Urea Complexation for the Rapid, Ecologically Responsible Fractionation of Fatty Acids from Seed Oil

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ABSTRACT: Urea complex formation is a classic method for fractionating fatty acids from seed and other oils. The method's simplicity, ease of scaling, and ecological friendliness suggest its reevaluation in regard to modern fractionation challenges. In keeping with this, a simple, quick, inexpensive, robust, and environmentally friendly procedure was developed for reducing the saturated free fatty acid (FFA) content of saponified low-erucic acid rapeseed oil (LEAR). The process involves formation of a homogeneous 65°C solution of FFA and urea in 95% ethanol (5% water), followed by cooling of the resultant urea complex slurry to room temperature. The urea complex and liquid phases are separated by gravity filtration, and the urea isolated in each phase is removed by extraction with 60°C water. Saturated LEAR oil FFA preferentially formed urea complexes easily separated from the noncomplexed, mostly unsaturated FFA, the main product of interest. The effects of single- vs. double-stage fractionations and several other variables (component mass or volume ratios, temperature, ethanol solvent to water ratio) were preliminarily evaluated. Results demonstrated the robustness, reproducibility, and simplicity of the method.
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KEY WORDS: Fractionation, free fatty acid, (low-erucic acid) rapeseed oil, urea complex.

The use of free fatty acids (FFA) from seed oils for commercial applications requires various fractionation steps. For example, the use of unsaturated FFA from low erucic acid rapeseed (LEAR) oil in paints, foods, and pharmaceuticals necessitates the removal of saturated FFA, which if recovered can be employed in other applications such as the synthesis of lubricants. Other industrially significant lipid separation challenges include the isolation of polyunsaturated FFA, such as eicosapentaenoic ($20{:}5^{5c,8c,11c,14c,17c}$) and docosahexaenoic ($22{:}6^{4c,7c,10c,13c,16c,19c}$) acids (EPA and DHA, respectively) from fish and algae, and the isolation of hydroxy acids from oils such as castor (*Ricinus communis*), lesquerella (*L. fendleri*), and dimorphotheca (*D. pluvialis*). Most common lipid fractionation techniques, such as molecular distillation, require extreme temperatures which can degrade polyunsaturated and oxygenated FFA. Techniques such as membrane filtration, liquid chromatography, crystallization, or processes involving lipase enzymes are slow, inefficient, expensive, and often difficult to scale up. Many procedures also require nonrenewable organic solvents, whose negative environmental effects dictate rising costs.

The above indicates a need to investigate fractionation methods which involve ecologically friendly reagents and reagent recycling. Methods which are energy efficient, technically simple, and involve mild operating conditions may find use as unit operations augmenting existing processes, or as primary fractionation steps carried out in rural settings so as to reduce loss of regional biomass and the cost of transporting materials to finishing sites.

Under certain conditions, urea complexes form between urea molecules and "guest" molecules, typically linear alkyl chain-containing molecules. The latter act as templates with which urea molecules complex in spiral-shaped structures. The urea molecules bond together *via* hydrogen bonding, while strong van der Waals attractions exist between the urea molecules and the "guests" which are held in circular channels of diameter 0.4–0.6 nm. Most urea complexes have hexagonal crystalline structure as compared to the tetragonal structure of pure urea crystals (1–3). Urea complexes dissociate when raised above a critical temperature, which increases with guest chainlength and can approach the 135°C melting point of crystalline urea (4).

Research from the 1940s to 1960s demonstrated that urea complexation protects guest molecules from oxidation (5) and can be used to fractionate FFA and derivatives such as esters from various seed, fish, and other oils (5–10). Much of this early research was reviewed by Swern (4). Appropriate "guest" molecules possess long, continuous hydrocarbon chains. In contrast, branched and cyclic molecules, or substances with chainlength less than 6 to 8 carbon atoms, rarely form urea complexes. FFA of shorter chainlengths or containing constituents such as double bonds, as well as epoxy or hydroxy functional groups, are less likely to form complexes. As expected, the abundance of good "guest" molecules in a mixture may enhance the complexation of poorer "guests" (4–10).

Recent research has focused upon the physical chemistry, e.g., molecular structure (2,3) related to complexes formed with urea or urea analogs. Also of interest are complexation of linear polymer guests (11,12) and the phase diagrams as-

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sociated with various complex-forming systems (13). Practical studies are somewhat limited, and there is a scarcity of information useful in evaluating commercial use of this ecologically friendly fractionation method.

