Supercritical Fluid Extraction and Chromatography of Emulsifiers

William E. Artz* and Michelle R. Myers
The University of Illinois, Urbana, Illinois 61801-4726

ABSTRACT: Selected emulsifiers, which included acetylated monoglycerides, lactylated monoglycerides, hexaglycerol distearate, triglycerol mono/dioleate and decaglycerol decaoleate, were separated with capillary supercritical fluid chromatography on a 25% cyanopropyl stationary phase with a mobile phase of CO_2 at 100–150°C. In general, the density/pressure programs that produced the best separations were those with reduced pressure/density ramp rates, which encompassed the largest possible pressure or density range. Samples of acetylated monoglycerides were placed in a supercritical fluid extraction cell on a glass bead bed and extracted for 15 min at 50°C at 340, 408, 544 and 680 atm with CO_2. At 544 atm, 103.6 ± 1.0% of the emulsifiers was extracted. Acetylated monoglycerides were added during twin-screw extrusion of cornstarch (3% w/w emulsifier/corn starch). The acetylated monoglycerides were extracted from the cornstarch extrudate for 15 and 45 min, at 544 and 646 atm and 50–120°C with 0 and 5% added methanol. The percent acetylated monoglyceride extracted after 45 min at 120°C and 646 atm was 60.0 ± 5.7%, while the amount extracted during a 7.5-h Soxtec extraction ranged from 17.9 to 29.4%, depending upon the solvent used. *JAOCS 72,* 219–224 (1995).

KEY WORDS: Acetylated monoglycerides, decaglycerol decaoleate, emulsifiers, hexaglycerol distearate, lactylated monoglycerides, supercritical fluid chromatography, supercritical fluid extraction, triglycerol mono/dioleate.

Emulsifier analysis is important to both regulatory agencies and industry from the standpoint of quality control during preparation and quantitation of emulsifiers in food products. Methods for the gas chromatographic (GC) separation of a few emulsifiers, such as the acetylated and lactylated monoglycerides, have been developed (1). However, the analytes require derivatization. High-performance liquid chromatographic (HPLC) (2) separation of oligomeric emulsifiers, such as decaglycerol decaoleate, have been published, but resolution is limited. Supercritical fluid chromatography (SFC) offers several advantages over GC and HPLC. SFC analysis allows the separation of nonvolatile samples, such as the larger oligomeric emulsifiers. In addition, analytes that would require derivatization for GC analysis can usually be analyzed without derivatization with SFC.

*To whom correspondence should be addressed at Dept. of Food Science, University of Illinois, 382 Agr. Eng. Sci. Bldg., 1304 W. Pennsylvania Ave., Urbana, IL 61801-4726.

Method development for emulsifier extraction from food systems can be difficult due to emulsifier complexation with constituent starches and proteins present in food (3). The disadvantages of solvent extraction include lengthy extraction times, the expense of organic solvents, and the environmental and health hazards associated with many of the solvents that are used. Supercritical fluid extraction (SFE) can be an effective method for the analysis of intermediate and low-polarity analytes (4). Compounds similar in structure to emulsifiers (5) are soluble in supercritical CO_2 (6,7). The objective of this work was to evaluate SFC for the separation of emulsifiers and to evaluate the feasibility of SFE and SFC as potential techniques for the analysis of emulsifiers from an extruded food product model system.

EXPERIMENTAL PROCEDURES

Samples of acetylated monoglycerides and lactylated monoglycerides were obtained from Grindsted Products, Inc. (Industrial Airport, KS). Samples of decaglycerol decaoleate, hexaglycerol distearate, and triglycerol mono/dioleate were obtained from Karlshamns, USA Inc. (Janesville, WI). Lactylated monoglycerides, hexaglycerol distearate and decaglycerol decaoleate were dissolved in HPLC-grade methylene chloride (Fisher Scientific, Fairlawn, NJ) at final concentrations of 10.74, 2.64 and 11.36 mg/mL, respectively. Acetylated monoglycerides were dissolved in HPLC-grade acetone (Fisher Scientific) at a final concentration of 10.54 mg/mL. Triglycerol mono/dioleate was dissolved in HPLC grade isopropanol (Fisher Scientific) at a concentration of 6.67 mg/mL. All samples were filtered with 0.45-μm Teflon filters (National Scientific, Co., Lawrenceville, GA) into amber Teflon-capped vials (National Scientific).

