Prostaglandins, Leukotrienes and Essential Fatty Acids ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

The application of ultrasound energy to increase lipid extraction throughput of solid matrix samples (flaxseed)

Adam H. Metherel [a], Ameer Y. Taha [b,c], Hamid Izadi [a], Ken D. Stark [a,*]

[a] Laboratory of Nutritional and Nutraceutical Research, Department of Kinesiology, University of Waterloo, 200 University Avenue West, Waterloo, Ontario, Canada, N2L 3G1
[b] Certo Labs, Inc., 5 Adrian Avenue, Toronto, Ontario, Canada, M6N 5G4
[c] Department of Pharmacology and Toxicology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada, M5S 1A8

ARTICLE INFO

Article history:
Received 8 June 2009
Received in revised form
21 July 2009
Accepted 24 July 2009

Keywords:
Flaxseed
Ultrasound
Sonication
Lipid extraction
Fatty acids
High throughput

ABSTRACT

Ultrasound may reduce lipid extraction times and increase analysis throughput of food materials. Ground flaxseed (25 mg aliquots) were extracted in quadruplicate in 2:1 (v:v) chloroform:methanol, 3:2 hexane:isopropanol, 1:1 diethyl:petroleum ether or hexane with exposure to sonication at low frequencies of 20 kHz with a 600 W ultrasonic processor. Power was automatically varied to maintain constant amplitudes of 20%, 60% and 100% of 240 μm for sonication exposures for 5, 10 and 20 min, respectively. Total lipid dry weights and quantitative and qualitative fatty acids were determined. Results were compared to a standard 24-h, Folch-based, 2:1 chloroform:methanol extraction. Longer time exposures and higher sonication amplitudes were associated with increases in lipid recoveries. In particular, ultrasound-assisted extraction in 3:2 hexane:isopropanol for only 10 min resulted in lipid and fatty acid recoveries similar to the 24-h standard method. Comprehensive testing on a variety of sample matrices and food products is required, but lipid extraction by ultrasound has potential to reduce sample processing time.

1. Introduction

Standard analytical methods for the determination of fatty acids in foods are time-consuming and a significant financial burden with mandated labeling of food products. The standardized method of the Association of Official Analytical Chemists (AOAC) [1] and the International Organization for Standardization (ISO) [2] can require between 4 and 14 h. Ultrasonic energy has previously been utilized to assist the extraction of phenols [3], ginsenosides [4], anthraquinones [5] and polycyclic aromatic hydrocarbons [6,7]. Sonication results in cavitation or the formation and collapse of microscopic bubbles that can release tremendous energy as heat, pressure and mechanical shear [8]. Ultrasonic energy has been shown to improve extraction from vegetal tissues through the action of accelerating the rehydration or swelling of plant cells that is accompanied by the fragmentation of the tissue matrix [9]. This involves the mass transfer and penetration of the solvent into the cell promoting absorption of cell contents into the solvent [10].

Ultrasound-assisted lipid extractions from solid matrix samples have been reported with fatty acid profiles qualitatively similar to conventional techniques and significantly reduced sample extraction times (approximately 1 h) [2,11–13]. These reports largely examined the application of ultrasound during extraction in a Soxhlet apparatus [2,11,12], which is bulky and requires large sample and solvent volumes. Cravotto et al. [13] also examined ultrasound-assisted extraction immersion and cup horns and a novel cavitating tube with small volumes of sample and solvents. Fatty acid determinations, however, were qualitative and not quantitative, making it difficult to evaluate ultrasound-assisted and conventional methods.

In the present study, the application of ultrasound for the fast extraction of lipids prior to the determination of fatty acids in flaxseed is examined. This includes an examination of the use of ultrasound to assist extraction at 20%, 60% and 100% of total probe amplitude and with different solvents including hexane, hexane:isopropanol, chloroform:methanol and diethyl ether:petroleum ether. Ultrasound-assisted exposures of 5, 10 and 20 min were examined. Dry extraction weight and qualitative and quantitative determinations of individual fatty acids were determined and compared to conventional techniques. Samples were also examined by gas chromatography mass spectrometry (GCMS) and malonaldehyde levels were determined to assess ultrasound-mediated oxidation of fatty acids. Ultrasound-assisted extraction may have the potential to increase analytical throughput while keeping solvent volumes and sample masses low.

* Corresponding author. Tel.: +1 519 888 4567x37738; fax: +1 519 885 0470.
E-mail address: kstark@uwaterloo.ca (K.D. Stark).

0952-3278/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.plefa.2009.07.003

2. Methods

2.1. Experimental design

In all analyses, 25 mg of ground flaxseed (Bob's Red Mill Natural Foods, Inc., Milwaukie, OR, USA) previously stored in a refrigerator were extracted in quadruplicates. We have previously compared unassisted ISO, AOAC standardized methods with 2:1 (v:v) chloroform:methanol extractions up to 48 h (manuscript in progress) with maximal recoveries of fatty acids determined by 24 h of exposure to 2:1 chloroform/methanol. For the present study, the 24-h, 2:1 chloroform/methanol extraction was utilized as the standard for comparison of novel ultrasound-assisted extraction techniques. All extractions were completed with solvents totaling 3 mL in volume and the solvents examined were: 2:1 (v:v) chloroform:methanol [14]; 3:2 (v:v) hexane:isopropanol [15]; 1:1 (v:v) diethyl ether:petroleum ether [1] and hexane only [2]. The Misonix ultrasonic processor S-4000 (Misonix Inc., Farmingdale, NY) operates with a 20 KHz electrical signal supplied to the converter, which is equipped with a 1/8″ (3.2 mm) microprobe having a maximal amplitude of 240 μm. The sonication probe was placed directly into the solvent containing the flaxseed at 20% (48 μm), 60% (144 μm) or 100% (240 μm) of maximal probe amplitude and sonicated for 5, 10 or 20 min. The set amplitude was constant as the power (wattage) was automatically adjusted in response to detected probe load. Ultrasound probe parameters for extraction were determined based on pilot work leading up to the current study. Samples were reconstituted with the appropriate amount of solvent if evaporation was noticeable. Solvent evaporation was particularly evident by 20 min for some samples.