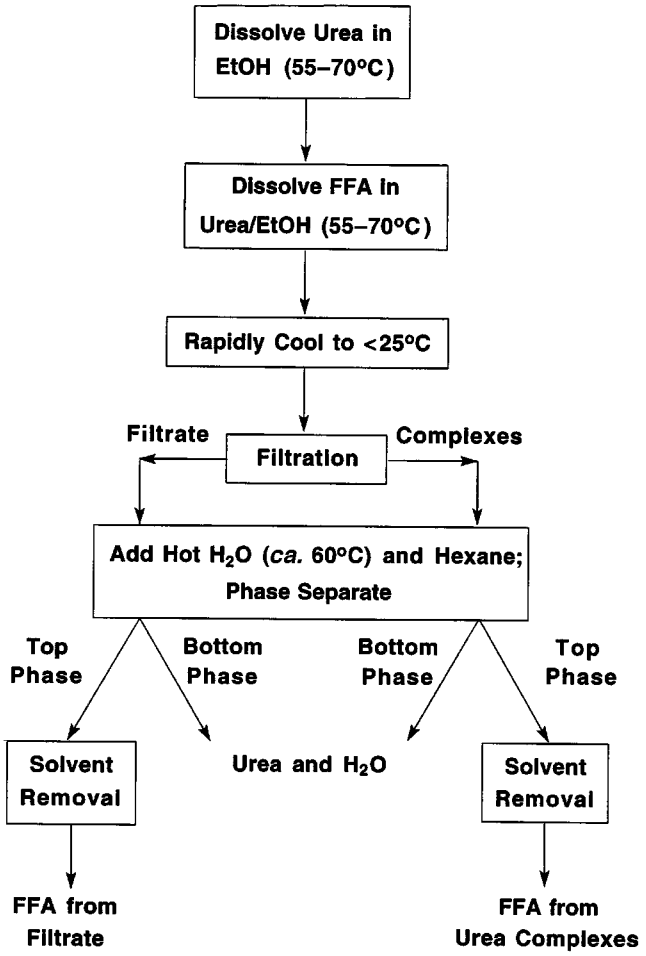
The present study involved development of a simple, ecologically responsible, easily adaptable method for FFA fractionation *via* urea complex formation, and its testing in regard to a modern challenge—reducing the saturated FFA content of LEAR oil FFA. The process was preliminarily investigated with regard to reproducibility, and several variables related to its commercial potential (e.g., component mass or volume ratios, temperature, ethanol solvent water content, single- vs. double-stage fractionation).

EXPERIMENTAL PROCEDURES

LEAR oil samples were purchased from a commercial food supplier (Konsum, Stockholm, Sweden). All chemicals and solvents were of ACS purity and used without further purification. Deionized water was employed throughout. FFA were obtained by saponifying oil with 0.5 N KOH in methanol (the latter present at ≥50% molar excess) in a hot water bath under reflux for at least 1.5 h. The resultant solution was treated with aqueous concentrated HCl, aqueous saturated NaCl, and hexane to release FFA. Thin-layer chromatographic (TLC) analysis indicated only trace amounts of non-FFA material.

The urea complexation procedure used in the present study is summarized in Scheme 1. Typically 6.0 g (100 mmol) of urea was dissolved in 40 mL of 95% (vol/vol) ethanol at 65 ± 5°C. While solution temperature was maintained, a determined mass of FFA was added and the solution allowed to achieve homogeneity. Once FFA and urea were dissolved, the solution was rapidly cooled (20–25°C) by shaking the reaction flask under cold tap water. This promoted formation of urea complexes, and when the resultant slurry reached room temperature (20–25°C), gravity filtration was applied to separate the complexes from the filtrate. At such bench-scale solubilization, complex formation and filtration steps require 5 to 10 min. Depending on the relative amounts of urea and FFA, further complexes could form in the filtrate, but were not removed by further filtration. Recovered complexes were washed with isooctane (a noncomplexing solvent) to remove all traces of the filtrate. Isooctane "rinsates" were then combined with the filtrate. Slightly acidified, warm water (pH ≈ 6, 65°C, 400 mL) was applied to recover FFA from both filtrate and isolated complexes. A small amount of hexane (5 mL) was added to improve the recovery of FFA (see below). The mass of FFA recovered was determined gravimetrically after the evaporation of solvent. Percentage retention (Figs. 1 to 3) relates to compound weight (g) recovered from the filter retained complexes as a percentage of that in the original sample.

