Polyunsaturated Fatty Acid Concentrates from Borage and Linseed Oil Fatty Acids

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ABSTRACT: A modified low-temperature solvent crystallization process was employed for the enrichment of polyunsaturated fatty acids (PUFA) in borage and linseed oil fatty acids. The effects of solvent, operation temperature, and solvent to free fatty acid (FFA) ratio on the concentration of PUFA were investigated. The best results were achieved when a mixture of 30% acetonitrile and 70% acetone was used as the solvent. With an operation temperature of –80°C and a solvent to FFA ratio of 30 mL/g, γ-linolenic acid (GLA) in FFA of saponified borage oil can be raised from 23.4 to 88.9% with a yield of 62.0%. At a yield of 24.9%, α-linolenic acid in linseed oil can be increased from 55.0 to 85.7%. The results of this work are comparable to the best results available in the literature.

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KEY WORDS: ALA, borage oil, GLA, linseed oil, low temperature solvent crystallization.

Polyunsaturated fatty acids (PUFA) such as γ-linolenic acid (GLA) (all-*cis*-6,9,12-octadecatrienoic acid) and α-linolenic acid (ALA) (all-*cis*-9,12,15-octadecatrienoic acid) have been the subject of intensive research due to their special nutritional and physiological roles (1–3). Chemical synthesis of the thermally labile PUFA is difficult due to their all-*cis* and polyunsaturated structure. Currently, PUFA are obtained from natural sources. The free fatty acid (FFA) composition in acylglycerols of natural oils must be modified, or the PUFA contents in FFA must be enriched for pharmaceutical and dietary purposes.

Methods commonly employed for the enrichment of PUFA include urea fractionation (4), supercritical fluid extraction (5), HPLC (high-performance liquid chromatography) (6), and enzyme-catalyzed reactions (7–9). In urea fractionation, saturated and monounsaturated fatty acids tend to form complexes of hexagonal crystals with urea and can be separated from PUFA in a supersaturated organic solvent, usually methanol. Part of the FFA may be converted into the corresponding methyl ester, and approximately 10% of the PUFA may be lost during the extractive separation of acid and ester (10). Supercritical fluid extraction is effective in the separation of ester from fatty acids that differ in chain length. This method performs poorly for esters of fatty acids with the same chain length but differing in the numbers of double bond (11). Both HPLC and enzymatic methods are suitable for the purification of PUFA. However, large-scale applications of these methods are not yet practical.

Low-temperature solvent crystallization was developed decades ago for the separation of fatty acids or their esters (12). Fatty acids can be separated by their solubility difference in organic solvent (13). The low operation temperature involved keeps thermal degradation of PUFA to a minimum. Solvent, which is acetone in most cases, can easily be recovered and reused. In this work, acetone, acetonitrile, or a mixture of the two was used as the solvent in the low-temperature crystallization of borage and linseed oil fatty acids. The effects of temperature, solvent to FFA ratio, and composition of the solvent mixture on the content and yield of PUFA were systematically studied.

EXPERIMENTAL PROCEDURES

Materials. Borage oil, linseed oil, and fatty acid standards were purchased from Sigma Chemical Company (St. Louis, MO). Hexane, ethanol, acetone, and acetonitrile were either HPLC- or analytical grade and were obtained from commercial sources.

Preparation of FFA. FFA obtained by the saponification of borage oil and linseed oil were prepared according to the method described by Huang *et al.* (14). Typically, a NaOH solution was prepared by dissolving 48 g NaOH and 0.5 g Na_2EDTA in 160 mL of water. To this solution, 160 mL of ethanol was added. A mixture containing 100 g oil and 200 mL NaOH solution was heated at 60°C with magnetic stirring at 550 rpm for 1 h. Forty milliliters of water and 400 mL of hexane were then added, and the solution was stirred for 1 h. The upper layer containing unsaponifiable matter was removed and discarded. Water (160 mL) was added to the lower layer, and 12 N hydrochloric acid was then added until the solution was at pH 1. The resulting lower layer was removed using a separatory funnel and discarded. The FFA-containing upper layer was dried with anhydrous magnesium sulfate, and solvent was evaporated in a vacuum rotary evaporator at 35°C.