2.2. Fatty acid analysis

All four extraction solvents included 50 μg/mL of butylated hydroxytoluene (Sigma-Aldrich, St. Louis, MO, USA) as an antioxidant. Ethyl esters of nonadecanoic acid (19:0) (Nuchek Prep, Elysian, MN, USA) were added as an internal standard. Following sonication, an aqueous buffer of either sodium phosphate (chloroform:methanol) or sodium sulfate (hexane:isopropanol and diethyl ether:petroleum ether) was added to the extraction solvents, gently mixed and centrifuged. The organic layer was collected and dried under nitrogen in a pre-weighed test tube to allow for dry lipid weight determinations. Total lipids were dissolved in hexane and stored at −80 °C until fatty acid analyses were completed. Fatty acid methyl esters were prepared from total lipid extracts with 14% boron trifluoride in methanol (Pierce Chemicals, Rockford, IL, USA) and hexane with convectional heating at 95 °C for 1 h [16]. The organic layer containing the fatty acid methyl esters were collected for analysis on a Varian 3900 gas chromatograph equipped with a DB-FFAP 15 m × 0.10 mm i.d. × 0.10 μm film thickness, nitroterephthalic acid modified, polyethylene glycol, capillary column (J&W Scientific from Agilent Technologies, Mississauga, ON) with hydrogen as the carrier gas. Samples (2 μL) were introduced by a Varian CP-8400 autosampler into the injector heated to 250 °C with a split ratio of 200:1. Initial temperature was 150 °C with a 0.25 min hold followed by a 35 °C/min ramp to 200 °C, an 8 °C/min ramp to 225 °C with a 3.2 min hold and then an 80 °C/min ramp up to 245 °C with a 15 min hold at the end. The flame ionization detector temperature was 300 °C with air and nitrogen make-up gas flow rates of 300 and 25 mL/min, respectively, and a sampling frequency of 50 Hz.

2.3. Gas chromatography mass spectrometry (GCMS) analysis

Fatty acids extracted by the 24-h standard Folch technique and for the four solvents after 20 min of sonication at 100% probe

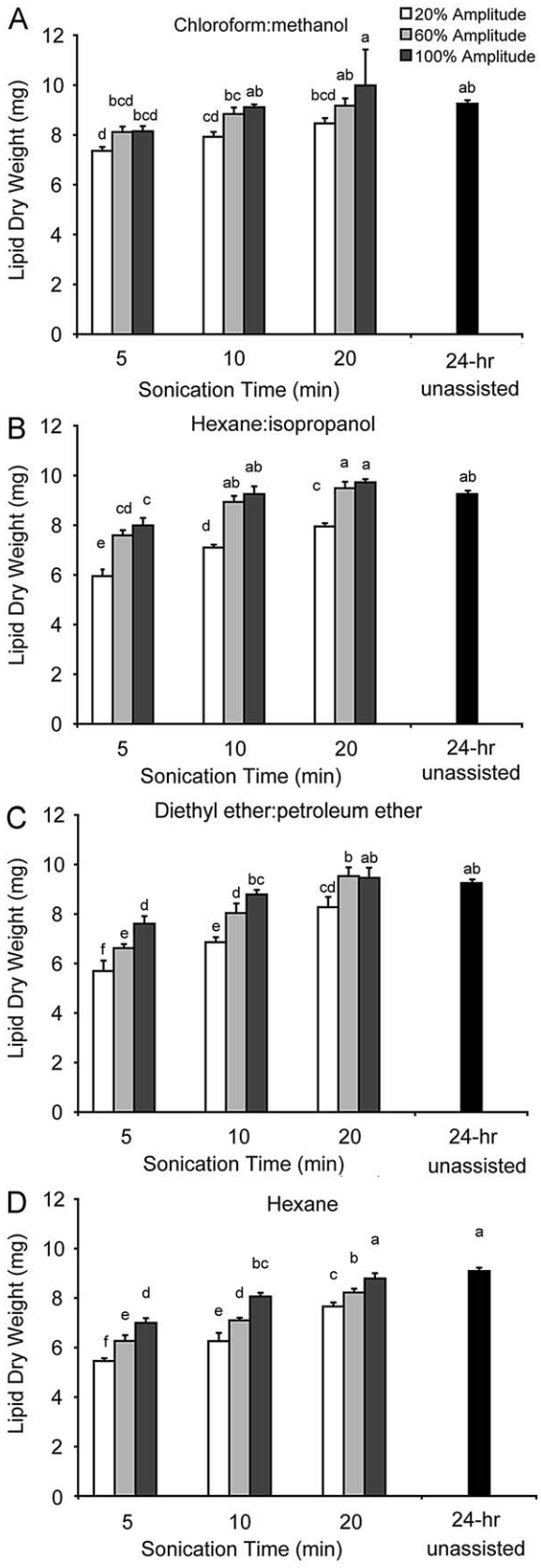

Fig. 1. Lipid dry weights following ultrasound-assisted extraction at different intensities with a 240 μm amplitude probe and time exposures in: (A) 2:1 (v:v) chloroform:methanol; (B) 3:2 (v:v) hexane:isopropanol; (C) 1:1 (v:v) diethyl ether:petroleum ether; (D) Hexane. Bars represent means with error bars representing the S.D., $n = 4$. Bars with different letters are significantly different by Tukey's HSD post hoc procedure ($P < 0.05$) after a significant F-value by one-way ANOVA ($P < 0.05$).

