Urea-Based Fractionation of Seed Oil Samples Containing Fatty Acids and Acylglycerols of Polyunsaturated and Hydroxy Fatty Acids

Douglas G. Hayes[a], James M. Van Alstine[b,c,*], and Fredrik Setterwall[c]

Departments of [a]Chemical and Materials Engineering and [b]Chemistry, University of Alabama in Huntsville, Huntsville, Alabama 35899, and [c]Department of Chemical Engineering and Technology, Royal Institute of Technology, SE-100 44 Stockholm, Sweden

ABSTRACT: The selectivity and efficiency of urea complex (UC) formation-based fractionation of free fatty acids (FFA) were examined. A rapid, simple, and inexpensive procedure recently developed for urea fractionation was applied to lipid mixtures containing various polyunsaturated and hydroxy FFA species. Urea treatment proved useful for isolating polyunsaturated FFA (PUFA) from FFA derived from fish, borage, and linseed oils by removal of saturated and monounsaturated FFA, but was not effective for isolating hydroxy FFA from the FFA derived from castor, *Lesquerella,* and *Dimorphotheca* oils. In situations where FFA within the crystalline or UC phase were rich in PUFA, the urea/FFA mole ratio of the UC was relatively higher, with lower recovery of FFA in this phase. The distribution of urea between the crystalline phase and the solvent was not significantly affected by the FFA composition of feed nor the overall ratio of FFA to urea. It was strongly dependent on the overall mass fraction of solvent. Phospholipids and mono-, di-, and triacylglycerols were poor templates for UC formation relative to FFA. Their inclusion in acylglycerol mixtures containing FFA reduced UC formation.

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The formation of complexes between urea and linear hydrocarbon templates is a well-known and potentially valuable separation technique for the fractionation of free fatty acids (FFA) (1). Urea molecules readily form solid-phase complexes with saturated FFA; however, the presence of double bonds, branching, or bulky constituents in the hydrocarbon chains greatly reduces the propensity for urea complex (UC) formation (1). Polyunsaturated FFA (PUFA) and branched FFA have been isolated through removal of saturated and monounsaturated FFA by UC formation. Examples include the isolation of $20{:}5^{5c,8c,11c,14c,17c}$ (eicosapentaenoic acid, or EPA) and $22{:}6^{4c,7c,10c,13c,16c,19c}$ (docosahexaenoic acid, or DHA) from fish oil FFA (2–5), $18{:}3^{6c,9c,12c}$ (γ-linolenic acid, or GLA) from black currant and borage oil FFA (6,7), and $18{:}3^{9c,12c,15c}$ (α-linolenic acid) from linseed oil (8,9). In addition, UC formation effectively discriminated against cyclic FFA, such as malvalic (*cis*-8,9-methyleneheptadec-8-enoic) and sterculic (*cis*-9,10-methyleneoctadec-9-enoic) FFA derived from *Bombax munguba* (cotton) and *Sterculia foetida* (kapok) seed oils, respectively (10). Most of the cited studies employed the process on a gram scale, although Ackman and coworkers operated on a kilogram (pilot) scale (3). For all of the cited examples, UC formation was allowed to proceed by slowly cooling the medium over several hours, as would occur during crystallization.

Our group recently determined that UC formation was very effective in removing saturated FFA from rapeseed oil FFA, which should improve the FFA mixture's nutritional value (11). The procedure designed and employed in our laboratory occurred within a few minutes and was simple, inexpensive, and reproducible (11). It utilized abundant and inexpensive resources, namely, urea and 95% ethanol. UC were formed by heating a mixture of urea, lipid, and solvent (95% ethanol) to 30–70°C to achieve homogeneity. The mixture was rapidly cooled (within 1 min) to room temperature to produce a biphasic system consisting of solid "raffinate" phase (the UC) and a liquid "extract" phase. The primary role of the solvent was to promote contact between urea and FFA in order to induce UC formation. An increase of the relative amount of solvent lowered the temperature required to achieve miscibility; however, UC formation (hence removal of saturated FFA) decreased linearly with solvent concentration (11). The formation of UC was also reduced by increasing the water content of the solvent, or complex formation temperature (11). The major concern is the possible formation of carbamates between ethanol and urea (12), a future topic of research for our group.

