Effect of Temperature Programming on the Performance of Urea Inclusion Compound-Based Free Fatty Acid Fractionation

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ABSTRACT: The effect of cooling rate on the degree of removal of saturated acyl groups from FFA derived from canola oil and the isolation of di- and polyunsaturated acyl groups from FFA derived from vegetable and fish oil, respectively, during urea inclusion compound (UIC)-based fractionation was investigated. Traditionally, slow cooling has been used (*ca.* –1°C min^{-1}). A more rapid cooling rate (–47°C min^{-1}) produced UIC crystals of similar morphology and thermodynamic properties, but of a size an order of magnitude smaller than the UIC formed during slow cooling. Fractionations used only renewable materials (urea, FFA, and 95% ethanol as solvent) and benign operating conditions (ambient pressure, 25–75°C, and neutral pH). When the recovery of FFA (in the solvent-rich phase) was relatively high (>60%), the selectivity of UIC-based fractionation toward the inclusion of saturated FFA and against polyunsaturated FFA was not affected by the cooling rate. In contrast, when the FFA recovery was low, representing cases in which an increase of the PUFA purity is a more important economic goal, a slower cooling rate resulted in a significantly greater discrimination against PUFA groups, hence to a FFA product with a measurably greater purity.

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KEY WORDS: Canola oil, DHA (docosahexaenoic acid), DSC, EPA (eicosapentaenoic acid), FFA (fractionation of), fish oil, PUFA, urea inclusion compounds, vegetable oil.

Urea inclusion compounds (UIC), hexagonal clanthrate materials consisting of hydrogen-bonded networks of urea that form a series of linear, parallel, narrow channels of diameter 0.55–0.58 nm, are well-known vehicles for fractionating or purifying FFA or FAME (reviewed in Ref. 1). For a given mixture of FFA or FAME, UIC will selectively remove long-chain saturated acyl groups, while acyl groups with branching and polyunsaturation do not partition as strongly to the UIC solid phase. Thus, UIC have been used to isolate PUFA from FFA derived from fish, linseed, and borage oils and to remove saturated acyl groups from FFA derived from edible oils such as low erucic acid rapeseed (LEAR) (reviewed in Refs. 2–4).

UIC-based fractionation has potential value as a large-scale and robust prefractionation step because of its low temperature and environmentally friendly operating conditions, and its use of inexpensive renewable materials (urea and ethanol or methanol as solvent). To be a viable choice, a process that uses UIC-based fractionation must occur within a short time period. In contrast, a typical UIC-based fractionation procedure consists of slowly cooling a homogeneous solution of urea, FFA, and solvent for several hours. Recently, however, a rapid cooling process effectively fractionated FFA in a highly reproducible fashion, resulting in a simple and scalable purification process (5,6). But, as suggested recently by Lee (7), the use of a slower-temperature cooling program improves the selectivity by reducing the amount of tetragonal crystals of pure urea that form. The purpose of this paper is to quantify the effect of the temperature cooling program on the performance of UIC-based fractionation.

EXPERIMENTAL PROCEDURES

Canola, vegetable, and fish oils were obtained from a local grocer. Urea (>99%) was purchased from Sigma-Aldrich (St. Louis, MO). All other materials were of high purity and used without further purification. Deionized water was used throughout. FFA were formed from seed oils by saponifying the oils with KOH in methanol at reflux for *ca.* 2 h, then releasing FFA by treatment with concentrated HCl (aq.), a saturated NaCl solution, and hexane.

UIC fractionation was performed as described previously (5,6). Mixtures of 95% ethanol, urea, and FFA were placed in stoppered glass vials and heated until a single liquid phase formed (74–80°C). The one-phase solution was then cooled by one of four different programs (listed in order of decreasing heat transfer rate): (A) rapid cooling by convection, using flowing cold tap water to remove heat, (B) conductive heat transfer *via* storage in a water bath maintained at 23°C, (C) conductive heat transfer *via* storage in ambient air (23°C), and (D) conductive heat transfer in a water bath undergoing a slowly decreasing temperature program. Quantitative descriptions of the temperature programs are given in Table 1. Upon reaching 25°C, the liquid–UIC mixture was immediately filtered. Ethanol was then removed from the solvent-rich phase *via* evaporation. Urea was removed from both the solvent-rich and the UIC phases, i.e., the "extract" and "raffinate" phases, respectively, using mildly acidified (pH 5–6) warm (60°C) water, after which the FFA from both phases were isolated and their amounts determined gravimetrically. The distribution of urea between the raffinate and extract was determined from mass balances of urea and FFA based on the measured mass of UIC and of FFA in the raffinate and extract, as conducted previously (5,6).