Please cite this article as: A.H. Metherel, et al., The application of ultrasound energy to increase lipid extraction throughput of solid matrix samples (flaxseed), Prostaglandins Leukotrienes Essent. Fatty Acids (2009), doi:10.1016/j.plefa.2009.07.003

amplitude were analyzed on a Varian 3800 GC coupled to a Varian 4000 MS (gas chromatography mass spectrometer, GCMS) with a quadrupole ion trap (Varian Canada Inc., Mississauga, ON) in External EI mode as described previously [16]. Briefly, the GCMS was equipped with a DB-FFAP 30 m × 0.25 mm i.d. × 25 μm film thickness, capillary column (J&W Scientific from Agilent Technologies, Mississauga, ON) interfaced directly onto the ion source with helium as carrier gas. Initial column temperature was 50 °C with a 1 min hold, followed by a 30 °C/min ramp to 130 °C, an 8 °C/min ramp to 175 °C, a 1 °C/min ramp to 210 °C, a 30 °C/min ramp to 245 °C and a 30 min hold at the end. Additional temperature settings were as follows: injector, 260 °C; transfer line, 250 °C; source, 180 °C and manifold, 50 °C. Mass ranges between 50 and 400 m/z were examined with target ion counts of 20,000 with a maximum ionization time of 65,000 μs and an emission current of 25 μA. Each data point was generated by a 3 uScan average resulting in a 0.55 s maximum scan time. Fatty acid mass spectra were cross-referenced to the NIST 05 database for identification and confirmation.

2.4. Malonaldehyde determinations

The concentration of malonaldehyde, a major product of unsaturated fatty acid peroxidation [17], was determined in the 24-h standard Folch lipid extraction and in lipid extractions for the four solvents after 20 min of sonication at 100% probe amplitude by a modified thiobarbituric acid reactive species assay [18]. Briefly, lipid extracts were dissolved in 55 mM thiobarbituric acid, 3.5 M acetic acid, deionized H_2O and 0.28 M sodium dodecyl sulfate and heated in a water bath at 95 °C for 1 h. Samples were rapidly cooled and 5 mL of 15:1 (v:v) butanol:pyridine and 1 mL of deionized H_2O were added, vortexed and centrifuged. The absorbance at a wavelength of 532 nm was measured in the top organic butanol layer on a UV160U Spectrophotometer (Shimadzu, Columbia, MD) and quantified against a malonaldehyde standard curve.

2.5. Statistical analysis

Dry weight extraction values and qualitative and quantitative values for individual fatty acids were determined and are expressed as mean ± SD. All statistical analyses were performed with the SPSS System (SPSS Inc., Chicago, IL). Various extraction protocols were examined by one-way ANOVA with individual means compared following a significant F-value by Tukey's Honestly Significant Different (HSD) post hoc procedure. Significance was inferred at $p < 0.05$.

3. Results

3.1. Ultrasound-assisted extractions and dry weight

A lipid dry weight of 9.25 ± 0.14 mg was determined in 25 mg of ground flaxseed by the 24-h unassisted extraction with 2:1 (v:v) chloroform:methanol. With ultrasound assistance, lipid extractions dry weight recovery was maximal at 100% amplitude for 20 min with 2:1 (v:v) chloroform:methanol (9.98 ± 1.44 mg) (Fig. 1). Dry lipid weight recoveries with ultrasound assistance at 100% amplitude for 20 min also resulted in high recoveries in 3:2 (v:v) hexane:isopropanol (9.72 ± 0.13 mg), 1:1 (v:v) diethyl ether/

Table 1
Quantitative fatty acid profiles of flaxseed following ultrasound-assisted extraction in various solvents.

	24-h unassisted	Ultrasound-assisted with 240 μm amplitude probe			
		5 min at 100%	10 min at 100%	20 min at 60%	20 min at 100%
	mg/25 mg of ground flaxseed				
	2:1 (v:v) chloroform:methanol				
16:0	0.50 ± 0.02	0.37 ± 0.02*	0.41 ± 0.03*	0.44 ± 0.04*	0.41 ± 0.03*
18:0	0.33 ± 0.02	0.23 ± 0.01*	0.25 ± 0.02*	0.27 ± 0.03*	0.25 ± 0.02*
18:1n-9	2.07 ± 0.07	1.57 ± 0.09*	1.70 ± 0.14*	1.83 ± 0.20*	1.62 ± 0.12*
18:2n-6	1.45 ± 0.07	1.11 ± 0.05*	1.19 ± 0.09*	1.29 ± 0.14	1.14 ± 0.09*
18:3n-3	4.04 ± 0.14	3.10 ± 0.15*	3.34 ± 0.27*	3.63 ± 0.36	3.20 ± 0.27*
Total	8.62 ± 0.31	6.48 ± 0.33*	7.01 ± 0.54*	7.59 ± 0.77	6.74 ± 0.54*
	3:2 (v:v) hexane:isopropanol				
16:0		0.38 ± 0.02*	0.45 ± 0.03*	0.45 ± 0.03*	0.43 ± 0.03*
18:0		0.24 ± 0.01*	0.28 ± 0.02*	0.29 ± 0.03	0.27 ± 0.02*
18:1n-9		1.63 ± 0.09*	1.89 ± 0.11	1.84 ± 0.06*	1.87 ± 0.13
18:2n-6		1.14 ± 0.06*	1.33 ± 0.06	1.32 ± 0.06	1.30 ± 0.10
18:3n-3		3.21 ± 0.18*	3.72 ± 0.13	3.68 ± 0.18	3.68 ± 0.26
Total		6.72 ± 0.36*	7.79 ± 0.33	7.72 ± 0.36	7.68 ± 0.55
	1:1 (v:v) diethyl ether:petroleum ether				
16:0		0.32 ± 0.02*	0.38 ± 0.04*	0.42 ± 0.02*	0.43 ± 0.01*
18:0		0.21 ± 0.01*	0.24 ± 0.02*	0.26 ± 0.01*	0.27 ± 0.01*
18:1n-9		1.39 ± 0.07*	1.62 ± 0.14*	1.78 ± 0.09*	1.86 ± 0.05*
18:2n-6		0.99 ± 0.05*	1.15 ± 0.10*	1.26 ± 0.06*	1.30 ± 0.03*
18:3n-3		2.73 ± 0.16*	3.22 ± 0.30*	3.52 ± 0.15*	3.61 ± 0.08*
Total		5.73 ± 0.32*	6.73 ± 0.59*	7.36 ± 0.33*	7.60 ± 0.17*
	Hexane				
16:0		0.34 ± 0.05*	0.36 ± 0.02*	0.39 ± 0.04*	0.35 ± 0.03*
18:0		0.21 ± 0.03*	0.22 ± 0.02*	0.23 ± 0.02*	0.21 ± 0.01*
18:1n-9		1.42 ± 0.22*	1.53 ± 0.12*	1.62 ± 0.15*	1.47 ± 0.10*
18:2n-6		1.06 ± 0.15*	1.12 ± 0.08*	1.21 ± 0.09*	1.12 ± 0.08*
18:3n-3		3.10 ± 0.40*	3.30 ± 0.18*	3.61 ± 0.28	3.37 ± 0.27*
Total		6.24 ± 0.86*	6.62 ± 0.43*	7.18 ± 0.59*	6.62 ± 0.49*