Samples were injected *via* a Valco A90 injector (Houston, TX) with a 0.2-μL internal loop operated in a time split mode into a capillary SFC column in a β501 model SFC equipped with a flame-ionization detector (FID) (Lee Scientific, Div. of Dionex, Salt Lake City, UT). Density or pressure programming (Table 1) was used to separate the emulsifier samples in a 17-m capillary column with a stationary phase of SB-Cyano-25 (50 μm i.d., d_f = 0.25 μm; Lee Scientific). The mobile phase was SFE/SFC grade supercritical CO_2 (MG Industries, Valley Forge, PA). Chromatographic data were collected on a Hyundai 386 PC while using Baseline™ software (Waters Assoc., Milford, MA).

TABLE 1
Supercritical Fluid Chromatography Emulsifier Separation Conditions

Sample	Initial pressure (atm)	Pressure ramp (atm/min)	Final pressure (atm)
Acetylated monoglycerides[a]	150	5.0	410
Lactylated monoglycerides[a]	70	5.0	415
Decaglycerol decaoleate[a]	150	3.4	360
Triglycerol mono/dioleate[a]	70	2.6	300
	300[b]	3.9	415
Hexaglycerol distearate[a]	85	4.0	320

[a]17-m, 50-μm (i.d.) SB-Cyano-25 (Lee Scientific, Div. Dionex, Salt Lake City, UT), 120°C, flame-ionization detector @ 375°C, and injection times of 0.1–0.4 s.
[b]Two-ramp separation.

Extrusion of cornstarch and emulsifier. Food-grade hybrid cornstarch (78-0166) was obtained from National Starch and Chemical Co. (Bridgewater, NJ). Acetylated monoglycerides were obtained from Grindsted Products, Inc.

A Werner and Pfleiderer twin-screw extruder model ZSK 30 (Ramsey, NJ) was used for the extrusion. A Masterflex variable-speed (1–100 rpm) pump model #7553-30 with controller and Masterflex standard pumphead #7014-20 with Masterflex Norprene food tubing #6402-14 (Cole Parmer, Niles, IL) was used to pump the emulsifier into the extruder. A Masterflex model #7520-20 standard drive with a Masterflex standard pumphead #7016-20 and Masterflex Norprene food tubing #6402-14 (Cole Parmer) was used to pump distilled water into the extruder.

Pumps were calibrated by taking volume readings for the water and the emulsifier at time intervals for each pump setting and recording the flow rate in mL/min. The extrusion feeder was calibrated by taking weight readings of cornstarch at time intervals and determining the feed rate (g/min) for each setting of the feeder. From these data, calibration curves were determined for the water, emulsifier and cornstarch. Extrusion conditions were: screw speed (rpm), % torque, dry feed rate (g/min), water feed rate (g/min), emulsifier feed rate (g/min) 400, 45–46, 200, 325, 6.0—respectively; the temperature profile was 34, 67, 98, 107, 132°C in zones 1–5, respectively. The current state of extrusion research is presented in a text by Kokini *et al.* (8). A small sample of extrudate was collected, immediately after emerging from the extruder, in a plastic cup, and the cup was immediately capped. The extrudate sample was analyzed for moisture content. Larger samples of extrudate (approximately 600 g) were collected on trays for approximately 3 min and allowed to stand in the pilot plant on the trays for approximately 30 min. The extrudate samples were then frozen at −40°C for 7 h, then freeze-dried for 36 h. A freeze-dried sample of extrudate was collected in a plastic sample cup and capped for moisture analysis upon removal from the freeze-drier. The remaining sample was collected into large, double-thickness, sample bags, which were sealed and stored in the lab at room temperature. Samples of freeze-dried extrudate were ground in a Braun coffee grinder (Lynnfield, MA) to a particle size of 2.00–0.25 mm in diameter and stored in glass screw-capped jars. The ground extrudate was separated into three samples, based on particle size. One sample was 1.00–2.00 mm in diameter, a second sample was 0.50–1.00 mm in diameter and the third sample was 0.25–0.50 mm in diameter.

The moisture content was determined by the Association of Analytical Chemists method 14.003 (9) with the following modification: aluminum weighing pans were used instead of covered dishes. The pans were dried in a forced-air oven at 130°C for 1 h prior to sample addition. Extrudate samples were dried in a vacuum oven at 60°C for 8.5 h to constant weight.