The composition of FFA was determined using gas chromatography (GC) on a Vega 2 GC 6000 (Carlo Erba Instruments, Milan, Italy) after conversion of FFA into methyl esters (FAME) *via* heating with BF_3 (14%, w/w) in methanol (Kebo, Stockholm, Sweden) using a common, previously described procedure (14). The sample was injected onto a low polarity (25 m × 0.32 mm i.d.) CP Sil 19CB capillary column (Chrompack, Middleburg, The Netherlands). Helium was used as the carrier gas at a column flow rate of 1.0 mL/min. A split ratio of 100:1 was employed. The injector and flame ionization detector temperatures were maintained at 265 and 275°C, respectively. The GC oven temperature was programmed with a 2-min isothermal hold at 180°C, followed by a 4°C/min increase up to 235°C.



SCHEME 1

RESULTS AND DISCUSSION

General process evaluation. Urea complexes are often formed by adding FFA to a solution of urea in a warm cosolvent such as a polar alcohol. Exothermic complex formation is initiated by allowing the solution to cool. A low urea to FFA ratio (≤7:1 mol/mol) prevents indiscriminate FFA complexation. Most previous FFA fractionation studies (5–10,15) allowed solutions to slowly cool over a long period of time, e.g., 24 h (15), in order to maximize complex formation. This is not suitable for applications involving use of a complex formation-based unit operation in an ongoing industrial process, or in regional settings where efficient refrigeration is not pos-

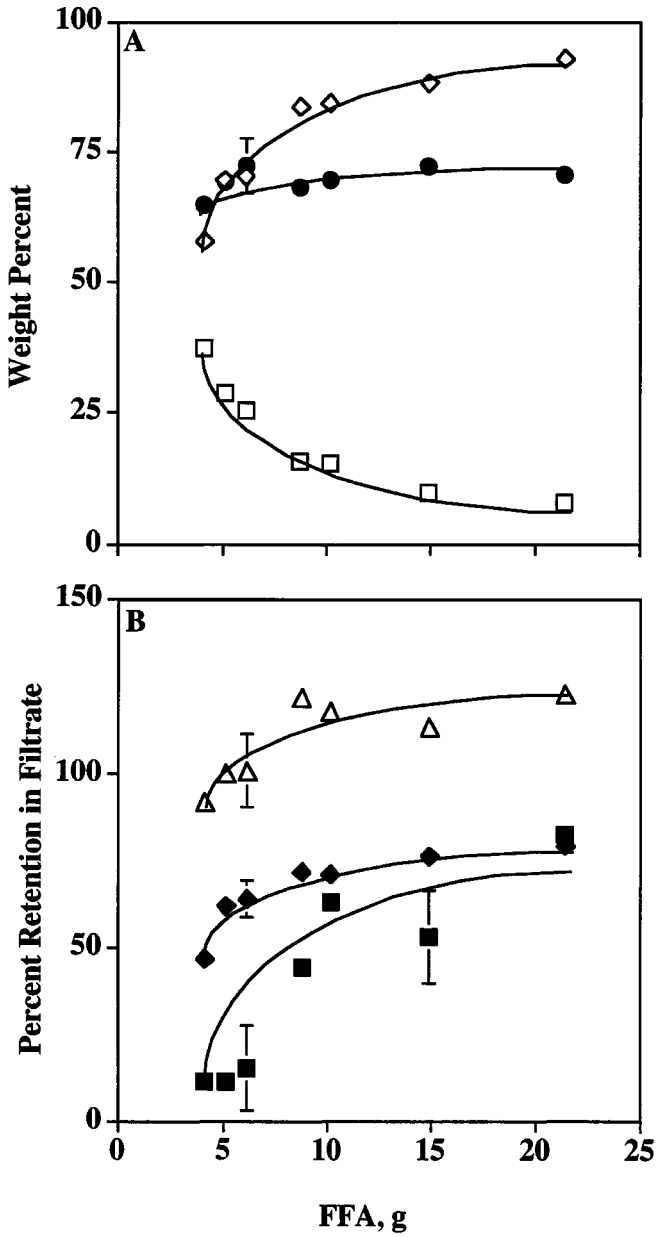


FIG. 1. (A) Effect of low erucic acid rapeseed oil (LEAR) free fatty acid (FFA) mass on urea complexation. Weight percentage of FFA (◇) recovered as product (in filtrate), FFA recovered in complexes (□), and urea recovered in complexes (●). Six grams (100 mmol) of urea and 40 mL of 95% ethanol were combined with the indicated amount of FFA. (B) Percent retention of FFA species in the filtrate (product) as a function of the FFA amount for 16:0 (■) 18:1^{9c} (◆), and 18:2^{9c,12c} (△). Under these conditions approximately 100% of 18:3^{9c,12c,15c} was recovered in the filtrate (not shown). Error bars relate to Table 1.