In the present study, the same fractionation procedure was applied to different sources of FFA mixtures containing PUFA (linseed, borage, and fish) or hydroxy FFA (castor, *Dimorphotheca,* and *Lesquerella*), as well as to acylglycerol- and phospholipid-containing mixtures. The results obtained

*To whom correspondence should be addressed at Royal Institute of Technology, Teknikringen 28, SE-100 44 Stockholm, Sweden.
Email: alstine@ket.kth.se

further understanding of process selectivity, the impact of impurities, and new applications of this technique.

EXPERIMENTAL PROCEDURES

Materials. Rapeseed oil (low erucic acid) was purchased from Konsum (Stockholm, Sweden). Fish oil was from Cardinova (Uppsala, Sweden). Seed oils from *Lesquerella fendleri*, *Limnanthes alba* (meadowfoam), and *Dimorphotheca pluvialis* were gifts from International Floratechnologies (Apache Junction, AZ), Dr. Thomas Abbott (USDA/ARS/NCAUR, Peoria, IL), and Dr. Johannes T.P. Derksen (ATO-DLO, Wageningen, The Netherlands), respectively. Oils from castor (*Ricinis communis*), borage (*Borago officinalis*), and linseed were purchased from Sigma (St. Louis, MO). Free fatty acid (FFA) from rapeseed oil was a gift from Karlshamns AB (Karlshamn, Sweden). The FFA sources employed were received as refined materials. Egg yolk phosphatidylcholine (*ca*. 60% pure) was purchased from Sigma. Lipozyme IM, *Rhizomucor miehei* lipase immobilized onto anion exchange resin, was donated by Novo Nordisk Bioindustrials (Copenhagen, Denmark). All other reagents were of high purity and were used without further purification. Distilled, deionized water was used throughout.

Preparation of starting materials. FFA were formed from seed oils by saponifying with KOH in methanol at reflux for *ca*. 3 h, then releasing FFA by treatment with concentrated HCl (aq.), saturated NaCl solution, and hexane. Fatty acid methyl esters (FAME) of *Dimorphotheca* oil were generated using NaOMe in methanol under reflux. *Lesquerella* oil was lipolyzed using the 1,3-positional selective Lipozyme IM catalyst (13). Released FFA were recovered from the remaining partial acylglycerols and triacylglycerol using a saponification/extraction technique, "SAPEX" (14). The FFA resulting from SAPEX also contained a small amount of partial acylglycerols (discussed below).

Procedures. UC formation was applied to FFA or acylglycerol mixtures as described previously (11). Mixtures of 95% ethanol and urea, FFA, or acylglycerols were heated until a single liquid phase formed. For mixtures rich in rapeseed triacylglycerol (TAG), 1-butanol was added dropwise until the TAG completely solubilized into ethanol. The one-phase solution was rapidly cooled, within 1 min, to 22–25°C, then immediately filtered. Urea was removed from both the filtrate and the UC, i.e., the extract and raffinate phases, respectively, using acidified warm (60°C) water, after which the FFA from both phases were isolated, and their amounts determined gravimetrically. Ethanol was evaporated from the filtrate prior to the addition of hot water in order to reduce the loss of hydroxy FFA to the warm water rinsate during the urea removal step. The distribution of urea between the raffinate and extract was determined from mass balances of the urea and the FFA, using the mass of UC measured gravimetrically, and the mass of FFA in the raffinate and extract.

The composition of FFA was determined using gas chromatography (GC) of FAME as described previously (11). To analyze FAME of meadowfoam and borage oils, a temperature program with a less steep temperature ramp [150°C (1 min hold), followed by a 2°C/min temperature ramp up to 265°C, followed by a 1 min hold at 265°C] was required to achieve the separation. *Lesquerella* oil FFA samples were silylated rather than methylated in order to separate lesquerolic (R-20:1^{11c}-OH^{14}) and auricolic (R-20:$2^{11c,17c}$-OH^{14}) acid chromatographic peaks (15). Owing to the difficulty of derivatizing *Dimorphotheca* for GC analysis (16,17), high-performance liquid chromatography (HPLC) was employed using a dual-pump system from Rainin Instruments (Woburn, MA) in series with an MKIII evaporative light-scattering mass detector from Alltech (Deerfield, IL). A C_{18}-reversed phase column (4.6 mm × 25 cm, Rainin), operated isothermally at 25°C, was utilized with an acetone/acetonitrile/acetic acid (45:45:10, vol/vol/vol) solvent system delivered at 1.0 mL/min. Lipid mixtures were separated into subclasses using normal-phase HPLC (18). A silica Microsorb-MV column (4.6 mm × 25 cm, Rainin) was employed using isooctane/2-propanol (90:10, vol/vol) as mobile phase under the following flow program: 0–17 min, flow rate at 0.3 mL/min; 17–25 min, increase flow rate to 1.5 mL/min at 0.2 mL/min/min; 25–27 min, decrease flow rate to 0.3 mL/min at 0.3 mL/min/min. The FFA content of lipid mixtures was also measured using a standard titration method (19).

