Microwave-Assisted Catfish Liver Oil Extraction and FA Analysis

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ABSTRACT: FA profiles of catfish liver oils were analyzed after microwave-assisted (without solvent extraction) and/or conventional (with solvent extraction) preparation methods. Microwave heating of the samples was performed at 100, 80, 60, or 40% power at 1,000 W and 2,450 MHz, each for 80, 60, 40, or 20 s. Significant differences in the content of recovered FA were observed among the microwave-heated samples, except for C20:0 and C20:4. Recovery of C16:0, C20:0, and C20:4 from the samples analyzed by the microwave-assisted method was lower than that of the samples analyzed by the conventional method. Much greater recovery was observed for C18:1, C18:2, and C22:6; however, the recovery was not different from or was only slightly lower than that of the conventional method when microwave heating was set at 40% power for 20 s. This was also observed for the total unsaturated or saturated FA. Compared to other microwave treatments, heating at 100% power for 80 s yielded the greatest recovery of C14:0, C18:0, C18:1, C18:2, C18:3, C20:1, C20:2, and C22:6.

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KEY WORDS: Catfish liver oil, fatty acid analysis, microwave heating.

The analysis of lipids and FA compositions of foods and ingredients is an important element in food chemistry. FA composition of foods, especially PUFA, is of interest to consumers as well as food scientists because of their nutritional and health benefits. Studies have shown the potential health benefits of long-chain PUFA, e.g., EPA (20:5n-3), arachidonic acid (20:4n-6), and DHA (22:6n-3). PUFA are important to human health and essential to the development of the fetus and infants. Dietary intake of PUFA reduces the incidence of coronary diseases and some cancers (1). A number of extensive research programs have been oriented toward development of enriched PUFA-containing products. This type of research requires routine FA analyses, which are time consuming and labor intensive. Thus, a faster, yet simple and reliable method is needed.

GC analysis is commonly used to determine FA profiles of lipids in biological materials and normally requires methyl esterification of FA (2). The preparation of FAME requires fat extraction from biological materials with organic solvents, followed by esterification of the fat to form FAME. Organic solvents commonly used for fat extraction include chloroform, dichloromethane, hexane, toluene, benzene, and methanol or a mixture of them. After extraction, the solvent is evaporated from the mixture by mild heating under nitrogen gas. The conventional fat extraction method requires a large volume of solvents, is a multistep procedure not suitable for handling a large number of samples, and may lead to introduction of contaminants and losses of esters. Therefore, it may not be practical in laboratories where a number of tests are required in a short period of time.

Several methods of FAME preparation have been developed to reduce the number of steps to one or two. For example, Ichihara *et al.* (3) rapidly transesterified a lipid extract with methanolic HCl and resolved major FA using high-temperature GLC. Shimasaki *et al.* (4) chemically removed water from brain and plasma samples with 2,2′-dimethoxypropane and then transesterified the lipids to produce FAME. Lepage and Roy (5–7) reported a one-step procedure for FAME preparation with acetyl chloride applied to several sources and classes of lipids. A similar approach was reported by Sukhija and Palmquist (8) but was applied to freeze-dried materials. Ohta *et al.* (9) applied plasma samples directly to TLC plates to separate lipids and then transesterified the silica-adsorbed FA with boron trifluoride in methanol. Lipid extraction procedures, for example, those reported by Folch *et al.* (10) and Bligh and Dyer (2), ideally separated all lipids from the bulk of the material, which were then partitioned to a hydrophobic phase for separation and subsequent purification. The use of microwave irradiation in conjunction with solvents has been suggested as an efficient technique for the extraction of lipophilic substances from biological tissue (11–13). Pare *et al.* (11) utilized petroleum ether with microwave-assisted extraction under atmospheric conditions to extract fat from meat, dairy, and egg products. Leray *et al.* (12) used chloroform/methanol mixtures in an open vessel with microwave-assisted extraction to isolate fat from dried animal food. A study by Batista *et al.* (13) showed that microwave-assisted extraction using ethyl acetate and cyclohexane to extract fat from cod liver and mackerel fillets resulted in a FA composition similar to that found using the method of Bligh and Dyer (2). No one has previously tried microwave extraction without solvent present during heating.