SFE of acetylated monoglycerides. Samples of acetylated monoglycerides of approximately 50 mg were accurately weighed (±0.01 mg) into a 9-mL high-temperature crystalline polymer extraction cell (ISCO, Inc., Lincoln, NE) loaded with 3-mm diameter solid glass beads (Kimble Glass, Inc., Vineland, NJ). The loaded extraction cells were placed into an ISCO model SFX 2-10 supercritical fluid extractor (ISCO, Inc.) equipped with dual model-DX syringe pumps. SFC/SFE-grade CO_2 (MG Industries) was used as the extraction solvent. Samples were extracted for 15 min at 50°C. A 38.1-cm polyimide-coated fused-silica capillary restrictor (ISCO, Inc.) with a 50 μm diameter was heated and maintained at 100°C. The extracted material was collected in HPLC-grade acetone (Fisher Scientific). Extractions were carried out at pressures of 340, 408, 544 and 680 atm. Each extraction was done in duplicate with the exception of the sample extracted at 408 atm, for which four replicates were done.

SFE of extruded cornstarch and emulsifier. Approximately 2 g of extrudate was accurately weighed into a 9-mL extraction cell (ISCO, Inc.). The SFE was an ISCO model SFX 2-10 extractor (ISCO, Inc.) equipped with dual model-100 DX syringe pumps. Samples were extracted for 15–45 min at 50–120°C (Table 2). A 55-cm (length) polyimide-coated fused-silica capillary restrictor (ISCO, Inc.) with a 50-μm internal diameter was heated to and maintained at 100°C. The extraction solvent was SFC/SFE-grade CO_2 (MG Industries).

For modifier addition, HPLC-grade methanol (Fisher Scientific) was added at a rate of 5% (vol/vol) with a second model-100 DX syringe pump (ISCO, Inc.). Samples were extracted at 646 atm, and the extracted analyte was collected in

TABLE 2
Supercritical Fluid Extraction Conditions for Extruded Cornstarch and Acetylated Monoglycerides

Conditions[a]	Extraction sample 1	2	3	4
Extraction temp. (°C)	50	90	120	120
Pressure (atm)	544	646	646	646
Extraction time (min)	15	45	45	45
Particle size (mm)	0.50–1.00	0.50–1.00	0.50–1.00	0.25–0.50
Modifier (% methanol)	0	0	5	5
Flow rates (mL/min)	2.4–2.8	2.5–2.7	2.7–2.8	2.6–2.7

[a]The 55 cm × 50-μm (i.d.) restrictor was maintained at 100°C.

HPLC-grade acetone (Fisher Scientific). Samples with a particle size of 0.50–1.00 mm and 0.25–0.50 mm were extracted.

SFC analysis of extracted samples. The internal standard, nonadecanol (Nu-Chek-Prep, Inc., Elysian, MN), was accurately weighed and prepared at a concentration of approximately 5 mg/mL in HPLC-grade acetone (Fisher Scientific). Extracted samples were transferred quantitatively to a 5-mL volumetric flask, 1 mL of the internal standard solution was added, and the contents were brought to volume with HPLC-grade acetone (Fisher Scientific) for a final internal standard concentration of approximately 1 mg/mL. Acetylated monoglyceride standards, as well as the samples extracted from the extrudate, were separated as outlined in Table 1. Two replicates of each SFE extraction of the acetylated monoglycerides were analyzed with SFC, for a total of four replicates at each extraction pressure, with the exception of the 408-atm sample, where a total of eight replicates were analyzed. Each SFE replicate of the extracted cornstarch and acetylated monoglyceride extrudate sample was analyzed in duplicate on SFC. The concentration of each component of the acetylated monoglycerides present in the extracted samples was calculated with Baseline^{TM} software as follows:

$$C_c = A_c(C_i/A_i) \quad [1]$$

where C_c = component concentration, A_c = area of component peak, C_i = internal standard concentration and A_i = area of internal standard peak.

The peak concentrations (excluding that of the internal standard) were added together to obtain the total concentration of acetylated monoglycerides, which was then adjusted with a response factor, obtained from the SFC quantitation of a sample of acetylated monoglycerides at a known concentration.