RESULTS AND DISCUSSION

Urea fractionation of PUFA and hydroxy FFA from seed oils. Urea fractionation was applied to FFA derived from several sources rich in PUFA (fish, borage, and linseed oils), hydroxy FFA (castor, *Dimorphotheca*, and *Lesquerella* oils), or in FFA of unusual double bond position, i.e., near the carbonyl group. Sources for the latter category include meadowfoam oil, which contains several long-chain Δ5 FFA; fish oil, which contains EPA and DHA; and borage oil, which contains GLA. In Tables 1 and 2 are lists of the amount of UC formed, and the partitioning of urea and FFA between the UC or "raffinate" phase and the noncomplex-forming, solvent-rich "extract" phase. The set of experiments within Table 1 employed

TABLE 1
Properties of Urea Complexes (UC) Formed from 3.0 g (12.0 wt%) Urea, 6.0 g (24.0%) FFA, and 20 mL (64.0%) of 95% Ethanol

Run no.	FFA source	UC yield[a]	Urea[a] (% recov.)	FFA[a] (% recov.)	Urea/FFA (mol/mol)
B1	Borage	0.120 (0.333)	74.3	12.5	13.9
C1	Castor	0.123 (0.341)	77.9	12.2	15.2
F1	Fish	0.116 (0.321)	76.7	9.8	18.2
Lq1	*Lesquerella*	0.110 (0.306)	68.0	12.0	14.0
Ln1	Linseed	0.124 (0.336)	78.2	9.8	19.6
M1	Meadowfoam	0.121 (0.336)	76.6	12.0	16.6
	Rapeseed[b]	0.114 (0.317)	69.2	13.7	13.8

[a]UC yield is reported in mass of UC produced per mass of entire system (solvent-free yield in parentheses). Other denoted table headings refer to recovery of urea and free fatty acid (FFA) mass in the raffinate (UC) phase.
[b]Inferred from data in Reference 11.

TABLE 2
Properties of UC Formed from 3.0 g (14.3 wt%) Urea, 2.0 g (9.52%) FFA, and 20 mL (76.2%) 95% Ethanol

Run no.	FFA source	UC yield[a]	Urea recovery[a] (%)	FFA recovery[a] (%)	Urea/FFA (mol/mol)
B2	Borage	0.132 (0.547)	71.5	29.6	16.7
D2	*Dimorphotheca*	0.136 (0.569)	77.3	26.5	20.4
F2	Fish	0.132 (0.553)	75.1	25.6	20.2
Lq2	*Lesquerella*	0.130 (0.544)	70.9	29.8	17.7
LLq2	Lipolysized *Lesquerella*[b]	0.110 (0.459)	56.8	29.9	14.3
Ln2	Linseed	0.128 (0.532)	73.5	22.4	23.0
M2	Meadowfoam	0.146 (0.617)	79.0	35.7	17.3
	Rapeseed[c]	0.129 (0.542)	65.0	37.3	13.8
B3	Borage[d]	0.132 (0.616)	62.1	60.1	16.2

[a]UC yield is reported in mass of UC produced per mass of entire system (solvent-free yield in parentheses). Other denoted table headings refer to recovery of urea and FFA mass in the raffinate (UC) phase.
[b]Lipid composition of initial, raffinate, and extract phases: 83.9% FFA/9.3% MAG/2.6% DAG, 4.3% TAG; 76.5% FFA/13.3% MAG/8.8% DAG/1.4% TAG; and 86.3% FFA/6.2% MAG/3.2% DAG/4.2% TAG, respectively.
[c]Inferred from data of Reference 11.
[d]10 g (16.5 wt%) urea, 3.0 g (4.93%) borage FFA, and 60 mL (78.6%) 95% ethanol. MAG, monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol; for other abbreviations see Table 1.