Catfish liver, a waste from the filleting process, is a potential source of health-promoting FA. The liver contains a high

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moisture content, which may interfere with lipid extraction; thus drying may be required before FAME are prepared. When a sample is microwave-heated, moisture in the tissues creates localized superheating, causing rapid release of moisture, expulsion of fat/oil from fat cells, and partial release of oil to the surface. It may be possible to prepare FAME from microwave-heated samples without solvent extraction. The objectives of this study were to investigate FA profiles of samples containing a high moisture content by microwave-assisted FA analysis without solvent extraction, to compare the FA profiles of samples analyzed by the microwave-assisted vs. conventional methods, and to determine microwave-heating power (%) and time (s) for maximal FA recovery. Catfish liver containing 75% moisture was used for demonstration.

EXPERIMENTAL PROCEDURES

Microwave-assisted FAME preparation. Fresh liver of farm-raised catfish obtained from a local seafood store in Baton Rouge, Louisiana, was finely ground, with a commercial blender. Approximately 5 g of finely ground, homogeneous catfish liver was placed in a 50-mL sample vial. The vials were placed in a microwave oven (Model R-508AK, Sharp Carousel, 1,000 watts and 2,450 MHz) and heated according to our experimental design. A 4×4 full-factorial design was employed. The concentration and type of FA were dependent variables, and the microwave power (100, 80, 60, 40% of 1,000 watts) and heating time (80, 60, 40, 20 s) were independent variables.

After microwave treatment, the vials were immediately sealed with Teflon-coated caps and cooled to room temperature. After cooling, each vial was weighed. The moisture loss of liver was calculated as [(initial weight of the sample + vial) − (weight of the sample after microwave heating + vial)] × 100/initial weight of sample. Methanolic sodium hydroxide (2% NaOH in methanol; 6 mL), 7 mL BF_3, and 5 mL heptane were added to each vial. The reaction mixture was stirred with a Teflon-coated magnetic stirrer bar in a 70°C controlled water bath for 30 min. The heptane containing FAME was collected from the upper layer, dehydrated with anhydrous sodium sulfate, and stored under nitrogen in a Teflon-capped 20-mL vial at –20°C until further analyzed for FA profiles. Each microwave-heated treatment was repeated three times, each using a different batch of catfish liver.

Fat extraction by a conventional method. Fat was extracted from catfish liver according to the method of Bligh and Dyer (2). Approximately 5 g of finely ground, homogeneous catfish liver sample was placed in a screw-capped test tube; then 5 mL of distilled water, 20 mL of chloroform, and 20 mL of methanol (1:4:4, by vol) were added to the tube, and the mixture was thoroughly mixed on a vortex for 10 min. The homogeneous mixture was filtered through Whatman No. 1 filter paper. The filtrate was placed in a separatory funnel. The bottom layer of the solution was collected. Anhydrous sodium sulfate (5.7 g) was added to the collected solution to remove water. The residual solvent was removed from the solution by the Meyer-N-Evaporator (an analytical evaporator; Organomation Associates Inc., West Berlin, MA) under nitrogen atmosphere. The evaporation was continued until the solution was free of chloroform. The extracted oil samples were kept at −20°C until analyzed. Fat extraction was repeated three times, each using a different batch of catfish liver.

Esterification of FA by a conventional method. FAME were prepared according to the AOAC procedure 969.33 (14). Each extracted catfish oil was placed into a 50-mL flat-bottomed boiling flask containing approximately 4 mL of methanolic sodium hydroxide (2 g of NaOH dissolved in 100 mL of methanol), and 10 boiling chips were added to the flask. The condenser and reflux units were attached to the flask, and refluxing took place for 12 min after 7 mL of boron trifluoride had been added through the condenser. The esterified FA were extracted from the mixture by adding 5 mL of heptane and refluxing for 1 min. The esterified solution was allowed to cool to room temperature. A saturated solution of sodium chloride was added and the flask gently rotated. Saturated sodium chloride solution was added until the heptane solution containing FAME reached the neck of the flask. The heptane solution containing FAME was recovered, dehydrated with 1.5 g anhydrous sodium sulfate, and stored under nitrogen in Teflon-capped vials at –20°C until analyzed.