Values are means ± SD, $n = 4$.

* Significantly different from 24-h unassisted by Tukey's HSD post hoc procedure ($P < 0.05$) after a significant F-value by one-way ANOVA ($P < 0.05$).

Please cite this article as: A.H. Metherel, et al., The application of ultrasound energy to increase lipid extraction throughput of solid matrix samples (flaxseed), Prostaglandins Leukotrienes Essent. Fatty Acids (2009), doi:10.1016/j.plefa.2009.07.003

petroleum ether (9.46 ± 0.41 mg) and hexane only (8.94 ± 0.22 mg). Dry lipid weight recoveries at 60% amplitude for 20 min were statistically similar to the 24-h unassisted and the 100% amplitude for 20 min in chloroform:methanol (9.17 ± 0.11 mg), hexane:isopropanol (9.49 ± 0.31 mg) and diethyl ether/petroleum ether (9.53 ± 0.18 mg) but not in hexane only (8.37 ± 0.16 mg). This dry weight recovery equivalency to the 24-h unassisted and the 100% amplitude for 20 min was also observed at 100% amplitude for 10 min in chloroform:methanol (9.11 ± 0.29 mg) and hexane:isopropanol (9.25 ± 0.26 mg).

3.2. Quantitative fatty acid analysis

Fatty acid concentrations in milligrams of fatty acid per 25 mg of ground flaxseed were determined using 19:0 ethyl ester as the internal standard in all extractions. Fatty acids with masses greater than 0.05 mg are presented for ultrasound-assisted extractions in the various solvents at 100% amplitude for 5, 10 and 20 min as well as 60% amplitude for 20 min (based on lipid dry weight recoveries similar or approaching lipid dry weight recoveries in the 24-h unassisted chloroform:methanol extraction) (Table 1). Fatty acids presented include palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1n-9), linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3), although myristic acid (14:0), palmitoleic (16:1n-7), vaccenic acid (18:1n-7), arachidic acid (20:0), eicosenic acid (20:1n-9), behenic acid (22:0), erucic acid (22:1n-9) and lignoceric acid (24:0) were also detected. Individual fatty acid determinations were most similar to the 24-h standard method with ultrasound assistance in the hexane:isopropanol with 10 min exposure at 100% amplitude, and with 20 min exposure at 60% and 100% amplitude. Ultrasound-assisted extractions in chloroform:methanol at 60% amplitude for 20 min resulted in a fatty acid profile similar to the 24-h standard but palmitic acid (16:0), stearic acid (18:0) and oleic acid (18:1n-9) were all statistically lower. Fatty acid concentrations were largely significantly lower than the 24-h standard with ultrasound-assisted extractions in diethyl ether:petroleum ether and hexane alone.

GCMS analyses combined with NIST 05 database searches confirmed the identification of fatty acid methyl esters across extraction techniques, thereby confirming quantitative measures. No differences in the spectra from the standard Folch extraction and the ultrasound-assisted extractions for 20 min at 100% amplitude are seen as demonstrated for alpha-linolenic acid (18:3n-3) (Fig. 2).

3.3. Qualitative fatty acid analysis

Fatty acid determinations were determined for all extractions and the results corresponding to Table 1 are presented herein (ultrasound-assisted extractions in the various solvents at 100% amplitude for 5, 10 and 20 min as well as 60% amplitude for 20 min) (Table 2). Ultrasound-assisted extractions in chloroform:methanol and hexane:isopropanol tended to be more similar to the 24-h standard as compared with the extractions in diethyl ether: petroleum ether and hexane alone. The number of significant differences in fatty acid determinations between ultrasound-assisted extractions and the 24-h standard was considerably less when the data were presented qualitatively (percentage weight of total fatty acids) as compared to when the data were presented quantitatively (mg/25 mg of flaxseed).

3.4. Lipid peroxidation

Lipid peroxidation after ultrasound extraction for 20 min at 240 μm (100%) amplitude in 3:2 hexane:isopropanol and 2:1 chloroform:methanol were similar to standard 24-h Folch extraction as estimated by malonaldehyde concentrations (Fig. 3). Ultrasound extraction in hexane resulted in significantly lower and barely detectable malonaldehyde concentrations. Ultrasound-assisted lipid extraction in 1:1 diethyl ether:petroleum ether did result in significantly higher levels of oxidation as compared with standard 24-h Folch extraction (0.98 ± 0.49 vs. 0.17 ± 0.09 nmol of malonaldehyde/25 mg flaxseed extraction).