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TABLE 1
Effect of Temperature Programming on Urea Inclusion Compound (UIC)-Based Fractionation of FFA from Canola, Vegetable, and Fish Oils[a]

Experiment number[b]	Temperature program	Urea/FFA (g g^{-1})	Yield of UIC (g $g_{urea+FFA}^{-1}$)	Urea/FFA ratio in UIC (raffinate) [g g^{-1} (mol mol^{-1})]
Can-1A	(A) −46.5°C min^{-1} to 25°C	1:1	0.558	2.68 (12.5)
Can-1D	(D′) −3.5°C min^{-1} to 56°C; −0.11°C min^{-1} from 56 to 25°C	1:1	0.551	2.38 (11.2)
Can-2A	(A) −46.5°C min^{-1} to 25°C	2:1	0.690	2.94 (13.7)
Can-2B	(B) −18.5°C min^{-1} to 40°C; −2.6°C min^{-1} from 40 to 29°C; −0.40°C min^{-1} from 29 to 25°C	2:1	0.678	3.00 (15.4)
Can-2C	(C) −4.1°C min^{-1} to 51°C; −1.0°C min^{-1} from 51 to 39°C; −0.13°C min^{-1} from 39 to 25°C	2:1	0.672	2.92 (13.1)
Can-2D	(D) −1.2°C min^{-1} to 52°C; −0.15°C min^{-1} from 52 to 25°C	2:1	0.671	3.09 (15.6)
Veg-1A	(A) −46.5°C min^{-1} to 25°C	1:1	0.572	2.39 (11.0)
Veg-1D	(D′) −3.5°C min^{-1} to 56°C; −0.11°C min^{-1} from 56 to 25°C	1:1	0.608	1.84 (8.47)
Veg-2A	(A) −46.5°C min^{-1} to 25°C	2:1	0.689	2.94 (13.8)
Veg-2D	(D′) −3.5°C min^{-1} to 56°C; −0.11°C min^{-1} from 56 to 25°C	2:1	0.648	3.00 (14.2)
Fish-1A	(A) −46.5°C min^{-1} to 25°C	1:1	0.564	2.67 (12.6)
Fish-1D	(D′) −3.5°C min^{-1} to 56°C; −0.11°C min^{-1} from 56 to 25°C	1:1	0.545	2.65 (12.4)
Fish-2A	(A) −46.5°C min^{-1} to 25°C	2:1	0.743	2.92 (13.7)
Fish-2D	(D′) −3.5°C min^{-1} to 56°C; −0.11°C min^{-1} from 56 to 25°C	2:1	0.709	3.09 (14.3)

[a]Fractionation conditions, 6.0 g (100 mmol) urea, 6.0 or 3.0 g (21.2 or 10.6 mmol) FFA, and 40 mL of 95% ethanol mixed and heated to 75–80°C to form a homogeneous solution; the solution was then cooled according to the temperature program indicated until 25°C was reached. Filtration was then applied to separate the liquid and solid phases. Further details are given in the Experimental Procedures section.
[b]Nomenclature consists of FFA source (Can, canola; Veg, vegetable; Fish, fish oil), followed by numeral corresponding to the overall urea/FFA mass ratio, followed by a letter that corresponds to the temperature program.