Soxtec extraction of extrudate containing acetylated monoglycerides. A method for the extraction of acetylated monoglycerides was obtained from Grindsted Products, Inc. This method was modified by substituting a Soxtec Extractor for the Soxhlet extractor. Extraction with the Soxtec System HT involves a two-stage process. During the first stage, the sample is placed in boiling solvent, and in the second, the sample is rinsed with the hot solvent. The American Association of Cereal Chemists (AACC) collaborative tests found Soxtec extraction times of 45 min were comparable to 4–16-h extractions with the Soxhlet procedure (AACC method 30-20). There were no significant differences between the AACC mean values (Soxhlet) and the Soxtec results when the procedure described in the application note is used (10).

For solvent extraction, extrudate samples were ground to a particle size of 0.50–1.00 mm with a Braun coffee grinder and stored in screw-capped glass jars. Soxtec aluminum extraction cups were dried at 105°C for 1 h and cooled in a desiccator. Approximately 3 g of extrudate was weighed into 26 mm × 60 mm cellulose extraction thimbles (Whatman, Hillsboro, OR). Thimbles were loaded into a Soxtec System HT 1043 Extraction Unit equipped with a Soxtec System HT 1046 Service Unit (Tecator, Inc., Herndon, VA). Approximately 37 mL of extraction solvent was loaded into aluminum extraction cups, which had been tared with boiling chips. Five samples were extracted at 160°C with 9:1 (vol/vol) chloroform/methanol and three samples were extracted at 160°C with 2:1 (vol/vol) chloroform/methanol. The samples were extracted by placing the thimbles in boiling solvent for 15 min, then rinsing with solvent for 45 min, followed by a solvent recovery step (10). Extraction cups were removed after solvent recovery and dried at 100°C for 30 min, cooled in a desiccator and weighed. A second series of extractions was performed, where two samples were extracted with 9:1 (vol/vol) chloroform/methanol, two samples with 2:1 (vol/vol) chloroform/methanol, and two samples with acetone. The procedure for these samples was the same as above, with the exception that the samples were extracted by placing the sample in the boiling solvent for 30 min, followed by rinsing with solvent for 7 h. The percent extractable material was determined by the following equation:

$$\% \text{ extractable material} = (W_3 - W_2)/W_1 * 100 \quad [2]$$

where W_1 = weight of the sample loaded into the thimble, W_2 = weight of extraction cup and boiling chips, and W_3 = weight of extraction cup + boiling chips + extracted material. The statistical analyses used are outlined in Miller and Miller (11).

RESULTS AND DISCUSSION

Acetylated monoglycerides. Enhanced separation of acetylated monoglycerides was achieved with the 17-m, 50-µm (i.d.) SB-Cyano-25 stationary phase (Fig. 1), as compared to a 20-m, 50-µm (i.d.) SB-Methyl-100 capillary column. Separations with a nonpolar stationary phase, such as 100% polymethyl siloxane, are based on the molecular weight of the analyte, whereas with a polar stationary phase, such as SB-cyano-25, separations are primarily based on the molecular weight, and secondarily, are a function of the polar groups

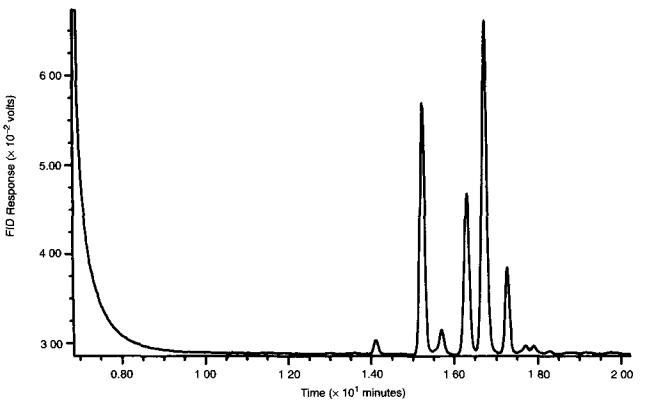

FIG. 1. Supercritical fluid chromatographic separation of acetylated monoglycerides (Grindsted Products, Inc., Industrial Airport, KS). Column, SB-Cyano-25; mobile phase, CO_2; 120°C; pressure ramp, 150–410 atm at 5.0 atm/min; flame-ionization detector (FID) at 375°C.

and/or double bonds present in the analyte (12). Acetylated monoglycerides can be separated with capillary GC (1), although derivatization is required. In the absence of derivatization, GC would be the preferred method of separation, because greater resolution can usually be achieved with capillary GC, as compared to capillary SFC.