FA analysis. The FAME obtained from microwave-heated and conventionally treated samples were quantified with a Hewlett-Packard 5890 Series II Gas Chromatograph equipped with a 7673A autosampler (Agilent Technologies, Palo Alto, CA) and interfaced to a 5970 mass selective detector (Agilent Technologies). The GC was equipped with an EZ-Flash fast temperature programmable column (Thermedics Detection, Inc., Chelmsford, MA). The column phase was RTX-2330 (90% biscyanopropyl/10% phenylcyanopropyl polysiloxane) with these dimensions: 5 m long, 0.25 mm i.d., 0.2-µm phase thickness. One microliter of FAME was injected in a split mode. The head pressure was set at 2 psi, and the split vent flow was 7 mL/min. The injector temperature was 260°C. The column flow rate at 2 psi was 0.68 mL/min, and the split ratio was set to 10.4:1. The column temperature was held at 50°C for 6 s, ramped from 50 to 260°C at 1°C/s, and held at 260°C for 84 s. Run time was 5 min. The transfer line temperature was 280°C. The mass selection detector was operated in the selected ion monitoring mode. FA were identified with retention times obtained from commercial FAME standards (Sigma Chemical, St. Louis, MO). The known concentration of commercial FAME standard was diluted in heptane to attain 3, 10, 100, 500, and 1,000 ppm. The standard curves were developed based on the peak areas of gas chromatograms for known concentrations of FAME standards. Concentrations (ppm) of individual FA in each sample were calculated from the standard curves. The internal standard (IS) solution used for quantification of FA contained 1 mg nonadecanoic acid (C19:0)/mL heptane. For the recovery studies, 1 mg nonadecanoic acid methyl ester/mL heptane was used as IS. The

calculated concentration of individual FA through the standard curves was quantified as mg of FA/g of wet sample, taking into account the recovery of IS and sample weight. Three experimental replications (batches) were conducted for both the microwave-assisted and conventional methods, each batch with three extractions and three GC injections per extraction. The FA content was reported as mg FA/g microwaved sample. The unit in this study was used to reflect the effect of the microwave heating on moisture and FA profile of samples.

Statistical analysis. All data were analyzed using SAS (15). ANOVA was performed ($\alpha = 0.05$) to determine differences in FA profiles among microwave-heated samples. Tukey's Studentized range test was performed for *post hoc* multiple comparisons ($\alpha = 0.05$). Pairwise comparison for each FA was performed ($\alpha = 0.05$) between each of the microwave-treated samples and the conventional (control) sample. Group differences, expressed in terms of differences in the mean vectors of the FA, were determined using multivariate analysis of variance (MANOVA). Principal component analysis (PCA) was used to group the samples based on similarity (correlation) in the FA profile. Descriptive discriminant analysis (16) was performed to identify FA that largely underlie group differences among FA profiles of microwave-heated and conventional samples.

RESULTS AND DISCUSSION

Moisture loss. The moisture loss increased with increased magnitude of microwave heating power and time. Pronounced effects were observed when the heating time was increased to 80 s. The initial moisture content of catfish liver tissue was 75%. Microwave heating at 60–100% power for 80 s removed over 50% of the water from the samples. Microwave energy is a rapid heating source. The presence of water in a sample facilitates increased fat extraction during microwave heat treatment. The moisture content in the samples to be fat-extracted is a critical factor, as water is very efficient at absorbing microwave radiation. Fat molecules in liver are surrounded by biological materials, particularly protein molecules. Rapid removal of water in the sample may disrupt the cell structure and support removal of lipids from their association with cell membranes and proteins (11,17). By denaturing the protein molecules, the fat molecules can be liberated to the surface of the biological materials.

FA profile. The FA composition of catfish liver oils analyzed by both the microwave-assisted (without solvent extraction) and conventional (with solvent extraction) methods are shown in Table 1. Significant differences in the content of recovered FA were observed among the microwave-heated samples, except for C20:0 and C20:4, and these were due to various heating conditions. MANOVA indicated that FA profiles of all microwave-heated samples were different ($P < 0.001$). C20:0 and C20:4 were not present in any microwave-treated samples, except for a minute amount found in the sample heated at 100% power for 80 s. Results showed that the FA contents (C14:0, C16:1, C18:0, C18:1, C18:2, and C22:6) recovered by the microwave-assisted method were generally greater than that of the conventional method. There were significant differences in saturated and unsaturated FA between microwave-heated and conventional samples. PUFA showed drastic differences among microwave treatments, which were mainly due to the differences in the content of C22:6n-3.

