreased solubility of the TAG. While in this work OOO was found to be more soluble than PPP at 40°C, the opposite was found at 60°C. In any case, solubility data for simple TAGs are apparently of limited value for predicting solubilities of related mixed TAG species. This appears to be especially true for multicomponent mixtures containing such species.

On the basis of data presented in Tables 4 and 5, some general conclusions can be made. First, it appears that to a first approximation, those TAG species of lower molecular weight have a somewhat larger PC. Alkyl esters are well-known to behave in a similar manner (4,20), and given that TAGs are essentially triesters, this is perhaps not a surprising finding. An exception to this rule may apply to longer-chain saturated TAGs such as PPP at temperatures approaching the critical temperatue of CO_2 (31.1°C). Pearce (16) measured the solubilities of PPP in CO_2 between 100 and 380 bar from 35–55°C. Nonretrograde behavior was observed at all but the lowest pressures investigated. Tristearoylglycerol solubility was also investigated at the same conditions and was never observed to exhibit retrograde behavior. If such tendencies also apply to mixtures involving these longer-chain saturated TAGs (as the data for PPP in Tables 4 and 5 suggest), a second-order temperature effect may also influence the magnitude of the PC for these saturated long-chain species.

For alkyl esters of equal chainlength, structural factors such as degree and position of unsaturation have been reported to influence the PCs for multicomponent mixtures in equilibrium with SF-CO_2 (20). The effect of similar structural characteristics upon PCs for systems including multicomponent mixtures of TAGs will be the subject of future investigation.

ACKNOWLEDGMENTS

The authors are grateful for the gift of Lypozyme™ IM-20 from Novo Nordisk Bioindustrials, Inc., Danbury, CT. Erich Gauglitz, Jr. provided a helpful review of this work.

REFERENCES

1. *Supercritical Fluid Technology in Oil and Lipid Chemistry,* edited by J.W. King, and G.R. List, AOCS Press, Champaign, in press.
2. J.P. Friedrich, G.R. List and A.J. Heakin, *J. Am. Oil Chem. Soc. 59*:288 (1982).
3. Temelli, F., *J. Food Sci. 57*:440 (1992).
4. Eisenbach, W., *Ber. Bunsenges. Phys. Chem. 88*:882 (1984).
5. Nilsson, W.B., E.J. Gauglitz, Jr., J.K. Hudson, V.F. Stout and J. Spinelli, *J. Am. Oil Chem. Soc. 65*:109 (1988).
6. Nilsson, W.B., E.J. Gauglitz, Jr. and J.K. Hudson, *Ibid. 66*:1596 (1989).
7. Krukonis, V.J., J.E. Vivian, C.J. Bambara, W.B. Nilsson and R.E. Martin, in *Seafood Biochemistry: Composition and Quality,* edited by G.J. Flick, Jr., and R.E. Martin, Technomic Publishing Company, Inc., Lancaster, 1992, pp. 169–179.
8. Passino, H.J., *Ind. Eng. Chem. 41*:280 (1949).
9. Dickinson, N.L., and J.M. Meyers, *J. Am. Oil Chem. Soc. 47*:235 (1952).
10. Zosel, K., *Angew. Chem. Int. Ed. Eng. 17*:702 (1978).
11. Ender, U., and R. Steiner, *Chem. Ing. Techn. 63*:727 (1991).
12. Krukonis, V.J., in *Supercritical Fluid Extraction and Chromatography,* ACS Symposium Serus 366, edited by B.A. Charpentier, and M.R. Sevenants, American Chemical Society, Washington, D.C., 1988, pp. 26–43.
13. Bamberger, T., J.C. Erickson, C.L. Cooney and S.K. Kumar, *J. Chem. Eng. Data 33*:327 (1988).
14. Santinelli, F., P. Damiani and W.W. Christie, *J. Am. Oil Chem. Soc. 69*:552 (1992).
15. P.A. Wells, N.R. Foster, K.K. Liong and R.P. Chaplin, *Sep. Sci. Techn. 25*:139 (1990).
16. Pearce, D.L., Ph.D. thesis, Solubility of Triglycerides in Supercritical Carbon Dioxide, Chemical Engineering Department, University of Canterbury, Canterbury, New Zealand, 1990.
17. Ergan, F., M. Trani and G. André, *Biotech. Biogeng. 35*:195 (1990).
18. Lortie, R., M. Trani and F. Ergan, *Ibid. 41*:1021 (1993).

19. Nilsson, W.B., V.F. Stout, E.J. Gauglitz, Jr., J.K. Hudson and F.M. Teeny, in *Supercritical Fluid Science and Technology*, ACS Symposium Series 406, edited by K. P. Johnson, and J.M.L. Penninger, American Chemical Society, Washington, D.C., 1989, pp. 434–448.
20. Nilsson, W.B., G.T. Seaborn and J.K. Hudson, *J. Am. Oil Chem. Soc. 69*:305 (1992).
21. Nilsson, W.B., E.J. Gauglitz, Jr. and J.K. Hudson, *Ibid. 68*:87 (1991).
22. King, M.B., T.R. Bott, M.J. Barr and R.S. Mahmud, *Sep. Sci. Techn. 22*:1103 (1987).
23. Gonçalves, M., A.M.P. Vasconcelos, E.J.S. Gomes de Azevedo, H.J. Chaves das Neves and M. Nunes da Ponte, *J. Am. Oil Chem. Soc. 68*:474 (1991).
24. Chrastil, J., *J. Phys. Chem. 86*:3016 (1982).

[Received March 12, 1993; accepted May 4, 1993]