Lactylated monoglycerides. The major components of the lactylated monoglycerides eluted between 220–295 atm (30–45 min) (Fig. 2). Soe (1) analyzed acetylated and lactylated monoglycerides with capillary GC. Samples were well separated with capillary GC. However, conversion of the samples to their trimethylsilyl derivatives was required prior to injection. Comparable separations were achieved with SFC, but without the need for sample derivatization.

Decaglycerol decaoleate. Elution of the major components occurred from 260–350 atm (30–60 min) (Fig. 3). Garti and Aserin (2) separated decaglycerol decaoleate with HPLC. Approximately six components were significantly resolved by HPLC with others present as shoulders. In comparison, the SFC separation showed improved resolution over HPLC with approximately ten components well resolved, and others present as shoulders. In addition, more of the low-concentration and early-eluting components were separated with SFC, as compared to HPLC. Sample preparation was comparable for both HPLC and SFC, with neither sample requiring derivatization.

Triglycerol mono/dioleate. The SFC separation of triglycerol monooleate and dioleate required a separation time of over 100 min because the pressure ramp rate was reduced to improve resolution (Fig. 4). Approximately 27 components were resolved. The majority of components eluted during the first ramp, between 200–300 atm (50–88.5 min). Several late-eluting compounds eluted during the second ramp, between 300–366 atm (88.5–105 min). Garti and Aserin (2) separated triglycerol monooleate and triglycerol dioleate by HPLC. Most peaks in the HPLC separation were present as shoulders and baseline resolution was not achieved.

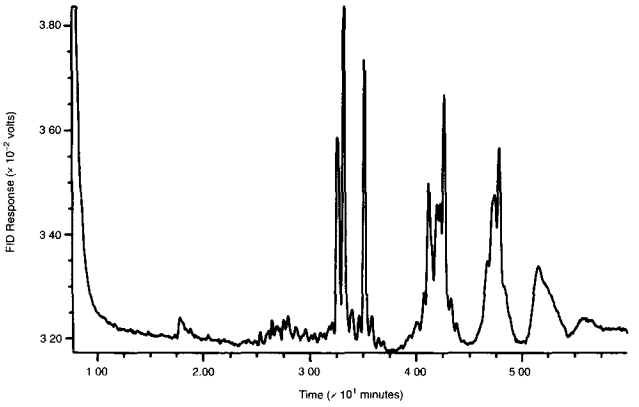

FIG. 3. Supercritical fluid chromatographic separation of decaglycerol decaoleate (Karlshamns, USA, Inc., Janesville, WI). Column, SB-Cyano-25; mobile phase, CO_2; 120°C; pressure ramp, 150–360 atm at 3.5 atm/min; FID at 375°C. See Figure 1 for abbreviation.

Hexaglycerol distearate. The SFC separation of hexaglycerol distearate on the SB-Cyano-25 stationary phase (Fig. 5) was improved relative to the separation with a 100% polymethyl siloxane stationary phase in a 20-m, 50-μm (i.d.) column. Separation of hexaglycerol distearate has not been reported by any other method.

For each emulsifier sample, several density/pressure ramp and temperature combinations were examined. In general, the most successful separation programs were those that encompassed close to the entire pressure or density range for the instrument. In addition, a reduced ramp rate usually resulted in improved resolution. These separations may not represent the optimum separations possible for these compounds, but the objective was to demonstrate the feasibility of SFC for the separation of these compounds, rather then to optimize separation conditions.

Extraction of acetylated monoglycerides. There are three important steps to consider to maximize the possibility of a successful SFE extraction. First, the analytes must be separated

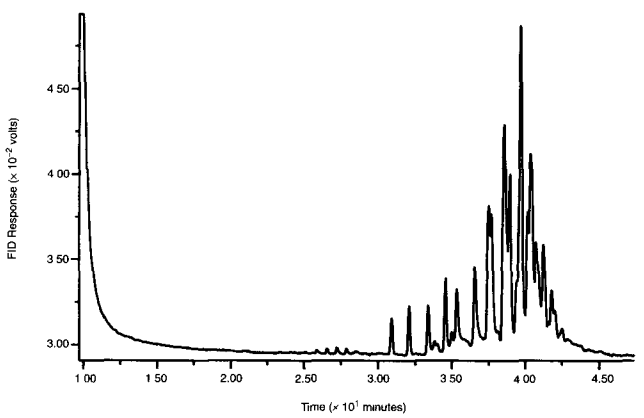

FIG. 2. Supercritical fluid chromatographic separation of lactylated monoglycerides (Grindsted Products, Inc., Industrial Airport, KS). Column, SB-Cyano-25; mobile phase, CO_2; 120°C; pressure ramp, 70–410 atm at 5.0 atm/min; FID at 375°C. See Figure 1 for abbreviation.