4. Discussion

Ultrasound assistance can dramatically increase lipid extraction throughput. In the present study, we demonstrate that increasing the amplitude of and the exposure time to sonication can increase lipid recoveries with extractions in all the solvents tested. However, the lipid dry weight recoveries were not equal in all the solvents with ultrasound assistance and when fatty acid determinations were also examined, ultrasound-assisted extractions in hexane:isopropanol appeared to best duplicate the 24-h standard procedure, followed by chloroform:methanol for lipid extraction and fatty acid determinations of flaxseed. Ultrasound-assisted extractions with diethyl ether:petroleum ether and hexane alone resulted in significantly lower fatty acid determinations in sonication exposures of 20 min or less.

In the present study, 3:2 hexane:isopropanol [15,19] and 2:1 chloroform:methanol [20] resulted in lipid extractions and fatty acid determinations that were similar to the 24-h standard method. The 1:1 diethyl ether:petroleum ether extractions had significantly lower lipid and fatty acid recoveries [1]. The addition of polar solvents such as isopropanol and methanol to nonpolar solvents such as hexane and chloroform increases the recovery of polar lipids such as phospholipids and lipoproteins [20]. The use of hexane:isopropanol as an extraction solvent also has as a significant advantage over chloroform:methanol with regard to toxicity and handling of extracts [19] and isopropanol can prevent lipid degradation in plant materials by phospholipase D [21–23], which can remain active in some solvents [24].

Hexane alone as a lipid extraction solvent was demonstrated to have the lowest recoveries in the present study. This is presently the solvent of choice for the ISO standard method [2], in which reflux-extraction is used to extract lipids from food. Ultrasound-assisted extractions in hexane with short time exposures resulted in poor recoveries. Nonpolar solvents, such as hexane and diethyl ether, can extract neutral lipids like triacylglycerols, which is the major lipid component of seeds [25]; however, polar lipids such as phospholipids can be lost [20]. While a loss of polar lipids during flaxseed extraction in the present study may partially explain the decreased lipid and fatty acid recovery, the use of polar and nonpolar solvents combined with ultrasonic energy has been reported to result in an emulsification–extraction process that results in the rapid and efficient extraction of total lipids from solid matrices [26].

Ultrasound-assisted extraction with the ultrasound probe in direct contact with the various extraction solvents resulted in levels of oxidation on the scale of the standard 24-h Folch extraction. Direct oil-ultrasound probe contact has been demonstrated to result in oxidation of olive oil in under 20 min [27], but direct sonication of sunflower oil has demonstrated little effect on fatty acid composition directly after ultrasound although peroxide levels increased [8]. In addition, sonication with a direct probe in the presence of KOH in ethanol to transesterify fish oil

Please cite this article as: A.H. Metherel, et al., The application of ultrasound energy to increase lipid extraction throughput of solid matrix samples (flaxseed), Prostaglandins Leukotrienes Essent. Fatty Acids (2009), doi:10.1016/j.plefa.2009.07.003