Recovery of C16:0, C20:0, and C20:4 from all samples analyzed by the microwave-assisted method was lower than that by the conventional method. Much greater recovery was observed for C18:1, C18:2, and C22:6; however, the recovery was not different from or was slightly lower than that of the conventional method when microwave heating was set at 40% power and 20 s. Similar results were also observed for the total unsaturated or total saturated FA. Compared to other microwave treatments, heating at 100% power for 80 s yielded the highest recovery of C14:0, C18:0, C18:1, C18:2, C18:3, C20:1, C20:2, and C22:6.

To differentiate among the microwave-heating effects on FA composition of catfish liver oils, univariate and multivariate statistical analyses were carried out (detailed results not shown). The model included 14 dependent variables, which were the 12 FA and the total unsaturated and total saturated FA shown in Table 1. The ANOVA results indicated that differences were found in each FA content among 16 microwave-heated samples. Considering all 14 variables simultaneously, MANOVA results indicated that there were overall differences among the FA profiles of all 16 microwave-heated samples. This was substantiated by the test statistics value for Wilk's lambda, Pillai's trace, Hotelling–Lawley and Roy's greatest root, all with $P < 0.001$. Discriminant analysis with an emphasis on canonical correlation was employed to determine which FA variables contributed the most to overall differences among 16 microwave-heated and the control samples. FA (C20:4, C16:0, and C20:0) of the first dimension of canonical variables (CAN1, with 59% of total variance explained) appeared to be the attributes contributing most to the overall differences. The canonical correlation values for C20:4, C16:0, and C20:0 were 0.67, 0.60, and 0.45, respectively. The second dimension of canonical variables included C20:1, C18:0, C18:2, C16:1, and C22:6, having a cumulative 88% variance explained.

The product mapping (PC1 vs. PC2) obtained from PCA based on 14 dependent variables (FA) is shown in Figure 1. The PCA plot shows three clearly differentiated groups: the first group, corresponding to the conventional control sample (Q), the second group, corresponding to samples microwave-heated at 100% power for 80 s (A) and 80% power for 80 s (E), and the third group, belonging to other microwave-heated samples. The PCA plot also indicated that samples microwave-heated for less than 80 s at a power of less than 80% were more alike, although some FA were significantly different as indicated by the ANOVA results (Table 1). The differences between the control (Q) and the microwave-heated samples (A and E) were due to the amounts of unsaturated FA (C18:1, C18:2, and C22:6) (Table 1).

TABLE 1
FA Profiles of Catfish Liver Oils as Affected by Microwave Heating Power and Time[a]

Power (%)	Time (s)	C14:0	C16:0	C16:1	C18:0
100	80	0.07 ± 0.03^{a,*}	0.23 ± 0.10^{a,*}	0.45 ± 0.17^{a,*}	1.77 ± 0.71^{a,*}
100	60	0.04 ± 0.01^{b,c,d,*}	0.07 ± 0.02^{b,c,d,*}	0.25 ± 0.04^{b,c,d,*}	0.91 ± 0.17^{c,d,*}
100	40	0.02 ± 0.01^{e,f}	0.03 ± 0.01^{d,e,f}	0.12 ± 0.03^{f,e}	0.44 ± 0.10^{e,f,g,h}
100	20	0.02 ± 0.01^{e,f}	0.02 ± 0.01^{f,*}	0.08 ± 0.07^{f,e}	0.26 ± 0.26^{g,h}
80	80	0.06 ± 0.02^{a,b,*}	0.10 ± 0.02^{b,*}	0.35 ± 0.09^{a,b,*}	1.35 ± 0.42^{b,*}
80	60	0.05 ± 0.01^{b,c,*}	0.07 ± 0.01^{b,c,d,e,*}	0.28 ± 0.03^{b,c,*}	0.98 ± 0.17^{b,c,*}
80	40	0.03 ± 0.01^{e,d}	0.03 ± 0.01^{d,e,f,*}	0.15 ± 0.07^{e,d,*}	0.41 ± 0.09^{e,f,g,h}
80	20	0.03 ± 0.02^{d,e,f}	0.02 ± 0.01^{e,f,*}	0.11 ± 0.06^{e,f}	0.31 ± 0.14^{f,g,h}
60	80	0.04 ± 0.01^{b,c,d,*}	0.08 ± 0.02^{b,c,*}	0.24 ± 0.0^{c,d,*}	0.72 ± 0.17^{c,d,e,*}
60	60	0.03 ± 0.00^{c,d,e,*}	0.04 ± 0.01^{c,d,e,f,*}	0.17 ± 0.03^{e,d,*}	0.53 ± 0.07^{d,e,f,g,h}
60	40	0.02 ± 0.00^{e,f}	0.02 ± 0.00^{f,*}	0.10 ± 0.03^{e,f}	0.33 ± 0.08^{e,f,g,h}
60	20	0.02 ± 0.00^{e,f}	0.02 ± 0.00^{f,*}	0.10 ± 0.01^{e,f}	0.30 ± 0.02^{f,g,h}
40	80	0.03 ± 0.00^{d,e}	0.04 ± 0.01^{c,d,e,f,*}	0.16 ± 0.02^{e,d,*}	0.60 ± 0.06^{c,d,e,f,g}
40	60	0.03 ± 0.01^{d,e,f}	0.03 ± 0.01^{d,e,f*}	0.12 ± 0.06^{e,f}	0.60 ± 0.15^{c,d,e,f,g}
40	40	0.03 ± 0.01^{c,d,e,*}	0.02 ± 0.00^{e,f,*}	0.17 ± 0.05^{d,e,*}	0.68 ± 0.17^{c,d,e,f}
40	20	0.01 ± 0.00^{f}	0.01 ± 0.00^{f,*}	0.04 ± 0.01^{f}	0.18 ± 0.02^{h,*}
Control		0.02 ± 0.01	0.48 ± 0.17	0.07 ± 0.02	0.45 ± 0.18