The composition of FFA was determined by RP-HPLC using an Altima HL C18 5-μm (250 × 4.6 mm i.d.) column from Alltech Associates (Deerfield, IL). The solvent flow rate was held constant at 1.0 mL min^{-1}. Canola and vegetable FFA fractionation experiments were analyzed using the following gradient program: acetone/acetonitrile/acetic acid 18:72:10 (by vol) held constant for 1 min, ramped linearly to 45:45:10 in a 9-min period, held at 45:45:10 for 10 min, then ramped linearly to 18:72:10 in a 1-min period, and held at 18:72:10 for 1 min. Fish FFA fractionation experiments were analyzed using the following gradient program: acetone/acetonitrile/acetic acid 10:90:10 (by vol) held constant for 5 min, ramped linearly to 45:45:10 in a 9-min period, held at 45:45:10 for 6 min, then ramped linearly to 10:90:10 in a 4-min period, and held at 72:18:10 for 2 min. Response factors for given FFA species were used to correct the ELSD detector signal. Chromatographic peaks were identified using FFA standards purchased from Sigma-Aldrich.

Microscopic images were obtained using an Eclipse 6600 microscope from Nikon (Melville, NY), equipped with phase-contrast imaging capabilities. A 10× magnification lens was used. Thermograms were obtained using a "Diamond" differential scanning calorimeter (DSC) from Perkin-Elmer (Shelton, CT). Prior to analysis, samples were allowed to dry at ambient conditions for several days until the sample mass reached a constant value. Samples of approximately 1 μg were placed in 40-μL crucibles, then subjected to two successive cycles of the following program: heating from −20 to 150°C at 20°C min^{-1}, then cooling to −20°C at −50°C min^{-1}. Thermograms were referenced with respect to an empty crucible undergoing the same temperature program. Morphological information on UIC was obtained *via* x-ray diffraction (XRD) using a Philips X'pert diffractometer (PANalytical B.V, Almelo, Netherlands) with a Ni-filtered Cu K-α radiation of wavelength 0.154 nm, Bragg angle range of $10 \le 2\Theta \le 100°$, step size of 0.02°, and dwell time of 2.0 s.

RESULTS AND DISCUSSION

Effect of cooling rate on physical properties of UIC. Slow rates of crystallization are known to yield long needle-like hexagonal crystalline UIC of nearly 30 μm diameter, as depicted in Figure 1B. The geometry and size of the UIC match those provided in the literature (8,9). In contrast, a rapid cooling process yields crystals of significantly smaller size but of fundamentally the same geometry (Fig. 1A).

XRD and DSC were used to verify that UIC formed, independent of cooling rate, to measure the thermodynamic properties of the UIC and to detect the presence of "free" FFA, i.e., FFA not serving as UIC "guests." XRD spectra for solid-phase material produced by both rapid and slow cooling are indistinguishable and are in strong agreement with previously published data for UIC (10), demonstrating that both rapid and

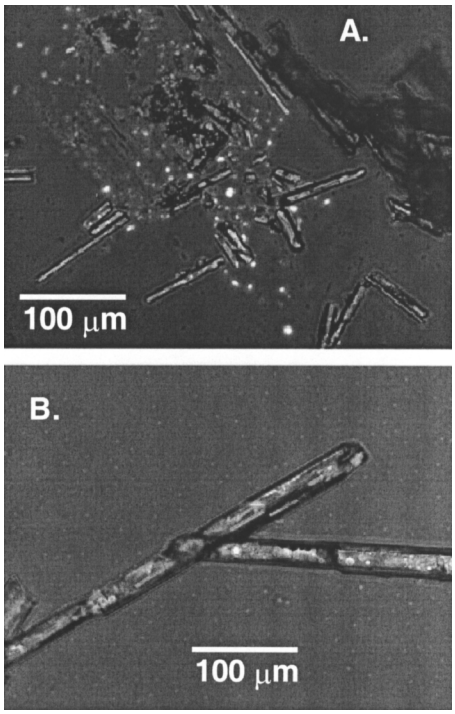

FIG. 1. Microscopic images of urea inclusion compounds (UIC) formed from technical grade oleic acid and urea using rapid convection (A) and a slow-temperature cooling program (B), corresponding to operating conditions and temperature cooling programs A and D′, respectively, given in Table 1.