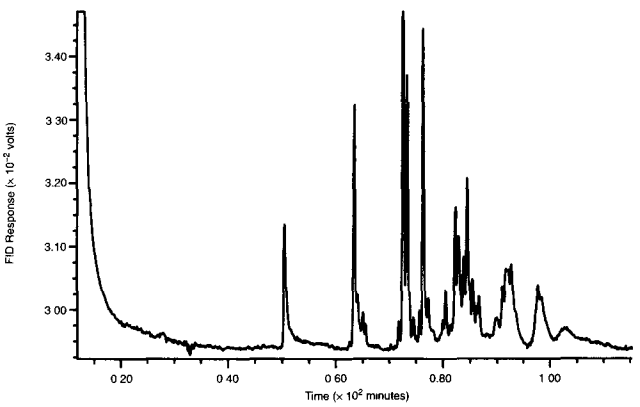

FIG. 4. Supercritical fluid chromatographic separation of triglycerol mono/dioleate (Karlshamns, USA, Inc., Janesville, WI). Column, SB-Cyano-25; mobile phase, CO_2; 120°C; pressure ramp, 70–300 atm at 2.6 atm/min, then 3.9 atm/min from 300–415 atm; FID at 375°C. See Figure 1 for abbreviation.

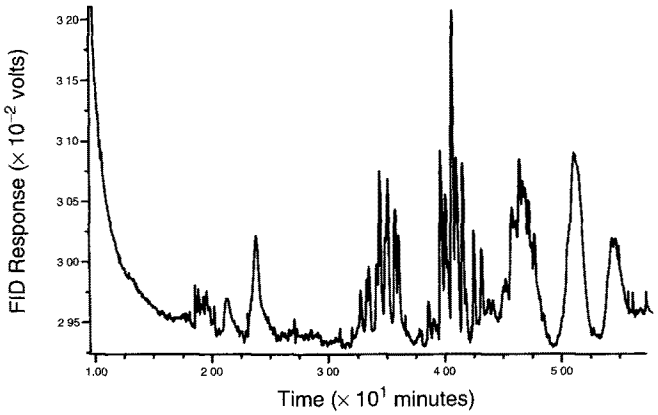

FIG. 5. Supercritical fluid chromatographic separation of hexaglycerol distearate (Karlshamns, USA, Inc., Janesville, WI). Column, SB-Cyano-25; mobile phase, CO_2; 150°C; density ramp, 0.140–0.620 g/mL at 0.008 g/mL, FID at 375°C. See Figure 1 for abbreviation.

from the matrix into the supercritical fluid. This step requires that: (i) the analyte be sufficiently soluble in the supercritical fluid; (ii) the analyte undergo sufficient mass transport by diffusion from the interior of the matrix in which it is located; and (iii) the matrix effects must be overcome (extraction difficulties as a result of matrix/analyte entrapment or binding). Secondly, the analytes must be carried away from the matrix and out of the extraction cell. The final step is the quantitative collection of the analytes in a solvent compatible with the subsequent analysis (13,14). The results of the SFEs of the acetylated monoglyceride standards are presented in Table 3. The improved reproducibility and accuracy at 544 atm and above indicate that an extraction pressure of 544 atm or greater is required. The incomplete extraction and large relative standard deviation values (>2.0%) at ≤408 atm indicate potential extraction problems with food samples at those pressures.

SFE of acetylated monoglycerides from extrudate. The results of the SFEs of extruded cornstarch, containing added acetylated monoglycerides, are in Table 4. For the first extraction series, 544 atm was selected because this was the lowest pressure that consistently resulted in complete extraction of the acetylated monoglycerides.

Because complete extraction was not achieved, the extraction temperature was increased from 50 to 90°C, the pressure was increased from 544 to 656 atm, and the extraction time was increased from 15 to 45 min (Table 4). However, extraction efficacy was not improved further.

To further increase the amount extracted, the temperature was increased from 90 to 120°C and 5% methanol was added as a modifier. Because most supercritical fluids used in SFE are somewhat weak solvents, particularly for intermediate polarity analytes, small amounts of compounds, such as polar organic solvents or compounds that interact with the analytes, can sometimes be added as modifiers to increase extraction efficacy (15). There was no increase, however, in the amount extracted with the addition of methanol (Table 4).