Low-temperature crystallization. A fixed amount of solvent, either acetone, acetonitrile, or a mixture of acetonitrile and acetone (usually 30:70 vol/vol unless otherwise specified), was added to a vial containing 3 g FFA. The vial was filled with nitrogen, put in a 35°C water bath, and stirred until all FFA were dissolved. The solution was then stored in a cold chamber for 24 h. Unless otherwise specified, the temperature of the cold

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chamber (Sanyo MDF-192, Gunma, Japan) was –40°C when acetonitrile was the solvent and –80°C when acetone or a mixture of acetone and acetonitrile was the solvent. Solid and liquid phases were separated immediately after removal from the cold storage by a Buchner funnel operating at room temperature. FFA in the liquid phase was obtained by removing the solvent in a vacuum rotary evaporator at 35°C. Some of the PUFA in the solid phase was recovered by adding acetone and acetonitrile (70:30 vol/vol) to the solid phase, storing the mixture at –80°C for 24 h, and collecting the resulting liquid phase. FFA composition was analyzed by gas chromatography. The yield of a fatty acid in the solvent crystallization is defined as the ratio of the amount of the fatty acid recovered in the liquid supernatant to that contained in the original FFA. All data were the average of at least three independent runs.

Determination of the FFA composition. FFA were transformed into corresponding methyl esters with trimethylsulfonium hydroxide. FFA composition was analyzed by a China Chromatography model 8700F (Taipei, Taiwan) gas–liquid chromatograph equipped with a flame-ionization detector. The column used was SP-2330 (30 × 0.25 mm i.d.; Supelco, Bellefonte, PA). The temperatures of the injector and the detector were set at 250 and 270°C, respectively. The column was operated under different temperature schemes for analyzing FFA-methyl ester derived from different oils. For borage oil, the column was held at 180°C for 10 min, then increased to 235°C at a constant rate of 15°C/min and kept at 235°C for 3 min. The column was held at a constant temperature of 180°C in the case of linseed oil.

RESULTS AND DISCUSSION

Enrichment of GLA in borage oil fatty acids. The effects of the solvent(acetonitrile)/FFA ratio and storage temperature on the GLA content and yield for the concentration of GLA in borage oil fatty acid are shown in Figure 1. At a fixed solvent/FFA ratio, a change in storage temperature results in a distinct change in GLA content as compared to the corresponding change in GLA yield. At a fixed storage temperature, GLA content decreases moderately with increasing solvent/FFA while the corresponding yield increases rapidly. For example, at solvent/FFA = 10 mL/g and a temperature of –40°C, GLA content can be raised from 23.4 to 87.4 wt% with a yield of 28.6%. If a twofold increase in solvent to FFA is used (i.e., solvent/FFA = 20 mL/g), the corresponding GLA content and yield will be 83.3 and 59.2%, respectively. As can be seen from Table 1, most saturated and monounsaturated fatty acids have been removed.

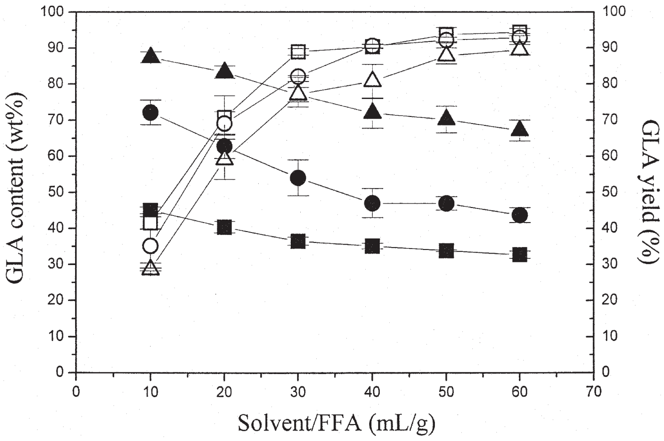

FIG. 1. The effects of storage temperature and solvent to free fatty acid (FFA) ratio on the γ-linolenic acid (GLA) content and yield of GLA-enriched FAA. Acetonitrile was the solvent. Open symbols: GLA yield, closed symbols: GLA content. FFA = 3 g. Storage temperatures: –40°C (▲,△), –30°C (●,○), –20°C (■,□).