Power (%)	Time (s)	C18:1	C18:2	C18:3	C20:0
100	80	6.13 ± 2.51^{a,*}	1.70 ± 0.66^{a,*}	0.05 ± 0.02^{a*}	0.01 ± 0.00*
100	60	3.71 ± 0.77^{b,c,*}	0.82 ± 0.15^{b,c,d,*}	0.02 ± 0.01^{c,d,e}	0.00 ± 0.00*
100	40	2.04 ± 0.40^{c,d,e,*}	0.41 ± 0.16^{e,f,g}	0.01 ± 0.00^{e,f,g,*}	0.00 ± 0.00*
100	20	1.32 ± 0.03^{d,e}	0.24 ± 0.03^{f,g}	0.01 ± 0.03^{f,g,*}	0.00 ± 0.00*
80	80	4.86 ± 2.08^{a,b,*}	1.20 ± 0.43^{b,*}	0.04 ± 0.01^{a,b,*}	0.00 ± 0.00*
80	60	3.70 ± 1.17^{b,c,*}	0.99 ± 0.13^{b,c,*}	0.03 ± 0.01^{b,c,*}	0.00 ± 0.00*
80	40	1.71 ± 0.15^{d,e}	0.47 ± 0.24^{d,e,f,g,*}	0.02 ± 0.01^{d,e,f,g}	0.00 ± 0.00*
80	20	1.71 ± 0.66^{d,e}	0.28 ± 0.12^{f,g}	0.01 ± 0.01^{f,g,*}	0.00 ± 0.00*
60	80	2.63 ± 0.66^{c,d,*}	0.76 ± 0.20^{c,d,e,*}	0.03 ± 0.01^{b,c,d}	0.00 ± 0.00*
60	60	2.43 ± 0.31^{c,d,e,*}	0.59 ± 0.13^{c,d,e,f,*}	0.02 ± 0.01^{c,d,e,f}	0.00 ± 0.00*
60	40	1.72 ± 0.35^{d,e}	0.33 ± 0.06^{f,g}	0.01 ± 0.00^{e,f,g,*}	0.00 ± 0.00*
60	20	1.66 ± 0.16^{d,e}	0.29 ± 0.03^{f,g}	0.01 ± 0.00^{e,f,g,*}	0.00 ± 0.00*
40	80	2.59 ± 0.19^{c,d,*}	0.57 ± 0.12^{d,e,f,*}	0.02 ± 0.00^{d,e,f,g}	0.00 ± 0.00*
40	60	2.54 ± 0.74^{c,d,*}	0.46 ± 0.11^{d,e,f,g,*}	0.01 ± 0.00^{d,e,f,g}	0.00 ± 0.00*
40	40	2.90 ± 0.70^{c,d,*}	0.54 ± 0.15^{d,e,f,g,*}	0.02 ± 0.01^{d,e,f,g}	0.00 ± 0.00*
40	20	0.83 ± 0.14^{e}	0.15 ± 0.03^{g}	0.00 ± 0.00^{g,*}	0.00 ± 0.00*
Control		0.81 ± 0.19	0.19 ± 0.08	0.02 ± 0.01	0.02 ± 0.01