a urea/FFA ratio of 1:2 (w/w), whereas those in Table 2 used a 3:2 ratio. (Run *B3* used an even higher ratio.) The FFA composition of the original materials plus the raffinate and extract phases for each experiment listed in Tables 1 and 2 are contained in Tables 3 to 8. FAME analyses of the various seed oil sources strongly agree with other reported analyses, as do values of the urea/FFA molar ratio (1).

The yield of UC and recovery of urea in UC are not strongly affected by the FFA source (Table 1). The recovery of FFA and the relative amount of FFA and urea in the UC are generally similar with different FFA sources. Linseed and fish oil FFA, which yielded lower FFA recoveries and larger urea/FFA ratios, are exceptions. This may be related to the relatively larger polyunsaturated (i.e., noncomplexing) FFA content of these samples.

Although the experiments of Table 2 employed a much higher urea/FFA ratio than those of Table 1, the recovery of urea in the raffinate phase, i.e., the partitioning of urea between the extract and raffinate, is very similar. Both sets of data share the same urea/solvent ratio. In agreement, the partitioning of urea was found to be independent of the relative amount of rapeseed FFA when the ratio of urea to solvent was held constant (11). As occurred in Table 1, experiments displayed in Table 2 (other than data set *B3*) exhibited nearly identical values for UC yield and FFA recovery. An exception to this is run *LLq2*, lipolyzed *Lesquerella* oil, which yielded a lower amount of UC owing to the presence of mono-, di-, and triacylglycerols (MAG, DAG, and TAG, respectively). Since MAG/DAG/TAG are less effective templates for UC formation relative to FFA, the yield of UC is lower and the recovery of urea is reduced (discussed below). Because of the higher urea/FFA ratio employed in Table 2 experiments relative to those of Table 1, a larger percentage of FFA was incorporated into UC, resulting in higher PUFA contents for the Table 2 raffinate phases (Tables 3 to 8). The increased PUFA content of the raffinate would require a higher urea/FFA ratio in this phase, which is consistent with Tables 1 and 2.

Run *B3* employed a very high urea/FFA ratio, 10:3. When rapeseed FFA was substituted for borage FFA and subjected to the same conditions as run *B3*, the extract was nearly devoid of FFA (11). The fact that run *B3* extract contained almost 40% of the FFA reflects the difficulty of forming UC with PUFA. This trend also explains the lower recovery of

TABLE 3
Purification of Eicosapentaenoic and Docosapentaenoic Acids (EPA and DHA, respectively) from UC Fractionation of Fish Oil FFA[a]

FFA	F-0	F1-r	F1-e	F2-r	F2-e	F3-r[b]	F3-e[b]
EPA	19.0	1.1	20.6	0.6	27.8	2.7	35.6
DHA	10.8	0.6	11.5	1.2	16.2	1.0	23.9

[a]Table headings: FFA = free fatty acid; other entries refer to run numbers contained in Tables 1 and 2 (*X*), where X-0 refers to original sample before urea treatment, and X-r and X-e refer to raffinate and extract phases, respectively. EPA and DHA refer to 20:5[5,8,11,14,17] and 22:6[4,7,10,13,16,19], respectively, where all indicated double bonds are in the *cis* configuration. For other abbreviations see Table 1.
[b]Run F3 was conducted using the same conditions as run B3 listed in Table 2. The yields and recoveries in the extract and raffinate phases were not determined.

TABLE 4
FA Analysis of Borage Oil FFA from UC Fractionation[a]

FFA	B-0	B1-r	B1-e	B2-r	B2-e	B3-r	B3-e
16:0	10.9	39.9	5.3	32.0	0.8	17.0	0.4
18:0	3.6	22.5	0.1	11.6	0.2	5.9	0.4
18:1	16.6	12.0	16.3	26.4	11.7	25.1	0.0
18:2	39.8	6.0	47.8	13.2	53.2	36.0	46.3
γ-18:3	21.7	0.6	24.8	0.6	31.6	3.5	52.6
20:1	3.9	4.8	3.5	8.8	1.7	6.2	Trace
22:1	2.0	5.0	1.5	5.5	0.3	3.2	0.0
24:1	1.2	5.8	0.5	0.3	0.0	2.0	0.0

[a]Table headings: FFA and other entries are identified in Tables 1 and 3. γ-18:3 (GLA), or γ-linolenate, refers to 18:3[6,9,12].