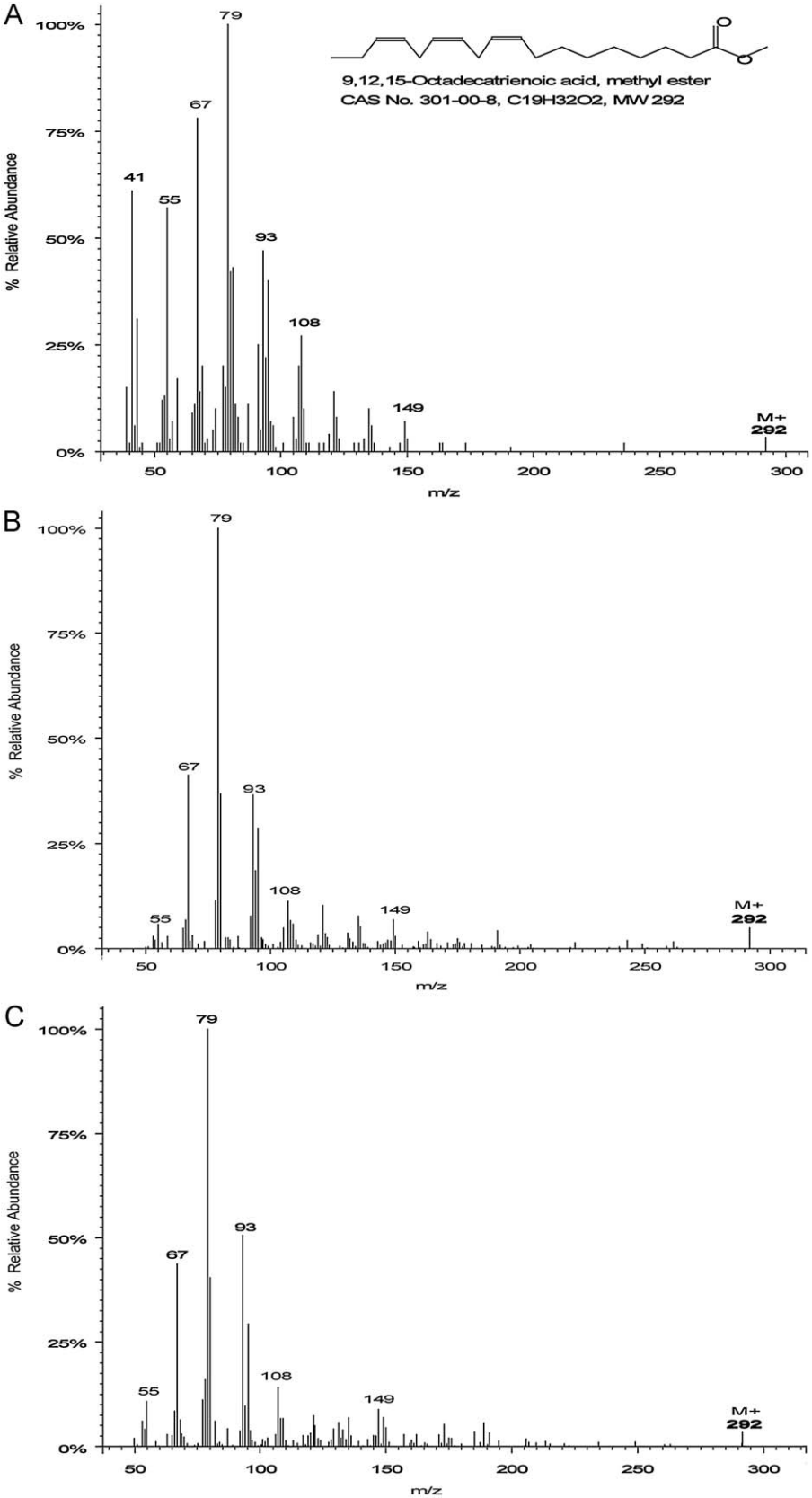

Fig. 2. Mass spectra of alpha-linolenic acid methyl ester (18:3n-3) obtained from (A) NIST 05 library database, (B) standard 24-h Folch extraction and (C) ultrasound-assisted extraction in 3:2 hexane:isopropanol for 20 min at 240 μm (100%) amplitude.

Please cite this article as: A.H. Metherel, et al., The application of ultrasound energy to increase lipid extraction throughput of solid matrix samples (flaxseed), Prostaglandins Leukotrienes Essent. Fatty Acids (2009), doi:10.1016/j.plefa.2009.07.003

Table 2
Qualitative fatty acid profiles of flaxseed following ultrasound-assisted extraction in various solvents.

	24-h unassisted	Ultrasound-assisted with 240 μm amplitude probe			
		5 min at 100%	10 min at 100%	20 min at 60%	20 min at 100%
	% weight of total fatty acids				
	2:1 (v:v) chloroform:methanol				
16:0	5.79±0.10	5.60±0.04	5.72±0.18	5.64±0.15	5.95±0.09*
18:0	3.74±0.10	3.48±0.03*	3.56±0.18	3.45±0.15*	3.67±0.06
18:1n-9	23.67±0.28	23.72±0.28	23.76±0.11	23.24±0.16	23.69±0.30
18:2n-6	16.57±0.30	16.85±0.29	16.64±0.29	16.46±0.13	16.55±0.11
18:3n-3	46.15±0.32	46.91±0.11	46.75±0.40	46.17±0.41	46.66±0.17
	3:2 (v:v) hexane:isopropanol				
16:0		5.52±0.06	5.64±0.14	5.66±0.18	5.52±0.04*
18:0		3.50±0.01	3.51±0.06	3.64±0.31	3.53±0.04
18:1n-9		23.77±0.13	23.80±0.46	23.31±0.36	24.12±0.08*
18:2n-6		16.66±0.08	16.72±0.08	16.70±0.29	16.67±0.07
18:3n-3		46.96±0.15	46.91±0.72	46.49±0.23	47.40±0.25*
	1:1 (v:v) diethyl ether:petroleum ether				
16:0		5.46±0.04*	5.60±0.02*	5.55±0.07*	5.59±0.07*
18:0		3.57±0.04*	3.56±0.10*	3.54±0.05*	3.55±0.05*
18:1n-9		23.69±0.19	23.58±0.15	23.76±0.24	24.01±0.24
18:2n-6		16.88±0.08	16.78±0.16	16.81±0.17	16.83±0.16
18:3n-3		46.58±0.23	46.79±0.21	47.02±0.20*	46.62±0.30
	Hexane				
16:0		5.39±0.09*	5.32±0.07*	5.31±0.07*	5.20±0.02*
18:0		3.27±0.06*	3.24±0.08*	3.11±0.06*	3.10±0.04*
18:1n-9		22.24±0.40*	22.79±0.28*	21.94±0.18*	21.90±0.31*
18:2n-6		16.67±0.05	16.66±0.07	16.45±0.26	16.72±0.10
18:3n-3		48.54±0.47*	49.30±0.62*	49.12±0.52*	50.10±0.30*

Values are means±SD, $n = 4$.

* Significantly different from 24-h unassisted by Tukey's HSD post hoc procedure ($P<0.05$) after a significant F-value by one-way ANOVA ($P<0.05$).