Power (%)	Time (s)	C20:1	C20:2	C20:4	C22:6	Sat	Unsat
100	80	0.22 ± 0.08^{a,*}	0.17 ± 0.09^{a,*}	0.01 ± 0.00*	0.96 ± 0.35^{a,*}	2.08 ± 0.81^{a,*}	9.68 ± 3.68^{a,*}
100	60	0.10 ± 0.02^{c,d,*}	0.09 ± 0.04^{c,d,e,*}	0.00 ± 0.00*	0.54 ± 0.11^{c,d,*}	1.03 ± 0.18^{c,d}	5.53 ± 1.04^{b,c,*}
100	40	0.04 ± 0.01^{e,f,g}	0.04 ± 0.01^{f}	0.00 ± 0.00*	0.26 ± 0.10^{d,e}	0.49 ± 0.11^{e,f,g,h}	2.93 ± 0.69^{d,e,f}
100	20	0.02 ± 0.02^{g,*}	0.02 ± 0.02^{f}	0.00 ± 0.00*	0.18 ± 0.19^{e,f}	0.29 ± 0.28^{g,h}	1.87 ± 1.69^{e,f}
80	80	0.15 ± 0.04^{b,*}	0.15 ± 0.04^{a,b,*}	0.00 ± 0.00*	0.83 ± 0.19^{a,b,*}	1.48 ± 0.44^{b,*}	7.58 ± 2.75^{b,a,*}
80	60	0.11 ± 0.01^{b,c,*}	0.11 ± 0.02^{b,c,*}	0.00 ± 0.00*	0.67 ± 0.09^{b,c,*}	1.10 ± 0.17^{b,c}	5.90 ± 1.34^{b,c,*}
80	40	0.40 ± 0.02^{e,f,g}	0.06 ± 0.03^{d,e,f}	0.00 ± 0.00*	0.31 ± 0.14^{d,e,f}	0.47 ± 0.11^{e,f,g,h,*}	2.76 ± 0.58^{d,e,f}
80	20	0.02 ± 0.01^{f,g,*}	0.03 ± 0.02^{f}	0.00 ± 0.00*	0.21 ± 0.09^{e,f}	0.36 ± 0.16^{f,g,h,*}	2.38 ± 1.04^{d,e,f}
60	80	0.08 ± 0.02^{c,d,e}	0.10 ± 0.03^{c,d,*}	0.00 ± 0.00*	0.52 ± 0.12^{c,d,*}	0.84 ± 0.18^{c,d,e}	4.35 ± 1.04^{c,d,*}
60	60	0.05 ± 0.01^{e,f,g}	0.05 ± 0.02^{d,e,f}	0.00 ± 0.00*	0.38 ± 0.08^{d,e}	0.60 ± 0.08^{d,e,f,g,h,*}	3.69 ± 0.36^{c,d,e,*}
60	40	0.03 ± 0.01^{f,g,*}	0.03 ± 0.01^{f}	0.00 ± 0.00*	0.24 ± 0.07^{e,f}	0.38 ± 0.09^{f,g,h,*}	2.46 ± 0.53^{d,e,f}
60	20	0.02 ± 0.00^{f,g,*}	0.03 ± 0.00^{f}	0.00 ± 0.00*	0.23 ± 0.02^{e,f}	0.35 ± 0.02^{f,g,h,*}	2.35 ± 0.22^{d,e,f}
40	80	0.06 ± 0.01^{d,e,f,g}	0.05 ± 0.01^{d,e,f}	0.00 ± 0.00*	0.34 ± 0.06^{d,e}	0.66 ± 0.06^{c,d,e,f,g}	3.78 ± 0.34^{c,d,e,*}
40	60	0.05 ± 0.01^{e,f,g}	0.04 ± 0.01^{f,e}	0.00 ± 0.00*	0.33 ± 0.10^{d,e}	0.66 ± 0.16^{d,e,f,g}	3.56 ± 0.95^{c,d,e,*}
40	40	0.06 ± 0.02^{e,d,f}	0.05 ± 0.01^{d,e,f}	0.00 ± 0.00*	0.40 ± 0.13^{d,e}	0.74 ± 0.18^{c,d,e,f}	4.14 ± 1.06^{c,d,e,*}
40	20	0.02 ± 0.00^{g,*}	0.01 ± 0.00^{f,*}	0.00 ± 0.00*	0.09 ± 0.01^{f,*}	0.20 ± 0.02^{h,*}	1.15 ± 0.16^{f}
Control		0.06 ± 0.02	0.05 ± 0.02	0.43 ± 0.16	0.27 ± 0.06	0.96 ± 0.36	1.90 ± 0.52