TABLE 5
FFA Analysis of Linseed Oil FFA from UC Fractionation[a]

FFA	Ln-0	Ln1-r	Ln1-e	Ln2-r	Ln2-e	Ln1E-r[b]	Ln1E-e[b]
16:0	5.3	30.0	2.1	16.8	0.6	6.0	0.3
18:0	3.8	22.4	1.2	10.7	0.4	3.1	0.6
18:1	25.0	30.7	23.4	41.4	14.2	41.3	12.4
18:2	16.6	4.6	18.4	9.2	20.1	15.4	22.1
18:3	49.4	9.6	55.0	20.7	62.3	33.2	64.6

[a]Table headings: FFA and other entries are identified in Tables 1 and 3.
[b]FFA from the extract of experiment Ln1 was subjected to UC formation by combining 6.0 g (23.5 wt%) urea, 3.7 g (14.3%) Ln1-e, and 40 mL (62.2%) 95% ethanol. The yield of UC was 0.197 g per g of overall mass (0.521 g/g on a solvent-free basis), with 67.6 and 26.4% of urea and FFA, respectively, being incorporated into the UC. The molar ratio of urea to FFA in the UC was 19.72.

TABLE 6
FFA Analysis of Meadowfoam Oil FFA from UC Fractionation[a]

FFA	M-0	M1-r	M1-e	M2-r	M2-e
16:0	0.6	1.1	0.1	0.6	0.3
$18:1^{5}$	0.5	0.3	0.7	0.2	0.7
18.1^{9}	1.8	0.7	2.0	0.8	2.4
18.2	2.5	0.3	2.8	0.3	3.6
20:0	0.7	3.4	0.4	1.5	0.0
$20:1^{5}$	62.8	75.4	59.3	72.7	54.9
$22:1^{13}$	12.3	13.4	12.9	16.9	10.6
$22:2^{5,13}$	15.7	3.5	18.6	5.3	24.7

[a]Table headings: FFA and other entries are identified in Tables 1 and 3.

urea in the raffinate even though the urea/solvent ratio for run *B3* is similar to those employed in Tables 1 and 2.

In agreement with the literature, UC fractionation strongly discriminated against the PUFA of fish oil, EPA and DHA (Table 3) (2–5). When the conditions of run *B3* were applied to fish oil FFA, an extract product containing 35.6% EPA and 23.9% DHA was recovered. GC analyses indicated that several peaks overlapped in the C_{16}, C_{18}, and C_{20} saturated plus mono-, di-, and triunsaturated regions, making it very difficult to monitor the partitioning of the individual components.

The other sources of PUFA, namely, borage and linseed FFA, were also successfully fractionated using UC (Tables 4 and 5). The GLA content of borage FFA can be increased more than twofold when the conditions of run *B3* are employed (Table 4). However, linoleic (18:2) acid is concentrated in the extract along with GLA. A second step must therefore be applied to separate 18:2 and GLA so as to obtain a highly concentrated GLA product. A candidate for the second step would be selective esterification using lipases, which have a strong substrate preference for 18:2 over GLA (20). Lipase biocatalysis and UC fractionation would be complementary techniques since, unlike UC fractionation, biocatalysis does not separate GLA from palmitic (16:0) and stearic (18:0) acids (20). The α-linolenic acid content of linseed FFA increased significantly, from 49 to 62–64% using urea (Table 5); however, similar to borage FFA processing, a second separation step would be required to remove 18:1 and 18:2 from the extract. In contrast, the linseed FFA raffinate phases contained much higher amounts of α-linolenic acid compared to the GLA content of borage raffinate phases. This suggests that the double bond position in the latter is discriminated against in UC formation. In agreement, α-linolenic acid was more strongly incorporated in UC than γ-linolenic acid during UC fractionation of blackcurrant oil FFA (6). Note that the extract of run *Ln1* was successfully treated with an additional UC step to yield a FFA extract highly concentrated in α-linolenic acid (Table 5, run *Ln1E-r*).