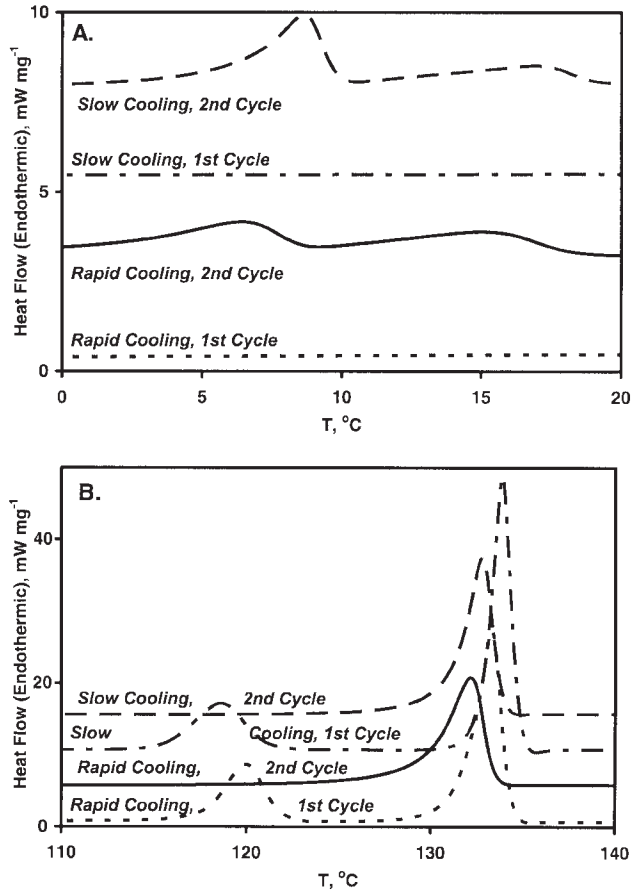

FIG. 2. DSC thermograms of UIC samples formed from technical grade oleic acid and urea *via* rapid- and slow-temperature programs (Programs A and D′, respectively, given in Table 1), heated at a rate of 20°C min^{-1} from –20 to 150°C, then cooled to –20°C at a rate of –50°C min^{-1}. (A) FFA region of the thermogram; (B) UIC and free urea region of the thermogram. Thermograms have been offset by 2.5 and 5.0 mW mg^{-1} for Figures A and B, respectively, to aid in visualization. For abbreviation see Figure 1.

slow-temperature programming yield UIC of similar crystalline structure (data not shown). The two solid-phase samples also yielded similar thermograms (Fig. 2) that shared common trends with previously published DSC data for UIC containing FFA or hexadecane guests (9,11). Upon an increase of temperature during the first cycle, a first peak appeared at *ca.* 119°C, reflecting the dissociation of UIC, and a second peak (*ca.* 133°C) corresponded to "free" tetragonal urea released from the UIC after dissociation. DSC analysis was not carried out beyond 150°C because of the irreversible degradation of urea that occurs at *ca.* 165°C (12,13), as detected using thermogravimetric analysis (Hayes, D.G., unpublished data). After cooling, the resultant physical mixture of FFA and urea yields peaks between 5 and 20°C and at *ca.* 133°C for "free" FFA and urea, respectively, during a second temperature ramping cycle, with the absence of the UIC dissociation peak (*ca.* 119°C), as displayed in Figure 2. During the first cycle, only trace levels of "free" FFA peaks between 5 and 20°C were detected for both samples. The occurrence of the peak maximum, or dissociation temperature, for the UIC formed from slow cooling at 118.6°C is in strong agreement with previously published data (11). The dissociation temperature for UIC formed using rapid cooling, 120.0°C, is slightly higher in comparison, perhaps suggesting a minor difference of physicochemical behavior for UIC formed from rapid cooling, in agreement with the difference in appearance suggested in Figure 1. However, the specific heats of UIC formation, calculated from the UIC dissociation peak of the thermograms contained in Figure 2, are in strong agreement, yielding a value of 67.6 J g_{UIC}^{-1} (equivalent to 74.3 kJ mol_{FFA}^{-1}, 5.46 kJ mol_{urea}^{-1}), which is slightly lower than reported values: 87–95 J g_{UIC}^{-1} (equivalent to 95–105 kJ mol_{FFA}^{-1}, 7.0–7.7 kJ mol_{urea}^{-1}) (9,11,14) for reasons unknown. The values for the m.p. temperature and heat of fusion for "free" urea determined from the thermograms of Figure 2 (first cycle) were both approximately 133.5°C and 12.2 J mol_{urea}^{-1}, respectively, near the reported values of 133.4°C and 14.4 kJ mol_{urea}^{-1} (9,11), with the slightly lower values for the heat of fusion reported herein perhaps due to the small loss of mass that occurred between 122 and 130°C, as detected by thermogravimetric analysis (Hayes, D.G., unpublished data). In summary, the DSC results demonstrate that the UIC formed using rapid and slow-temperature cooling had nearly the same thermodynamic properties and shared similar properties with previously published values for UIC.