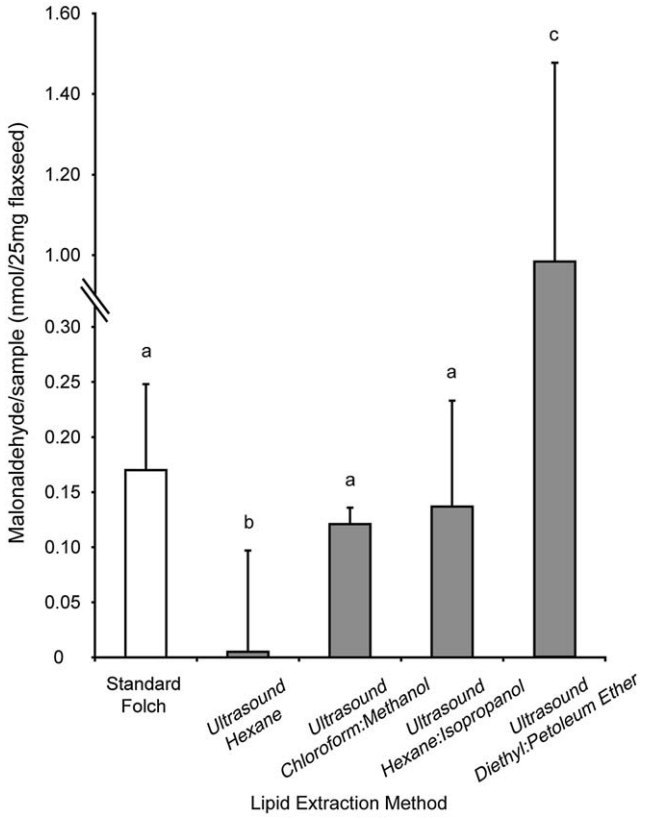

Fig. 3. Malonaldehyde concentrations in standard and sonication for 20 min at 240 μm (100%) amplitude lipid extractions in various solvents. Bars represent means±SD, $n = 4$. Bars with different letters are significantly different by independent t-test ($P<0.05$).

triacylglycerols to fatty acid ethyl esters has no effect on fatty acid quality [28]. Cavitation is likely the main cause of oxidation with ultrasound as micro-bubbles form and collapse, resulting in areas of high temperature and pressure. In addition to thermal and shear force-induced oxidation, free radicals may also be generated by sonolysis [8].

Presently, GCMS analysis confirmed the peak identification of fatty acid methyl esters after the various extraction techniques, confirming fatty acid quantitation by gas chromatography. The levels of malonaldehyde were significantly lower in the hexane-only extraction and may suggest that extraction in nonpolar, organic solvents may limit oxidation. Unfortunately extraction recoveries with hexane were limited. In contrast, sonication during extraction with diethyl ether:petroleum ether resulted in significantly higher levels of malonaldehyde. Diethyl ether is susceptible to peroxide formation that can be accelerated by heat, light, air and moisture [29], and it is possible that sonication may accelerate peroxidation of diethyl ether. Interestingly, the two most promising extraction solvents (2:1 chloroform:methanol and 3:2 hexane:isopropanol) showed no more lipid peroxidation than the standard Folch method. Malonaldehyde and conjugated dienes may not be as sensitive as hydroperoxides and their decomposition products including aldehydes, ketones and low molecular weight acids for determining lipid peroxidation induced by ultrasound [8]. However, the present oxidation results and the fatty acid composition data suggest that quantitative losses of fatty acids are minor during the ultrasound-assisted lipid extraction examined herein.

The present study is limited to the examination of ultrasound-assisted lipid extraction with various solvents and fatty acid determinations of flaxseed. In addition, the conditions for sonication were held constant across the experiments and attempts to optimize sonication through variables such as extractant volumes, probe position and duty cycle were not

Please cite this article as: A.H. Metherel, et al., The application of ultrasound energy to increase lipid extraction throughput of solid matrix samples (flaxseed), Prostaglandins Leukotrienes Essent. Fatty Acids (2009), doi:10.1016/j.plefa.2009.07.003

completed. However, the present findings of more efficient extractions with nonpolar/polar solvent combinations are likely transferable to other solid matrices, particularly food product analyses that can involve multiple matrices and higher proportions of polar lipids. An examination of ultrasound-assisted extractions of samples of animal origin with hexane:isopropanol and chloroform:methanol is required to assess efficiencies in extracting polar lipids. The present study also demonstrates the importance of using quantitative determinations of fatty acids to evaluate methods. The use of qualitative determinations of fatty acids for method comparisons can mask incomplete recovery of fatty acids, if the extraction efficiency for each individual fatty acid is similar. For example, in the present study fatty acid determinations at 5 min exposure at 100% amplitude were largely similar to the 24-h standard method when compared qualitatively as relative weight percentages, but quantitative comparisons reveal that total fatty acid recoveries at 5 min exposure at 100% amplitude were 22–34% less than the 24-h standard.

In conclusion, the present findings of efficient ultrasound-assisted extractions of lipids with hexane:isopropanol at greatly reduced time exposures can contribute to efforts to develop high-throughput analytical methods for fatty acid determinations. In addition to reductions in time of extraction, the use of hexane:isopropanol in place of chloroform:methanol can significantly reduce the environmental impact and burden of lipid extraction and fatty acid analyses. Further research is required to optimize solvent types and ratios as well as sonication parameters. Combining ultrasound-assisted lipid extraction with rapid derivitization techniques [16] and fast gas chromatography techniques [30] can significantly increase sample throughput. The applicability of these methodological advancements needs to be verified across a range of matrices and food samples.

Acknowledgements

Operating funds were provided by an Ontario Centres of Excellence Interact award to K.D.S. and Certo Labs, Inc. Infrastructure was purchased through Canada Foundation of Innovation and the Ontario Research Fund matching grants.