TABLE 7
FFA Analysis of Oils Rich in C_{18} Hydroxy FFA from *UC* Fractionation[a]

FFA	C-0	C1-r	C1-e	D-0[b]	D2-r	D2-e
16:0	1.6	10.4	0.5	1.9	2.5	0.0
18:0	0.9	9.4	0.1	1.7	3.3	0.0
18:1	6.0	13.6	4.5	20.9	22.1	1.2
18:2	10.5	5.7	9.1	10.8	2.2	20.1
18:3	0.4	0.2	0.7			
Ricinoleic	78.1	54.6	83.2			
Dimorphecolic				61.8	45.9	68.3

[a]Table headings FFA and other entries are identified in Tables 1 and 3. Ricinoleic and dimorphecolic acids refer to *R*-$18:1^{9c}$-OH^{12} and *S*-$18:2^{10t,12t}$-OH^{9}, respectively.
[b]FFA analysis from Reference 17.

The FFA distribution of meadowfoam is interesting given its abundance of long-chain and Δ5 acids. The isolation of Δ5 FFA has received considerable attention because of their potential applications (21). The challenge is to isolate the two most abundant FFA species, $20:1^{5}$ and $22:2^{5,13}$, present at 62.8 and 15.7%, respectively, from the third-most abundant species, erucic ($22:1^{13}$) acid, present at 12.3%. These three FFA species account for over 90% of meadowfoam FFA. UC formation did not demonstrate any discrimination against the Δ5 mono- and diunsaturated FFA of meadowfoam. Surprisingly, both $20:1^{5}$ and erucic acid were more highly concentrated in the raffinate phase than the extract (Table 6). However, UC fractionation discriminated against $22:2^{5,13}$, shown by the increase of its percentage in the extract phases relative to the starting material (Table 6). Perhaps urea treatment may be used to separate $22:1^{5}$ from $22:2^{5,13}$, with the former and latter occurring predominantly in the rafffinate and extract phases, respectively, once erucic acid is removed, e.g., using lipase catalysis (21).

UC fractionation was less discriminatory against hydroxy FFA relative to PUFA. Although the hydroxy FFA content of castor, *Dimorphotheca*, and *Lesquerella* increased slightly in the extract phases relative to the starting materials, the percentage of hydroxy FFA in the raffinate phases was significant (Tables 7 and 8). A strong discrimination against dimorphecolic (*S*-$18:2\Delta^{10t,12t}$-OH^{9}) acid was expected due to its possession of two double bonds and a hydroxyl group, with all three substituents being close together in the molecule. Perhaps dimorphecolic acid readily formed UC because of the *trans* configuration of its two double bonds, in contrast to the *cis* double bond configuration present in all of the other FFA examined. In agreement, a preference for UC formation of elaidic ($18:1\Delta^{9t}$) over oleic ($18:1\Delta^{9c}$) acid has been observed (1,22). Furthermore, UC fractionation was more discriminatory against auricolic (*R*-$20:2\Delta^{11c,17c}$-OH^{14}) acid than either lesquerolic (*R*-$20:1\Delta^{11c}$-OH^{14}) or dimorphecolic acid, presumably owing to the two *cis* double bonds of the former (Tables 7 and 8).

Although UC fractionation did not discriminate strongly against hydroxy FFA, it was useful in removing the saturated and monounsaturated FFA species that accompany castor and the 1,3-selective lipolysate of *Lesquerella* oil (Tables 7 and 8). When UC fractionation is performed under the conditions employed in Tables 1 and 2, the loss of hydroxy FFA to the raffinate phase is reasonably small; moreover, the recovery of ricinoleic and lesquerolic acids in the extract for experiments *C1* and *Llq2* were 87.9 and 73.4%, respectively.