The final extraction series with a smaller particle size did not result in improved extraction efficacy. Reducing particle size can significantly increase extraction efficacy (15). The shape and dimensions of the matrix particles determine the time scale for diffusion (14). Because a reduction in particle size did not result in an increase in the amount extracted, it indicates that diffusion was not the limiting factor (13).

It has been reported that lipid material may become bound during extrusion cooking (16). In a study to evaluate lipid–protein and lipid–carbohydrate interactions during extrusion of cornmeal, Ho and Izzo (17) found that extrusion of a cornmeal system drastically decreased lipid extractability. The study used the amount of hexane-extractable lipid in cornmeal samples as an indication of the free lipid available. Lipid (which could be extracted with hexane from unextruded starch samples) that is not extractable from extruded samples would be indicative of some type of interaction, either on a molecular level or by physical entrapment. Ho and Izzo (17) reported that triglycerides can become entrapped within the starch. Fatty acids were found to associate with the starch to a much greater extent than triglycerides, most likely by a direct molecular interaction during extrusion between fatty acids and amylose or amylopectin. This mechanism could involve hydrogen bonding and/or hydrophobic interactions by helical inclusion complex formation. The affinity of diglyc-

TABLE 3
Supercritical Carbon Dioxide Extraction of Acetylated Monoglycerides from a Glass-Bead Bed[a,b]

	Percent extraction (%)			
Pressure (atm)	340	408	544	680
Flow rate (mL/min)	2.1–2.3	2.6–2.7	3.1–3.4	4.0–4.4
Replicate				
1	76.1	65.9	104.5	99.0
2	71.3	73.8	102.6	97.5
3		93.5		
4		79.7		
Average ± standard deviation	73.7 ± 2.4	78.2 ± 10.1	103.6 ± 1.0	98.3 ± 0.8
RSD (%)	3.2	12.9	0.92	0.76

[a]15-min extractions at 50°C.
[b]Values for each supercritical fluid extraction replicate are from two supercritical fluid chromatography analyses.

TABLE 4
Supercritical Fluid Extraction Results of Extruded Cornstarch and Acetylated Monoglycerides

	Extraction sample[a] (% extraction)			
Replicate	1	2	3	4
1	35.0	54.4	58.3	68.0
2	58.4	55.3	53.3	55.8
3			54.2	56.1
Average ± standard deviation	46.7 ± 11.7	54.9 ± 0.5	55.3 ± 2.2	60.0 ± 5.7
RSD (%)	25.1	0.8	3.9	9.5

[a]Same conditions as listed in Table 2, e.g., sample 1 was extracted at 50°C, 544 atm, for 15 min with a particle size of 0.50–1.00 mm without added modifier.

TABLE 5
One-Hour Soxtec Extraction[a]

	Sample (% extraction)	
	1[b]	2[c]
1	21.5	15.0
2	17.8	10.5
3	23.8	
4	17.6	
5	14.3	
Average ± standard deviation	19.0 ± 3.3	12.8 ± 2.3
RSD (%)	17.4	17.6

[a]1-h total extraction, 15 min in solvent and 45-min rinse.
[b]Extracted with 9:1 (vol/vol) chloroform/methanol.
[c]Extracted with 2:1 (vol/vol) chloroform/methanol.

erides for starch was less than that of fatty acids, but more than that of triglycerides, and appeared to interact by a combination of both entrapment and molecular interaction. Results indicated that, although screw speed or shear did not have a significant effect on lipid–macromolecule interaction, moisture content and barrel temperature had a significant effect on lipid binding, with conditions of low moisture–high temperature producing the most interaction.

Although the extraction of lipids bound during extrusion can be facilitated by using α-amylase digestion to disrupt lipid–starch interactions (17), this was not investigated during this project because the objective of this study was to examine the feasibility of SFE for the extraction of emulsifiers from an extruded product with a minimum of sample pretreatment. Extraction strategies for improving the extraction efficacy of acetylated monoglycerides could possibly include enzyme addition, the use of a different modifier, a substantial increase of the extraction temperature, or a further reduction in particle size. In some cases, such as extraction from a polymer, chain entanglements can make extraction by any method, including SFE, extremely difficult. However, SFE recoveries substantially less than 100% may still be of interest for such concerns as the migration of additives from polymers in foodstuffs (14).