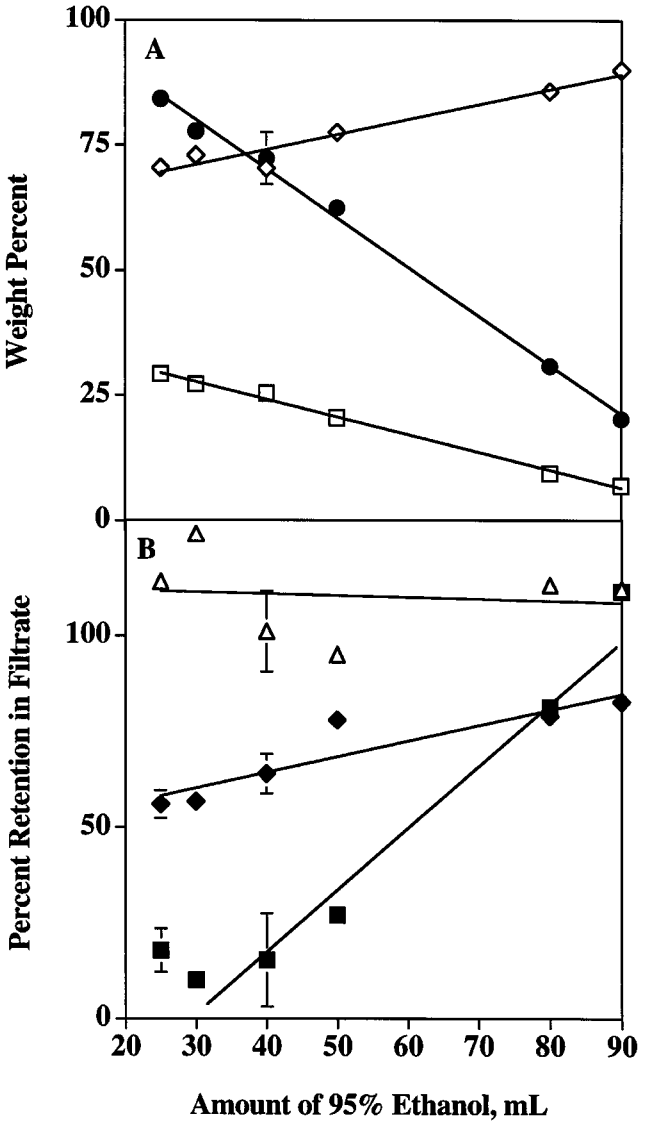


FIG. 2. (A) Effect of 95% ethanol volume on extent of urea complexation and (B) percentage retention of FFA species in the filtrate (product). Six grams (100 mmol) of urea and 6 g (21.3 mmol) of LEAR FFA were combined with the indicated amount of 95% ethanol. Under these conditions approximately 100% of 18:3^{9c,12c,15c} was recovered in the filtrate (not shown). See Figure 1 for symbols and abbreviations. Error bars relate to Table 1.

sible. Initial studies by the authors (not shown) indicated that urea complex crystals formed rapidly and reproducibly at room temperature (20–25°C). A process which included active cooling reduced the time for bench-scale urea complexation (in the present studies) to a few minutes. Further complexes which formed in the filtrate were not recovered so as to better evaluate the fractionation efficiency expected under commercial conditions (see below).