Urea fractionation of acylglycerols and acylglycerol/FFA mixtures. TAG of rapeseed and *Lesquerella* formed minor

TABLE 8
FFA Analysis of *Lesquerella* Oil FFA from UC Fractionation[a]

FFA	Lq-0	Lq1-r	Lq1-e	Lq2-r	Lq2-e	LLq2-0	LLq2-r[b]	LLq2-e[b]
16:0	1.5	7.2	0.4	4.3	0.1	2.2	5.7	0.2
16:1	0.7	0.4	0.5	0.5	0.4	0.4	0.2	0.4
18:0	2.2	12.5	0.3	6.0	0.1	3.0	7.9	0.1
18:1	15.5	24.5	12.7	29.2	10.2	7.1	9.9	5.1
18:2[c]	9.9	5.8	12.3	6.9	11.5	6.6	4.5	6.6
18:3	11.9	3.2	14.0	3.3	14.5	6.2	1.8	7.4
20:1	1.1	1.7	0.6	1.7	0.6	1.2	2.5	0.6
Lesquerolic	53.5	24.7	54.1	47.3	57.7	69.8	65.5	75.3
Auricolic	3.1	1.3	4.9	0.6	5.0	3.4	1.4	4.3

[a]Table headings FFA and other entries are identified in Tables 1 and 3. Lesquerolic and auricolic acids refer to *R*-20:1^{11c}-OH^{14} and *R*-20:$2^{11c,17c}$-OH^{14}, respectively.
[b]Lipid composition of initial, raffinate, and extract phases: 83.9% FFA/9.3% MAG/2.6% DAG, 4.3% TAG; 76.5% FFA/13.3% MAG/8.8% DAG/1.4% TAG; and 86.3% FFA/6.2% MAG/3.2% DAG/4.2% TAG, respectively.
[c]Contains also vaccenic (18:1^{11}) acid. For abbreviations see Tables 1 and 2.

amounts of UC (Table 9). In addition, the fatty acid compositions of TAG in both the raffinate and extract phases were identical to that of the original source, indicating no fractionation of TAG species occurred. The difficulty in forming UC of TAG may be due to the bulkiness of these molecules reducing their ability to fit into the narrow 5.5-Å diameter channel of UC (1). These results suggest that urea treatment could be used to isolate FFA from a FFA/acylglycerol mixture. This hypothesis was examined using mixtures of rapeseed oil FFA and TAG (Fig. 1). Experiment A, which employed a low percentage of FFA and a high urea/FFA ratio, resulted in the formation of UC of composition similar to that of the original material. Based on studies of rapeseed FFA, the conditions of Experiment A should lead to nearly 100% incorporation of FFA in the absence of TAG (11). Thus, the addition of TAG appears to greatly reduce the yield of UC and the inclusion of FFA in UC. When the FFA/TAG ratio was increased (and the urea/FFA ratio reduced), UC fractionation was more successful in selectively removing FFA (Fig. 1).

The UC fractionation of various acylglycerol mixtures of *Lesquerella* was examined under similar experimental conditions as the rapeseed FFA/TAG mixtures, with urea, lipid, and 95% ethanol being present at *ca*. 12, 19–24, and 64–68%, respectively. The presence of 11.5% MAG/DAG/TAG in the lipid feed did not affect the yield of UC nor the recoveries of urea and FFA in the UC. However, 16% MAG/DAG/TAG reduced the yield of UC at the conditions of Table 2 as noted above. The experiments of Table 2 employed a much higher urea/FFA ratio than those of Table 9, allowing for higher UC incorporation for partial acylglycerols. As the FFA composition of the aclyglycerol mixture was lowered, the UC yield generally decreased, as did the recoveries of urea and FFA in the UC (Table 9). In the absence of FFA, only a small portion of the partial acylglyerols were incorporated into UC

TABLE 9
Properties of UC Formed from Lipid Mixtures of *Lesquerella* Oil

Urea/lipid/EtOH	Composition	UC yield[a]	Urea recovery[a] (%)	Lipid recovery[a] (%)
12.0/24.0/64.0	FFA (100.0%)[b]	0.110 (0.306)	68.0	12.0
12.2/19.8/68.0	FFA (78.5%)[c]	0.113 (0.354)	72.1	12.8
12.8/19.2/68.0	FFA/TAG[d,e]	0.103 (0.322)	60.2	13.7
12.0/24.0/64.0	FFA/MAG/DAG/TAG[f]	0.089 (0.247)	55.4	9.4
12.0/24.0/64.0	MAG/DAG/TAG[g]	0.080 (0.220)	58.9	3.3
13.7/13.7/72.7	TAG (100.0%)	0.073 (0.270)	51.8	1.7