References

[1] AOAC Official Method 996.06, Official Methods of Analysis of AOAC International, 18th ed., AOAC International, Gaithersburg, 2005.
[2] J.L. Luque-Garcia, M.D. Luque de Castro, Ultrasound-assisted Soxhlet extraction: an expeditive approach for solid sample treatment. Application to the extraction of total fat from oleaginous seeds, J. Chromatogr. A 1034 (2004) 237–242.
[3] Y.G. Ahn, J.H. Shin, H.Y. Kim, J. Khim, M.K. Lee, J. Hong, Application of solid-phase extraction coupled with freezing-lipid filtration clean-up for the determination of endocrine-disrupting phenols in fish, Anal. Chim. Acta 603 (2007) 67–75.
[4] J. Wu, L. Lin, F.T. Chau, Ultrasound-assisted extraction of ginseng saponins from ginseng roots and cultured ginseng cells, Ultrason. Sonochem. 8 (2001) 347–352.
[5] S. Hemwimol, P. Pavasant, A. Shotipruk, Ultrasound-assisted extraction of anthraquinones from roots of Morinda citrifolia, Ultrason. Sonochem. 13 (2006) 543–548.
[6] A. Christensen, C. Ostman, R. Westerholm, Ultrasound-assisted extraction and on-line LC-GC-MS for determination of polycyclic aromatic hydrocarbons (PAH) in urban dust and diesel particulate matter, Anal. Bioanal. Chem. 381 (2005) 1206–1216.
[7] P. Richter, M. Jimenez, R. Salazar, A. Marican, Ultrasound-assisted pressurized solvent extraction for aliphatic and polycyclic aromatic hydrocarbons from soils, J. Chromatogr. A 1132 (2006) 15–20.
[8] F. Chemat, I. Grondin, P. Costes, L. Moutoussamy, A. Shum, A.S.C. Sing, J. Smadja, High power ultrasound effects on lipid oxidation of refined sunflower oil, Ultrason. Sonochem. 11 (2004) 281–285.
[9] M. Toma, M. Vinatoru, L. Paniwnyk, T.J. Mason, Investigation of the effects of ultrasound on vegetal tissues during solvent extraction, Ultrason. Sonochem. 8 (2001) 137–142.
[10] M. Vinatoru, M. Toma, O. Radu, P.I. Filip, D. Lazurca, T.J. Mason, The use of ultrasound for the extraction of bioactive principles from plant materials, Ultrason. Sonochem. 4 (1997) 135–139.
[11] J. Ruiz-Jimenez, F. Priego-Capote, M.D. Luque de Castro, Identification and quantification of trans fatty acids in bakery products by gas chromatography–mass spectrometry after dynamic ultrasound-assisted extraction, J. Chromatogr. A 1045 (2004) 203–210.
[12] F. Wei, G.Z. Gao, X.F. Wang, X.Y. Dong, P.P. Li, W. Hua, X. Wang, X.M. Wu, H. Chen, Quantitative determination of oil content in small quantity of oilseed rape by ultrasound-assisted extraction combined with gas chromatography, Ultrason. Sonochem. 15 (2008) 938–942.
[13] G. Cravotto, L. Boffa, S. Mantegna, P. Perego, M. Avogadro, P. Cintas, Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves, Ultrason. Sonochem. 15 (2008) 898–902.
[14] J. Folch, M. Lees, G.H.S. Sloane Stanley, A simple method for the isolation and purification of total lipides from animal tissues, J. Biol. Chem. 226 (1957) 497–509.
[15] A. Hara, N.S. Radin, Lipid extraction of tissues with a low-toxicity solvent, Anal. Biochem. 90 (1978) 420–426.
[16] J.M. Armstrong, A.H. Metherel, K.D. Stark, Direct microwave transesterification of fingertip prick blood samples for fatty acid determinations, Lipids 43 (2008) 187–196.
[17] S. Gabor, Z. Anca, Effect of asbestos on lipid peroxidation in the red cells, Br. J. Ind. Med. 32 (1975) 39–41.
[18] K.M. Wilbur, F. Bernheim, O.W. Shapiro, The thiobarbituric acid reagent as a test for the oxidation of unsaturated fatty acids by various agents, Arch. Biochem. 24 (1949) 305–313.
[19] N.S. Radin, Extraction of tissue lipids with a solvent of low toxicity, Methods Enzymol. 72 (1981) 5–7.
[20] W.W. Christie, Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids, 3rd ed., The Olly Press, Bridgwater, 2003.
[21] M. Kates, Chromatographic and radioisotopic investigations of the lipid components of runner bean leaves, Biochim. Biophys. Acta 41 (1960) 315–328.
[22] B.W. Nichols, Separation of the lipids of photosynthetic tissues: improvements in analysis by thin-layer chromatography, Biochim. Biophys. Acta 70 (1963) 417–422.
[23] W.W. Christie, S. Gill, J. Nordback, Y. Itabashi, S. Sanda, A.R. Slabas, New procedures for rapid screening of leaf lipid components from arabidopsis, Photochem. Anal. 9 (1998) 53–57.
[24] M. Heller, R. Arad, Properties of the phospholipase D from peanut seeds, Biochim. Biophys. Acta 210 (1970) 276–286.
[25] M.F. Ramadan, J.T. Morsel, Determination of the lipid classes and fatty acid profile of Niger (*Guizotia abyssinica* Cass.) seed oil, Phytochem. Anal. 14 (2003) 366–370.
[26] J.A. Perez-Serradilla, F. Priego-Capote, M.D. Luque de Castro, Simultaneous ultrasound-assisted emulsification–extraction of polar and nonpolar compounds from solid plant samples, Anal. Chem. 79 (2007) 6767–6774.
[27] M.P. Cañizares-Macías, J.A. García-Mesa, M.D. Luque de Castro, Fast ultrasound-assisted method for the determination of the oxidative stability of virgin olive oil, Anal. Chim. Acta 502 (2004) 161–166.
[28] R.E. Armenta, M. Vinatoru, A.M. Burja, J.A. Kralovec, C.J. Barrow, Transesterification of fish oil to produce fatty acid ethyl esters using ultrasonic energy, J. Am. Oil Chem. Soc. 84 (2007) 1045–1052.
[29] W.L.F. Armarego, C.L.L. Chai, Purification of Laboratory Chemicals, Butterworth–Heinemann, Boston, MA, 2003.
[30] K.D. Stark, N. Salem Jr., Fast gas chromatography for the identification of fatty acid methyl esters from mammalian samples, Lipid Technol. 17 (2005) 181–185.

Please cite this article as: A.H. Metherel, et al., The application of ultrasound energy to increase lipid extraction throughput of solid matrix samples (flaxseed), Prostaglandins Leukotrienes Essent. Fatty Acids (2009), doi:10.1016/j.plefa.2009.07.003