One of three methods is commonly employed to decompose urea complexes (4). Complexes can be decomposed by increasing their temperature (often above 100°C). Another method involves the use of relatively large amounts of noncomplexing, lipophilic solvents (e.g., isooctane, benzene, or carbon tetrachloride) in order to remove FFA from the complexes (4). The third and obvious choice for the present study was to use warm (70°C) water to solubilize urea and the complexes, leaving the complexed FFA in one phase of an aqueous-organic two-phase system. In the process used (Scheme 1), 7 mL of 65°C water was used per gram of complex. The water was slightly acidic (pH 6) to prevent the formation of FFA ammonium salts, which can induce emulsification. The

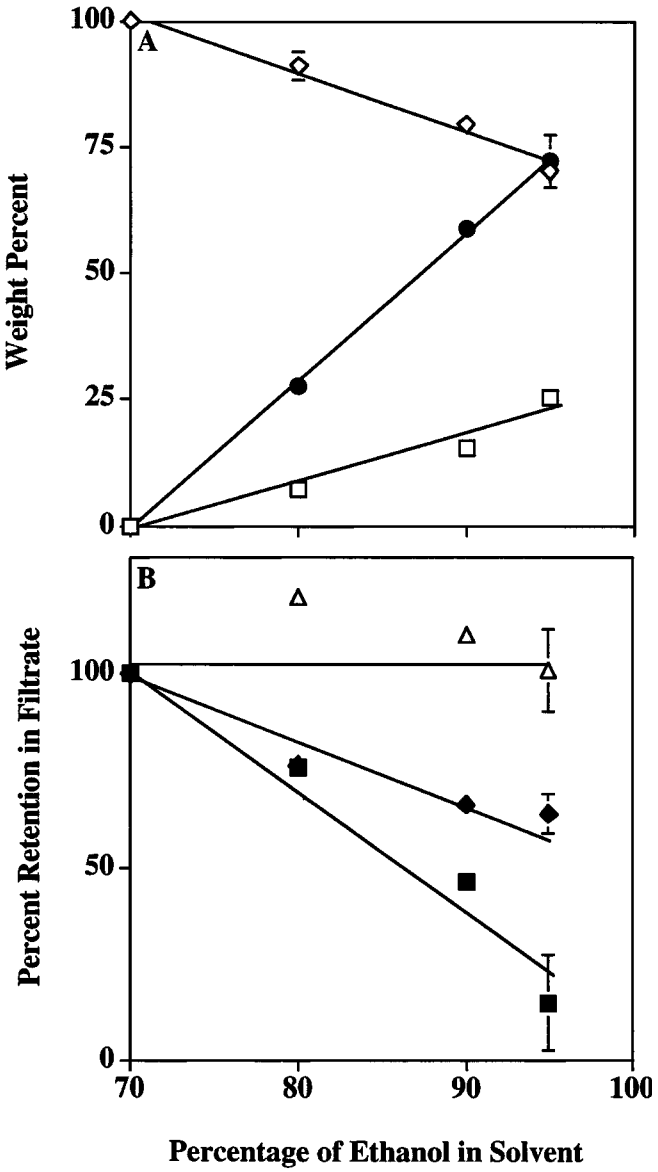


FIG. 3. Effect of ethanol content in solvent (ethanol/water binary system) on (A) extent of urea complexation and (B) percentage retention of FFA species in the filtrate (product). Six grams (100 mmol) of urea and 6 g (21.3 mmol) of LEAR FFA were combined with 40 mL of solvent. Under these conditions approximately 100% of 18:3^{9c,12c,15c} was recovered in the filtrate (not shown). See Figure 1 for symbols and abbreviations. Error bars relate to Table 1.

filtrate, which contained significant amounts of urea in addition to FFA, was also treated with 10 mL of warm pH 6 water per mL of filtrate. A few milliliters of hexane was included in these extraction steps to aid analysis by promoting FFA transfer from the aqueous phase.

The entire Scheme 1, from heating and dissolving of urea, ethanol, and FFA to separation of urea and FFA from both filtrate and complexes, required less than 15 min. The rate-limiting steps were the heating of the three-component mixture to 65 ± 5°C to achieve homogeneity, and rapid cooling to 25°C (under tap water) at which time slurry filtration was initiated. Complex-associated filtrate was washed away with a small amount (5–10 mL) of isooctane. Isooctane was used because unbranched solvents such as hexane or heptane may displace FFA molecules from the complexes (16).

Scheme 1 yields reproducible results and error bars in the supplied figures are often obscured by the data point symbols. It is inexpensive and primarily uses renewable, nontoxic materials such as urea, 95% ethanol, and water. Small amounts of hexane or isooctane were used in some steps to optimize quantitative analysis of the results. As discussed below, it should be possible to reduce or eliminate their use in large-scale applications. Although the present study dealt with small-scale separations, the ability of urea complex-based technology to function at larger scale and utilize inexpensive fertilizer-grade urea is well established, (e.g., 15) as is the potential to recycle urea and ethanol (17).