[a]UC yield is reported in mass of UC produced per mass of entire system (solvent-free yield in parentheses), excluding any added 1-butanol. Other denoted table headings refer to recovery of urea and lipid mass in the raffinate (UC) phase.
[b]Same as run Lq1 of Tables 1 and 8.
[c]78.5% FFA/18.3% MAG/3.0% DAG, resulting from application of a saponification/extraction method to lipolysate (Ref. 14).
[d]Lipids of rapeseed oil.
[e]33.3% FFA, 66.7% TAG.
[f]25.3% FFA/7.4% MAG/33.6% DAG/33.7% TAG, resulting from the 1,3-selective lipolysis of *Lesquerella* oil.
[g]<0.5% FFA/10.5% MAG/50.1% DAG/39.3% TAG, resulting from application of a saponification/extraction method to lipolysate (Ref. 14). For abbreviations see Tables 1 and 2.

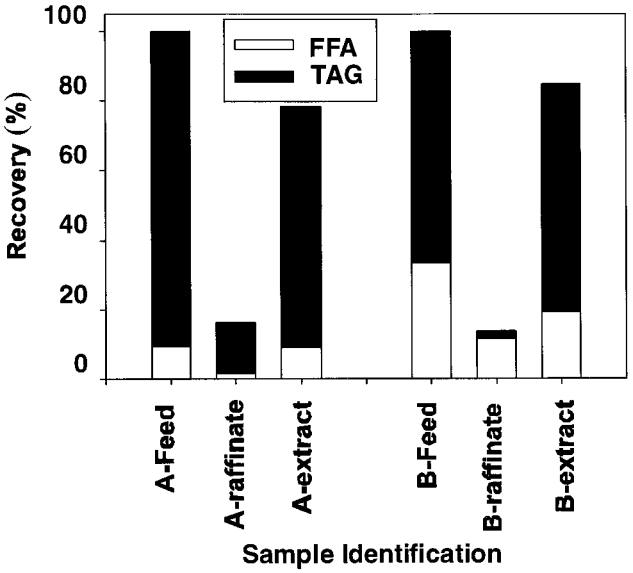

FIG. 1. Recovery of mass in extract and raffinate phases from urea treatment of oleic acid/rapeseed oil triacylglycerol (TAG) mixtures. X-0, X-e, and *X*-r refer to initial sample before urea treatment, and the extract and raffinate phases after urea addition, respectively, for each given experiment. Experiment A: 21.4% urea, 9.5% lipid, and 69.1% of 95% ethanol; Experiment B: 12.8% urea, 19.2% lipid, and 68.0% of 95% ethanol. Note: significant amounts of 1-butanol were required to form homogeneous liquid phases for Experiment A. FFA, free fatty acid.

(Table 9). MAG and DAG were not much better UC templates than TAG. Thus, the presence of glycerol esterified to FFA appears to deter UC formation significantly. Knight *et al.* (23) reported that 1-monopalmitin formed UC but required a much larger amount of urea than an equivalent mass amount of palmitic acid. For the experiments depicted in Table 9, with the exception of the rapeseed FFA/TAG mixture, the changes in composition of the extracts and raffinates from their respective original acylglycerol mixtures were modest (data not shown).

Urea fractionation of phospholipids and phospholipid/FFA mixtures. Phospholipids are common impurities encountered in processed seed oils and FFA mixtures. Under the conditions studied, phosphatidylcholine (PC) formed little or no UC. To check the effect of PC as impurity, we repeated run *F*1 (Table 1), but added 0.10 g PC/g FFA. The presence of PC greatly reduced UC formation (UC yield of 0.053 g/g or 0.141 g/g on a solvent-free basis) and drastically reduced the recovery of FFA in the UC to approximately 1%. The addition of only 0.036 g PC/g FFA to a system containing 13.6% each of urea and rapeseed FFA and 72.8% of 95% ethanol reduced the UC yield from 0.125 g/g (0.456 g/g on a solvent-free basis) to 0.009 g/g (0.032 g/g solvent-free). PC therefore strongly inhibited the formation of UC in a manner similar to partial acylglycerols and TAG. These substances should be removed from the lipid source before urea fractionation is applied. Other inhibitors of UC formation, such as sulfur compounds, are discussed in the literature (24). The mechanism for PC inhibition of UC formation is a subject for further study.

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