Effect of cooling rate on UIC-based fractionation of FFA. UIC-based fractionation was applied to FFA mixtures derived from three different oils, one of which was enriched in mo-

TABLE 2
Effect of Cooling Rate on the Fatty Acyl Composition of Products Resulting from UIC-Based Fractionation of FFA from Canola Oil[a]

Experiment number: Can-…[b]	Canola oil	1A-R	1B-R	2A-R	2B-R	2C-R	2D-R	1A-E	1D-E	2A-E	2B-E	2C-E	2D-E
16:0	6.1	7.5	5.4	7.6	11.5	3.9	4.6	0.0	0.0	4.4	0.0	0.0	0.0
18:0	0.7	1.6	1.7	1.0	1.0	1.0	1.2	0.0	0.0	0.5	0.0	0.0	0.0
18:1	61.2	77.8	80.7	82.2	77.1	83.1	79.4	47.0	47.2	45.5	48.3	45.2	44.5
18:2	18.9	7.0	6.6	2.7	5.4	4.6	6.2	36.0	36.0	33.0	34.3	32.8	35.5
18:3	11.8	4.4	4.2	4.3	3.6	5.7	6.8	17.1	16.8	16.1	17.1	22.0	20.1
20:1	1.2	1.5	1.5	1.6	1.4	1.7	1.7	0.0	0.0	0.5	0.3	0.0	0.0

[a]Fractionation conditions are given in Table 1. For abbreviation see Table 1.
[b]Nomenclature consists of FFA type, followed by numeral corresponding to overall urea/FFA mass ratio, followed by a letter that corresponds to the temperature program (as provided in Table 1), followed by either R (raffinate, or UIC, solid, phase) or E (extract, or solvent-rich, phase).

nounsaturated FFA (canola), one rich in diunsaturated FFA (vegetable), and the other in long-chain PUFA (fish). The utility of UIC-based fractionation is to reduce the saturated FFA content for canola oil-derived FFA and to enhance the PUFA content for the latter two FFA mixtures by removing saturated and monounsaturated FFA. Thus, in all three cases, the main product will reside in the solvent-rich, or extract, phase. Two different urea/FFA mass ratios were used, 2:1 and 1:1 g g^{-1}. The former represents conditions that would lead to a high percentage of urea and FFA incorporation into UIC, hence to a product with high purity obtained at a low recovery. The latter will produce a FFA product at a lower purity but higher recovery.

The effect of temperature programming on the yield and composition of UIC is summarized in Table 1, whereas the fatty acyl composition of solid or UIC (raffinate) and extract phases for the fractionation of FFA from canola, vegetable, and fish oils are provided in Tables 2–4, respectively. Use of a more rapid cooling program generally led to a slight increase in the yield of UIC (hence resulting in a slight loss of FFA product yield), with a greater difference obtained when larger fractions of the FFA groups were incorporated into the UIC, as well as when a 2:1 urea/FFA mole ratio was used in comparison with a 2:1 mole ratio (Table 1). The greatest difference in yield among the three FFA sources was for FFA derived from fish oil. The urea/FFA ratio of the UIC (Table 1) was not affected by temperature programming. Urea/FFA mole ratios are in agreement with previously published values of UIC-based fractionation of similar FFA sources (6,15). Other trends, such as the near-independence of the urea/FFA ratio of UIC with respect to FFA source (with differences attributed to differences in the FFA composition of the UIC, as provided in Tables 2–4) and the increase in UIC yield with an increase of the UIC/FFA ratio, are consistent with previous reports (5,6).