Solvent extraction of extrudate. The results for the one-hour Soxtec extraction are presented in Table 5. Extraction efficacies were much less than that obtained with SFE. A second Soxtec extraction series was done (Table 6) with increased extraction times and an additional solvent. There was an increase in extraction efficacy with an increase in extraction times. The extraction with acetone also resulted in an increase in the amount extracted, as compared to the chloroform/methanol solvent mixtures.

Although there were no significant differences between the samples extracted under different SFE conditions, all of the SFEs resulted in significantly greater extraction percentages than all of the extractions done with the Soxtec system, including both the 1-h and 7.5-h extractions. Extrusion induces strong interactions between the fatty acids and starch, generally characterized by physical entrapment of the lipid. SFE can cause physical changes (swelling) in the sample matrix, which may be one reason for the enhanced extraction efficacy (18).

TABLE 6
Soxtec Extraction (7.5-h)[a]

	Sample (% extraction)		
	1[b]	2[c]	3[d]
1	6.3	19.5	30.5
2	29.4	19.5	28.3
Average ± standard deviation	17.9 ± 11.6	19.5 ± 0.0	29.4 ± 1.1
RSD (%)	64.7	0.0	3.7

[a]7.5-h total extraction, 30 min in solvent and 7-h rinse.
[b]Extracted with 9:1 (vol/vol) chloroform/methanol.
[c]Extracted with 2:1 (vol/vol) chloroform/methanol.
[d]Extracted with acetone.

ACKNOWLEDGMENTS

This work was supported in part by Project No. 50-0134 of the Agricultural Experiment Station, College of Agriculture, University of Illinois at Urbana–Champaign and the University of Illinois Research Board.

REFERENCES

1. Soe, J.B., *Fette Seifen Anstrichm. 85*:72 (1983).
2. Garti, N., and A. Aserin, *J. Liquid Chromatogr. 4*:1173 (1981).
3. Baur, F.J., *J. Am. Oil Chem. Soc. 50*:85 (1973).
4. King, J.W., *J. Chromatogr. Sci. 28*:9 (1990).
5. McIntyre, R.T., *J. Am. Oil Chem. Soc. 56*:835A (1979).
6. Liong, K.K., N.R. Foster and S.S.T. Ting, *Ind. Eng. Chem. Res. 31*:400 (1992).
7. Nilsson, W.B., E.J. Gauglitz, Jr. and J.K. Hudson. *J. Am. Oil Chem. Soc. 68*:87 (1991).
8. Kokini, J.L., C-T. Ho and M.V. Karwe, in *Food Extrusion Science and Technology*, Marcel Dekker, Inc., New York, 1992, p. 740.
9. *Official Methods of Analysis of the Association of Analytical Chemists*, 14th edn., edited by S. Williams, Association of Analytical Chemists, Arlington, 1984, p. 218.
10. *Soxtec System HT6 Manual.* Manual Part No. 1000 1590, Tecator, Inc., Herndon, 1983.
11. Miller, J.C., and J.N. Miller, in *Statistics for Analytical Chemistry, Second Edition*, John Wiley & Son, New York, 1988, pp. 33–52.
12. Artz, W.E., in *Handbook of Chromatography*, edited by K.D. Mukherjee, N. Weber and J. Sherma, CRC Press, Boca Raton, 1993, pp. 83–87.
13. Hawthorne, S.B., in *Supercritical Fluid Extraction and Its Use in Chromatographic Sample Preparation*, edited by S.A. Westwood, New York, 1993, pp. 39–64.
14. Clifford, A.A., in *Ibid.*, edited by S.A. Westwood, New York, 1993, pp. 1–38.
15. Andersen, M.R., J.T. Swanson, N.L. Porter and B.E. Richter, *J. Chromatogr. Sci. 27*:371 (1989).
16. Smith, O.B., in *Fabricated Foods*, edited by G.E. Inglett, Avi Publishing, Westport, 1975.
17. Ho, C.T., and M.T. Izzo, in *Food Extrusion Science and Technology*, edited by J.L. Kokini, C.T. Ho and M.V. Karwe, Marcel Dekker, Inc. New York, 1992, pp. 415–425.
18. McHugh, M.A., and V.J. Krukonis, in *Supercritical Fluid Extraction*, Butterworths, Boston, 1986.

[Received June 6, 1994; accepted October 23, 1994]