Figure 1 indicates that a lower storage temperature is preferred for the solvent crystallization process. The melting point of acetonitrile is –45°C, hence an operation temperature lower than –40°C is impractical if acetonitrile is the solvent. With mixtures of acetone and acetonitrile as the solvent, the effects of storage temperature and volumetric ratio of acetonitrile to acetone on GLA content and yield at solvent/FFA = 30 mL/g are shown in Figure 2. Data for the low storage temperature cases (–70 and –80°C) are incomplete due to solvent solidification at high acetonitrile to acetone ratios. At a storage temperature of –80°C and with acetonitrile/acetone = 30:70 (vol/vol), a GLA content of 88.9 wt% with a corresponding yield of 62.0% can be achieved. As shown in Figure 2, with acetone as the solvent, under the same operating conditions, the corresponding GLA content and yield were 81.0 and 74.4%, respectively. The addition of acetonitrile to acetone is beneficial with regard to the increase in GLA content with a sacrifice in GLA yield. Syed Rahmatullah *et al.* (7) reported a GLA content of 91.8 wt% with a yield of 67.5% in the enrichment of GLA from borage oil fatty acids by lipase-catalyzed esterification. Shimada *et al.* (8) used a two-step enzymatic

TABLE 1
Fatty Acid Compositions (wt%)[a] of Borage Oil Before and After Enrichment

Fatty acid	Before	After[b]	After[c]	After[d]
16:0	10.49 ± 0.08	0.14 ± 0.01	0.08 ± 0.02	0.17 ± 0.02
18:0	3.71 ± 0.03	ND[e]	ND	ND
18:1	15.95 ± 0.10	2.90 ± 0.22	1.82 ± 0.07	3.15 ± 0.26
18:2n-6	37.11 ± 0.04	19.55 ± 1.57	8.29 ± 0.07	14.89 ± 0.84
18:3n-6 (GLA)[f]	23.36 ± 0.09	77.05 ± 1.96	88.94 ± 0.24	80.99 ± 1.13
20:1	4.25 ± 0.15	0.37 ± 0.17	0.80 ± 0.08	0.54 ± 0.03
22:1	2.94 ± 0.12	ND	0.08 ± 0.00	0.21 ± 0.03
24:1	2.18 ± 0.20	ND	ND	0.07 ± 0.02
Yield of GLA (%)	—	77.16 ± 3.49	61.95 ± 2.16	74.37 ± 1.08

[a] Mean absolute deviation of three independent determinations.
[b] Solvent = acetonitrile, solvent/FFA = 30 mL/g, temperature = –40°C.
[c] Solvent = 30% acetonitrile + 70% acetone, solvent/FFA = 30 mL/g, temperature = –80°C.
[d] Solvent = acetone, solvent/FFA = 30:1 (mL/g), temperature = –80°C.
[e] ND: not detected.
[f] GLA: γ-linolenic acid.

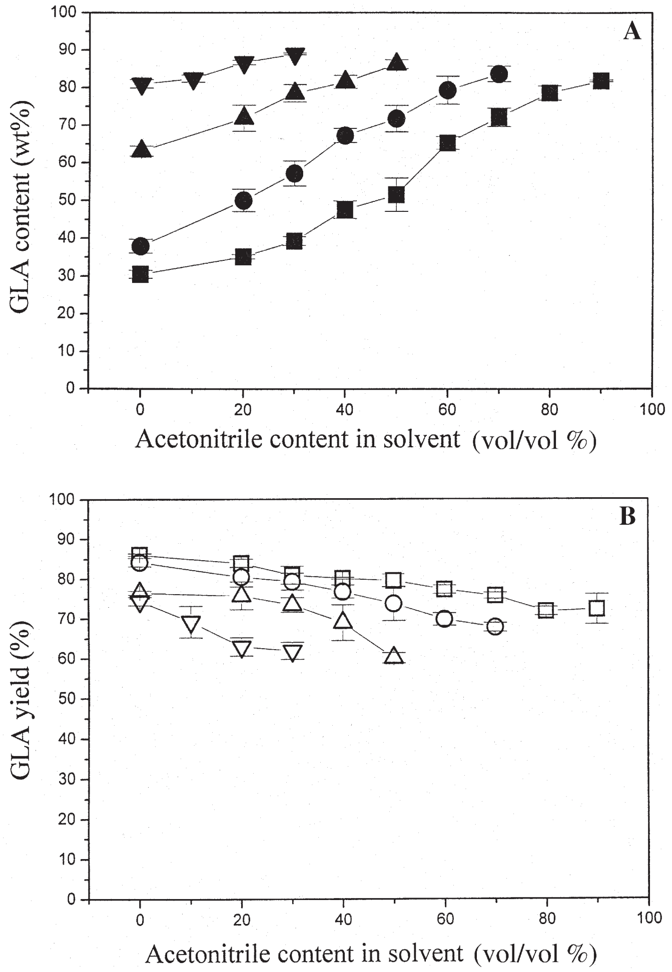

FIG. 2. The effects of storage temperature and composition of the solvent mixture on GLA content (A) and yield (B) of GLA-enriched FFA at a fixed solvent to FFA ratio of 30 mL/g. FFA = 3 g. Storage temperatures: −80°C (▼,▽), −70°C (▲,△) −60°C (●,○), −50°C (■,□). See Figure 1 for abbreviations.

method for the purification of GLA from borage oil in which GLA was purified to 93.7 wt% with a yield of 67.5%.