Generally, the use of a slower-temperature cooling program improved the purity of the FFA product in the solvent-rich (extract) phase under conditions that would result in a high-purity product with lower yield (i.e., a 2:1 mass ratio of urea to FFA), with only minor differences in purity occurring for conditions yielding a lower purity and higher recovery (1:1 ratio of urea to FFA). For instance, the canola FFA products produced from UIC-based fractionation with a 2:1 urea/FFA mass ratio subjected to slow cooling possessed a slightly higher 18:2 content (35.5% compared to 33.0%) and lower 16:0 content (0.0 compared to 4.4) than the fractionation that used rapid cooling (Table 2, experiments Can-2A-E and -2D-E). The FFA products for the UIC-based fractionation of canola FFA using a 1:1 urea/FFA ratio were indistinguishable (Table 2, experiments Can-1A-E and -1D-E). The FFA composition of the UIC phase was nearly identical for all fractionations performed on canola, independent of cooling rate and urea/FFA ratio (Table 2).

The cooling rate imposed a higher selectivity difference for the fractionation of vegetable FFA compared to canola FFA. Although the composition of raffinate and extract products

TABLE 3
Effect of Cooling Rate on the Fatty Acyl Composition of Products Resulting from UIC-Based Fractionation of FFA from Vegetable Oil[a]

Experiment number: Veg-…[b]	Vegetable oil	1A-R	1D-R	2A-R	2D-R	1A-E	1D-E	2A-E	2D-E
16:0	7.0	25.1	17.5	10.3	13.8	2.2	2.1	0.0	0.0
18:0	0.8	0.3	0.3	2.3	0.5	0.0	0.0	0.0	0.0
18:1	28.8	35.5	33.3	40.7	48.8	25.4	19.5	8.0	6.0
18:2	45.4	32.7	41.0	26.1	31.4	57.1	64.4	73.1	64.1
18:3	12.8	4.1	5.2	10.4	5.3	11.1	10.1	13.3	25.6

[a]Fractionation conditions given in Table 1. For abbreviation see Table 1.
[b]Nomenclature consists of FFA type, followed by numeral corresponding to the overall urea/FFA mass ratio, followed by a letter that corresponds to the temperature program (as provided in Table 1), followed by either R (raffinate, or UIC, solid, phase) or E (extract, or solvent-rich, phase).

TABLE 4
Effect of Cooling Rate on the Fatty Acyl Composition of Products Resulting from UIC-Based Fractionation of FFA from Fish Oil[a]

Experiment number: Fish-…[b]	Fish oil	1A-R	1D-R	2A-R	2D-R	1A-E	1D-E	2A-E	2D-E
16:0	9.8	27.8	27.2	10.6	16.5	4.5	2.5	0.0	0.0
18:0	2.1	7.0	8.5	1.7	3.5	0.0	0.0	0.0	0.0
18:1	16.5	19.1	18.6	19.1	30.0	16.1	14.1	5.1	5.2
18:3	15.1	7.5	8.8	17.6	12.7	14.8	17.0	24.4	23.1
20:1	2.5	3.5	4.0	5.7	6.0	1.8	1.5	0.0	0.0
20:5[c]	15.1	9.5	6.8	8.7	5.6	17.7	20.1	19.4	22.0
22:6[d]	23.2	15.1	11.7	21.1	11.2	24.1	25.7	27.4	41.5

[a]Fractionation conditions given in Table 1.
[b]Nomenclature consists of FFA type, followed by a numeral corresponding to the overall urea/FFA mass ratio, followed by a letter that corresponds to the temperature program (as provided in Table 1), followed by either R (raffinate, or UIC, solid, phase) or E (extract, or solvent-rich, phase).
[c]Eicosapentaenoic acid (EPA).
[d]Docosahexaenoic acid (DHA).

from fractionations using a 1:1 urea/FFA ratio were similar, significant differences occurred for runs using a 2:1 mole ratio. Moreover, the linoleic composition of the product was nearly doubled by using a slower cooling rate (Table 3, experiments Veg-2A-E and -2D-E), while the UIC for the slower cooling rate contained significantly larger amounts of 16:0 and smaller amounts of 18:2 (Table 3, experiments Veg-2A-R and -2D-R). In all cases, UIC-based fractionation was successful for increasing the polyunsaturated FFA content relative to the starting vegetable FFA mixture (Table 3).