Given that the process is rapid, inexpensive, eco-friendly, yields reproducible results, and should be easy to scale even in "low technology" settings, several variables related to commercial use of the technology were preliminarily investigated. These are discussed below.

Effect of FFA amount. The Scheme 1 process was evaluated in regard to removal of saturated FFA from LEAR FFA. The first parameter examined was FFA amount isolated at constant urea concentration (6.0 g, 100 mmol) and 95% ethanol (40 mL), with LEAR FFA in the range of 4–21 g (14.2–74.4 mmol). Ethanol and urea concentrations were selected based on earlier work noted by Swern *et al.* (4,18) while FFA concentration range was based on observation that under these conditions FFA amounts less than 4 g did not appreciably yield complexes. The same amount of urea complexes formed (5.6 ± 0.1 g) regardless of the FFA mass employed (4–21 g). The proportion of urea incorporated into the complexes (65–70%) was not significantly affected by FFA amount (Fig. 1A). Extraction of the filtrate with warm water (see above) was necessary to remove (and recycle) the remaining urea. The molar ratio of urea to FFA in the complexes (12.8 ± 1 mol/mol) is in agreement with literature values for urea complexation with pure oleic (18:1^{9c}) and palmitic (16:0) acids (5,18), two FFA species most common in LEAR FFA (see below). When the process was applied to "winterized" LEAR FFA, where the content of saturated palmitic (16:0), stearic (18:0), and eicosenoic (20:0) FFA was reduced from 7.2 to 1.7% by chilled storage (24 h, 4°C), the same weight amount of complexes formed.

As the mass of FFA increases, the weight amount of complex FFA remains constant (≈1.6 g). Therefore the relative proportion of complexed FFA decreases while the percentage of FFA recovered in the filtrate (or "product") increases (Table 1, Fig. 1A). Total recovery of FFA (in filtrate and complexed form) is typically greater than 95%. A small amount of FFA was detected in the warm water "stripping" solution after removal of urea (and ethanol) from the filtrate. This "lost" FFA, which is released from the solution after the ethanol evaporates, was greatest at lower FFA (3.4% for 4.1 g of FFA, vs. 1.4% for 5.1 g FFA or 1.2% for 6.1 g FFA). There

TABLE 1
FFA Composition (% w/w) of Both Product-Filtrates and Complexes (in brackets) as a Function of the Amount of LEAR FFA[a]

FFA (g)	16:0	18:0	18:1	18:2	18:3	20:0[b]	20:1
LEAR oil[c]	3.6 ± 2.9	1.5 ± 1.7	70.0 ± 8.7	19.4 ± 4.7	4.2 ± 2.0	0.5 ± 0.9	0.8 ± 0.8
4.1	0.7 (13.1)	0.4 (4.0)	56.4 (75.7)	30.8 (7.1)	10.3 (0.1)	(<0.1)	1.3 (<0.1)
5.1	0.6 (16.6)	0.2 (6.4)	62.3 (65.0)	27.8 (6.4)	8.4 (1.3)	(2.0)	0.7 (2.3)
6.0[d]	1.0 ± 0.2	0.2 ± .1	60.4 ± 1.2	29.3 ± 0.5	8.0 ± 1.1	<0.1	1.2 ± 0.1
	(16.0 ± 0.3)	(7.3 ± 0.8)	(66.5 ± 2.6)	(5.9 ± 1.1)	(0.9 ± 0.2)	(2.0 ± 0.3)	(1.4 ± 0.1)
6.0[e]	1.3 (16.6)	0.3 (8.3)	62.5 (62.5)	25.8 (6.1)	8.9 (1.2)	(2.9)	1.3 (2.3)
8.7	1.9 (21.2)	1.0 (10.6)	60.2 (56.3)	29.9 (6.1)	7.3 (1.1)	(3.2)	1.2 (1.5)
10.2	2.7 (25.1)	2.3 (10.8)	59.1 (52.6)	27.1 (5.0)	7.0 (0.9)	(3.7)	1.8 (1.8)
15.0[d]	2.3 ± 0.2	0.7 ± 0.1	61.4 ± 1.6	25.5 ± 0.5	9.0 ± 1.2	<0.1	1.2 ± 0.2
	(22.6 ± 0.5)	(13.7 ± 0.7)	(48.9 ± 1.6)	(5.4 ± 0.6)	(1.3 ± 0.5)	(5.9 ± 0.5)	(2.1 ± 0.5)
21.4	3.2 (31.6)	1.6 (14.9)	60.0 (40.2)	25.7 (4.0)	7.6 (1.2)	(6.7)	1.8 (1.4)
21.4[f]	2.7 (16.0)	0.6 (7.4)	60.7 (63.4)	25.6 (8.1)	8.4 (1.7)	(2.1)	2.0 (1.4)