Enrichment of α-linolenic acid in linseed oil fatty acids. At a storage temperature of −80°C and by using a mixture of 70% acetone and 30% acetonitrile as the solvent, the effects of the solvent/FFA ratio on ALA content and yield in the concentration of FFA derived from linseed oil are shown in Figure 3. The ALA yield increases with the increase of the solvent/FFA ratio, while the ALA content remains fairly independent of the solvent/FFA ratio. Using a mixture of acetonitrile and water as the solvent, Uksila and Lehtinen (15) obtained FFA with 85 wt% ALA and a yield of 15% from linseed oil FFA in a three-stage solvent crystallization operating at temperatures of 20, −20, and −40°C. The single-stage process developed in this work resulted in comparable ALA content but higher ALA yield when a high solvent/FFA ratio was used. The compositions of the FFA before and after the concentration of linseed oil are shown in Table 2.

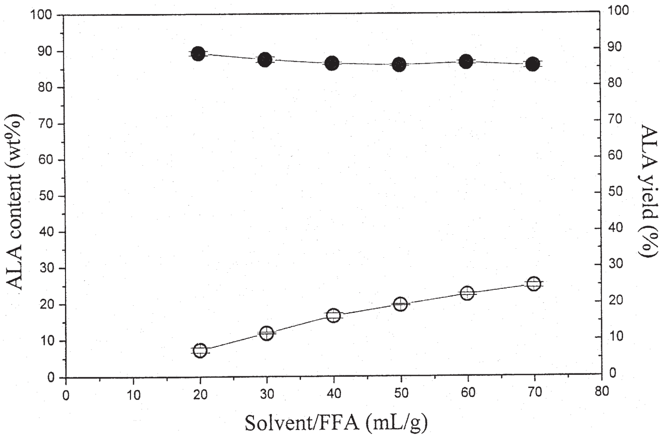

FIG. 3. The effects of solvent/FFA ratio on α-linolenic acid (ALA) content and yield in the concentration of linseed oil fatty acids. FFA = 3 g. Storage temperature = −80°C. The solvent was a mixture of 30% acetonitrile and 70% acetone. Open symbols: ALA yield, closed symbols: ALA content. For other abbreviation see Figure 1.

TABLE 2
Compositions (wt%)[a] of Linseed Free Fatty Acid (FFA) Before and After the Solvent Crystallization

Fatty acid	Before	After[b]	After[c]
16:0	5.25 ± 0.03	0.16 ± 0.02	0.20 ± 0.01
18:0	3.37 ± 0.10	0.10 ± 0.01	0.03 ± 0.00
18:1	21.43 ± 0.14	4.47 ± 0.38	4.06 ± 0.07
18:2n-6	14.98 ± 0.16	6.07 ± 0.22	10.06 ± 0.80
18:3n-3	54.96 ± 0.14	89.20 ± 0.61	85.66 ± 0.74
Yield (%)	—	7.25 ± 0.69	24.85 ± 0.63

[a] Mean absolute deviation of three independent determinations.
[b] Solvent = 30% acetonitrile + 70% acetone; solvent/FFA = 20 mL/g; temperature = −80°C.
[c] Solvent = 30% acetonitrile + 70% acetone; solvent/FFA = 70 mL/g; temperature = −80°C.

Recovery of PUFA from the solid phase. In the low-temperature solvent crystallization process, the discarded solid phase still contains a considerable amount of PUFA. For example, when a mixture of 30% acetonitrile and 70% acetone was used as solvent, at a solvent/FFA ratio of 30 mL/g and a storage temperature of −80°C, 38% of the GLA in the original FFA still remained in the solid phase having a GLA content of 9.1 wt%. This solid phase is basically a mixture of fatty acids. The solvent recrystallization process can be applied to recover part of the PUFA from the solid phase. At a storage temperature of −80°C using solvent/FFA = 70 mL/g, solvent = 30% acetonitrile + 70% acetone, FFA with a GLA content of 72.5 wt% and a corresponding yield of 88.8% was achieved, i.e., 88.8% of GLA in the solid phase was recovered. An overall GLA content of 81.9 wt% and a corresponding yield of 95.7% were obtained after FFA from the liquid phase and the PUFA-rich FFA recovered from the solid phase were combined.

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