The trends discussed above for the fractionation of vegetable oil are in strong agreement with those for the fractionation of fish oil, with the differences being greater for the latter. When comparing the effect of cooling on fractionation using a 2:1 urea/FFA ratio, the PUFA (20:5 + 22:6) content of the product was 63.5% when cooled slowly compared to 46.8% for rapid cooling (Table 4, experiments Fish-2A-E and -2D-E). Enhanced selectivity was also observed by a higher 16:0 and lower 20:5 and 22:1 fraction among the UIC-encapsulated FFA for the more slowly cooled fractionation (Table 4, experiments Fish-2A-R and -2D-R). In contrast, only small differences existed between raffinate and extract FFA materials derived from fractionation using a 1:1 urea/FFA ratio (Table 4, runs Fish-1A-R and -1A-E, Fish-1D-R and -1D-E).

The percentage recovery of total FFA in the extract, or product, for all experiments is depicted in Figure 3. Recovery refers to the ratio of the amount of a specific fatty acyl species contained in the solvent-rich or extract phase per the amount of the fatty acyl species contained in the overall system. Figure 3 indicates that the percentage recovery was nearly independent of the type of FFA mixture, in agreement with the near independence of the yield of UIC on FFA mixture type, as discussed above. The percentage recovery results also strongly agree with the values obtained for the UIC-based fractionation of LEAR FFA using a mathematical model we developed (16), with the exception of vegetable oil using a urea/FFA ratio of 2:1 for reasons unknown.

The mathematical model discussed above accurately predicted the UIC-based fractionation of LEAR FFA using rapid cooling; moreover, the amount of UIC formed and the overall and fatty acyl composition of both the raffinate and extract phases were predicted using triangular solvent–FFA–urea phase diagrams at several different temperatures and partition coefficients for the individual fatty acyl species as inputs (16). The latter input—more specifically, the percentage recovery of a fatty acyl species in the extract phase—varied consistently, often linearly, as a function of the percentage recovery of overall FFA in the extract for all fatty acyl species, independent of the overall proportions of solvent, urea, and FFA and of the composition of the binary solvent system (ethanol/water between 90:10 and 100:0, vol/vol) (16). Thus, the recovery of fatty acyl species, depicted in Figure 3 for the experiments displayed in Tables 1–4, provides a valuable measure of the selectivity of UIC-based fractionation. As expected, the value for a specific fatty acyl group increases as its degree of polyunsaturation increases (Fig. 3), reflecting the selective discrimination of UIC against encapsulation of acyl chains with multiple double bonds. Also, the values obtained for experiments using a 1:1 urea/FFA ratio are higher than corresponding experiments using a 2:1 ratio, reflecting the lower yield of UIC and the higher recovery of FFA in the extract, or product, phase (Table 1, Fig 3), in agreement with previously published results (16). In comparing the effect of the cooling rate, the differences between partition coefficients are greatest for saturated and polyunsaturated acyl species and for the experiments using a 2:1 urea/FFA ratio; for instance, the values for DHA (22:6) in the fractionation of fish FFA and for 18:3 in the fractionation of vegetable FFA differ by at least 60% (Fig. 3). Values for oleic and linoleic acids vary only slightly as a function of temperature program (Fig. 3). Worthy of note, values for the fractional recovery of a given acyl species and set of operating conditions (e.g., composition of the overall mixture and cooling rate) are highly dependent on the FFA source. For instance, experiments that used a 2:1 urea/FFA ratio suggest that the partitioning of oleic acid is strongly favored toward incorporation into the UIC for vegetable and fish FFA (indicated by low val-