[a]Indicated amount of free fatty acid (FFA) combined with 6 g (100 mmol) of urea and 40 mL of 95% ethanol. 18:1 refers to $18:1^{9c}$, 18:2 to $18:2^{9c,12c}$, 18:3 to $18:3^{9c,12c,15c}$, and 20:1 to $20:1^{11c}$. % w/w results for both filtrates and complexes (the latter in brackets).
[b]Filtrates only showed traces (<0.1%) of 20:0 FFA.
[c]Average composition of low-erucic acid rapeseed (LEAR) oil (before complexation) of 11 samples. $16:1^{9c}$ was also detected.
[d]Runs using 6.0 and 15 g FFA performed in three separate trials.
[e]Filtration was initiated when the slurry's temperature reached 35°C instead of below 25°C, the temperature used for the other experiments contained herein.
[f]Filtrate from a second stage of complexation (using 6 g urea and 40 mL of 95% ethanol) applied to the FFA recovered from the filtrate of the 21.4 g experiment.

appeared to be no need to recover the ≈1% of lost FFA when working with samples above 6 g.

Table 1 indicates typical FFA compositions of filtrates and complexes for a number of experiments. The mean FFA content of several LEAR oil samples compares reasonably well with literature data (19), although sample variation led to noteworthy standard deviation in the mean. Fractionation results reflect expected trends in the filtrates (4), including a large reduction in saturated FFA (16:0 and 18:0) content and increase in polyunsaturated FFA [i.e., linoleic ($18:2^{9c,12c}$) and linolenic ($18:3^{9c,12c,15c}$) acid] content. Complexed FFA samples are often 5 to 10 times richer in saturated FFA than starting samples and 10 to 30 times more than corresponding filtrate samples. As expected (see above) comparison of results (Table 1) for a homologous series of saturated (16:0, 18:0, and 20:0) and monounsaturated ($18:1^{9c}$ and $20:1^{11c}$) FFA suggests complex formation favors guests of longer chainlength. In keeping with earlier investigators' results involving FFA from other sources (4–10,15), urea complexation significantly reduced saturated FFA in LEAR FFA.

The error bars provided in Figures 1A and 1B represent studies involving different LEAR samples. Figure 1B indicates percentage retention in the filtrate (relative to the initial amount) vs. FFA mass for each FFA species. In keeping with Figure 1A, a larger percentage of each saturated FFA is retained in the filtrate as FFA amount increases. However, even the least-efficient separations (e.g., 10 times) are significant. Figure 1B indicates that FFA are complexed in the expected order 16:0 > 18:1 > 18:2 >18:3. As noted in the figure legend, ≥100% of 18:3 FFA was apparently recovered in the filtrate. This was also the case for 18:2 FFA in some experiments (Fig. 1B). As noted in the introduction, some incorporation of unsaturated FFA is expected, especially in the presence of good guest molecules such as saturated FFA (4–10,18). Average apparent recoveries of greater than 100% are therefore believed to reflect LEAR sample variation (Table 1).

In keeping with earlier investigators' results involving FFA samples from other sources (4–10,15), urea complexation significantly reduced saturated FFA in LEAR FFA. Under the conditions studied, there was a a lower threshold level of FFA (4 g) which could be effectively processed. This threshold will vary with ethanol and urea and is not expected to present a significant barrier to large- or small-scale application of the methodology. As FFA was increased, the ratio of urea to FFA and weight recovery of FFA was lowered, while the reduction in saturated FFA and increase in polyunsaturated FFA also diminished. Such trends may affect the oxidative stability of complexed material, and require use of added antioxidants (15).

Reproducibility. As indicated by Figure 1 error bars, Scheme 1 reproducibility was good. For example, at 6 and 15 g of FFA, three replicates of urea complexation yielded similar results for the amounts and composition of the complexes formed, as well as the FFA composition of the filtrate and complexes (Table 1).

Effect of filtration temperature. As cooling is expected to be a primary determinant of both process cost and time, Scheme 1 was studied with filtration being initiated when the 65°C slurry had cooled to 35 instead of 25°C. For the case of 6 g urea, 6 g LEAR FFA, 40 mL 95% ethanol fewer complexes were recovered at the higher temperature (4.9 vs. 5.7 g) and recovery of FFA in the filtrate was higher (79 vs. 72%), so that a lower percentage of saturated FFA was removed from the filtrate (Table 1). Although temperature variation may affect process efficiency, it is not so critical as to require the use of complex temperature-control equipment.

Stagewise operation of urea complexation. There appear to be few examples of the stagewise operation of urea complexation in FFA fractionation (e.g., 5). To explore whether multistage urea complexation is useful for LEAR FFA purification, the 5.9 g filtrate resulting from a 6 g urea, 21 g LEAR FFA, 40 mL 95% ethanol fractionation (Fig. 1, Table 1) was refractionated with an additional 6 g urea and 40 mL 95% ethanol. The second fractionation yielded the same amount of complexes (5.9 g) as the first stage, and further reduced the saturated FFA in the product (Table 1). Since the filtrate product is enhanced in unsaturated FFA (relative to the feed) in each successive complexation stage, further stages should be less efficient at removing saturated FFA. In addition, the FFA contained in the complexes will be less rich in saturated FFA. The expected behavior was found, e.g., the palmitic acid content in the complexes being lower for stage 2 (16.0%) relative to stage 1 (31.6%) (Table 1). So too, the composition of complexed winterized (4°C, 24 h) LEAR FFA was found to be lower in saturated FFA compared to untreated LEAR FFA when performed under identical conditions (data not shown).

Effect of solvent amount. In some experiments the volume of ethanol was varied while maintaining urea and LEAR FFA at 6 g each. As solvent volume increased, less complex formed, suggesting that larger proportions of both urea and FFA remained in the filtrate (Fig. 2A). In addition, larger amounts of solvent promoted the partition of urea to the solvent phase rather than the solid complex phase. This trend is also reflected in the partitioning of FFA species (Fig. 2B). As ethanol volume increased, the temperature required for solubilization of urea and FFA (before rapid cooling to form complexes) decreased from 76°C for 20 mL of ethanol to 61°C for 50 mL ethanol. Fortuitously, use of lower amounts of ethanol promotes greater complex formation with a larger percentage of urea (and FFA) partitioning to the complexes. However, the higher water temperatures then required for solubilization may promote the evaporation of ethanol and decrease the product sample's oxidative stability. Forty mL of ethanol was used in most experiments as a compromise between lower solubilization temperature and greater complex formation.

Effect of water content in ethanol. The process studied utilized 95% ethanol, as it is relatively inexpensive compared to purer grades of ethanol. The potential for process recovery and reuse of ethanol suggested investigation of the effect of ethanol water content on process efficiency. The experiments each involved 6 g of urea and 6 g LEAR FFA (100 and 21.3 mmol, respectively) plus 40 mL of ethanol containing varying amounts (5–30%) of water (i.e., 70–95% ethanol). As expected, complex formation decreased as water content increased, enhancing urea solubility in the solvent phase (Fig. 3A). Ethanol water content should have a profound effect on the partitioning of FFA species more likely to form complexes, so that removal of saturated FFA is strongly affected by the water content (Fig. 3B). At 30% water, no complexes formed until solvent volume was reduced from 40 to 25 mL. The latter provided 1.55 g of complex, which only contained 20% of the urea and 6% of the FFA added. The results agree with Figure 2. Although ethanol solvent volume and water content are important process variables, the process appears quite robust in terms of operating under varied conditions. Such robustness and the potential to operate with solvent recycling (17) further enhance the commercial potential of the process.

The above results suggest further research be undertaken in regard to the use of urea complexation for the rapid, ecologically friendly fractionation of seed oil and other fatty acids. Preliminary studies by the authors suggest a variation of Scheme 1 could be included as a unit operation in an ongoing rapeseed oil fractionation process at a cost of 35 U.S. dollars per metric ton of processed fatty acid (20). The authors intend to further investigate the potential use of urea complex-based processes in modern lipid fractionation challenges. In addition to various preliminary evaluations, such as comparing urea complexation to other methods (21) used for the fractionation of lesquerella oil, intended research includes processing factors such as scaling, design of continuous process equipment, and enhanced recycling of both urea and ethanol. In regard to the latter, it should be noted that steps to enhance phase separation in the filtration step *via* use of isooctane and in the decomplexation step *via* use of hexane may be replaced with low-speed centrifugation.

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