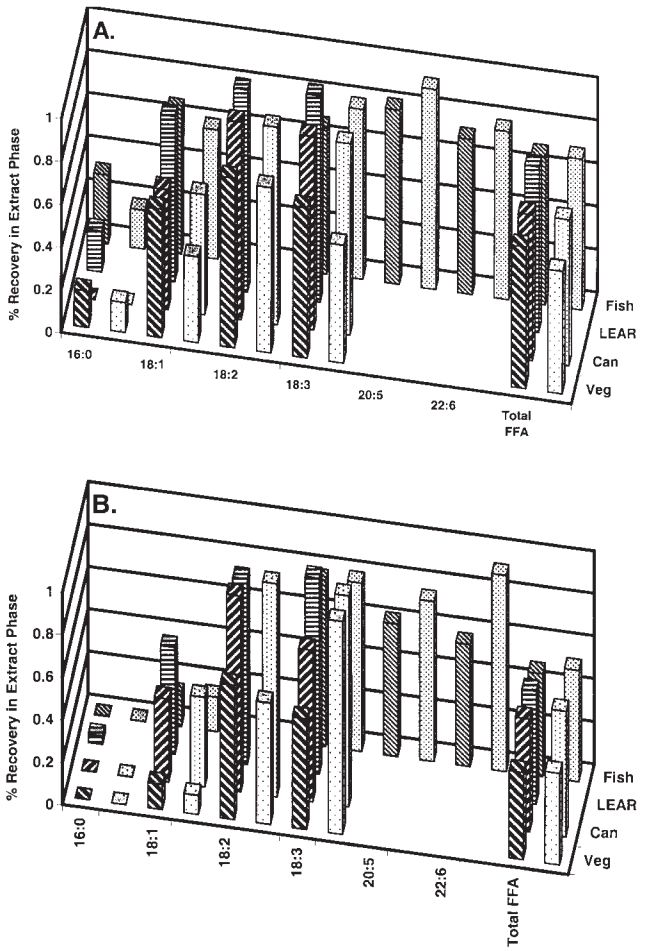

FIG. 3. Effect of temperature cooling rate on the percentage recovery of fatty acyl species in the solvent-rich, or extract, phase for overall urea/FFA mass ratios of (A) 1:1 and (B) 2:1, respectively. Striped and shaded bars represent results obtained using a rapid (–46.5°C min^{-1}, or "A" in Table 1) and slow (–0.11°C min^{-1}, or "D") cooling rate. Nomenclature for FFA type and separation conditions are given in Table 1. (LEAR refers to low erucic acid rapeseed oil; plotted values of recovery were calculated from data given in Ref. 16).

ues, between 0.09 and 0.16); in contrast, oleic acid partitions nearly equally between the extract and raffinate for LEAR and canola FFA (Fig. 3). Moreover, the presence of higher concentrations of di- and polyunsaturated acyl groups for vegetable and fish FFA make 18:1 a more viable candidate for incorporation into the UIC for the named FFA mixtures.

It appears that the temperature cooling rate used for UIC-based primary fractionation of FFA had little or no effect when employing conditions in which the recovery of overall FFA was greater than 60%, but was a more significant factor when the recovery was less than 50%. The latter cases refer to situations in which the purity, i.e., the concentration of PUFA in the solvent-rich phase, is of more economic significance than the recovery. Moreover, a trade-off exists between a high recovery of FFA and decreased loss of selectivity because of the use of a high-temperature cooling rate vs. high purity and a more significant loss of selectivity by use of a rapid cooling rate. The continuum of high to low recovery, low to high purity, and low to high loss of selectivity by the use of a rapid cooling program parallels the increase of UIC yield, controlled in turn by an increase of the urea/FFA ratio, a lower fraction of water in the solvent system, and a lower value of the final cooling temperature. The selection of the operating conditions and whether the decrease of productivity caused by a decrease in the cooling rate is outweighed by the increase of purity requires an economic analysis that accounts for market- and regulation-controlled FFA product composition and productivity targets and operating costs, among other factors. For instance, in comparing the fish FFA fractionation products produced using a 2:1 urea/FFA ratio, it is not clear whether an increase of the PUFA (EPA plus DHA) content from 68.1 to 81.5% (Table 4, products Fish-2A-E and -2D-E) is outweighed by the order of magnitude reduction of productivity resulting from a slower cooling rate.

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