[a]For each FA in each column, means (excluding that of the control sample) with the same superscript letter(s) are not significantly different ($P > 0.05$). Pairwise comparison between the microwave-heated and conventionally treated (control) samples was significant ($P < 0.05$) when indicated by an asterisk (*).

This research indicated that preparing FAME from microwave-heated samples without solvent extraction is feasible and practical. In addition to speed and ease of use, the advantages of microwave-assisted FA analysis were no solvent consumption during heating, low energy cost, and requirement for less labor. The total FAME preparation process takes a few minutes compared to a few hours when done using the conventional fat extraction methods, particularly for samples containing high moisture content. This technique also generates less chemical waste. Furthermore, the microwave-assisted FA

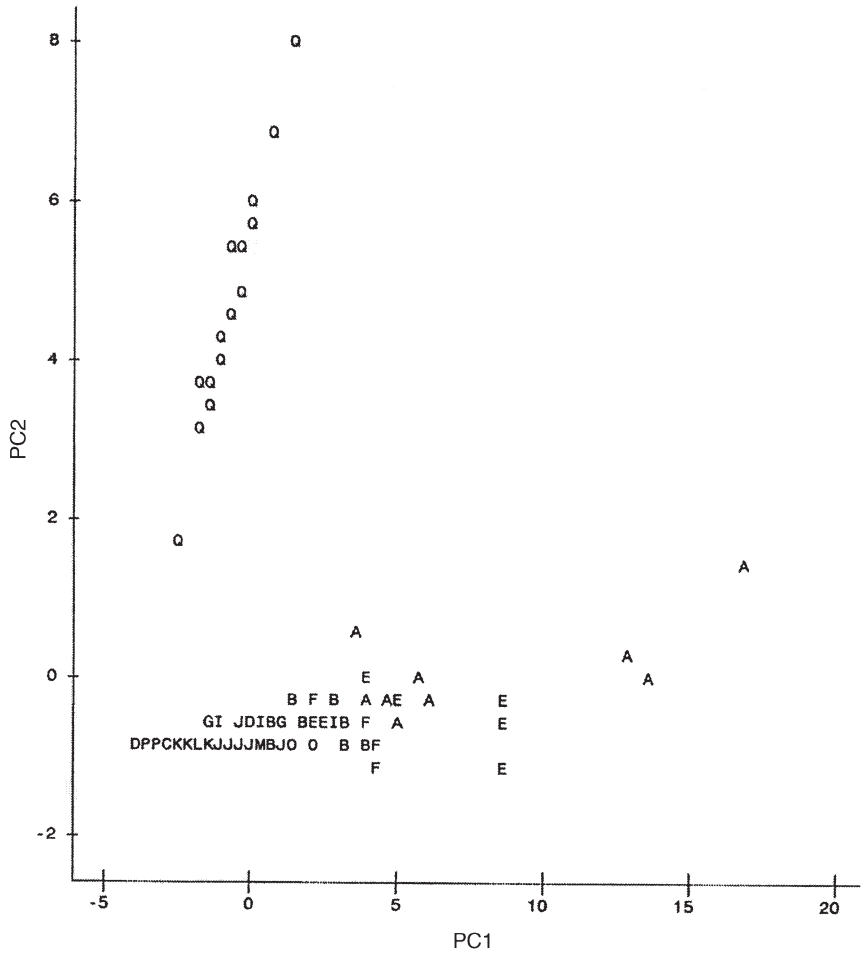

FIG. 1. A principal component mapping plot (PC1 and PC2) for all 16 microwave-heated samples and control samples. A = 100% power for 80 s; B = 100% power for 60 s; C = 100% power for 40 s; D = 100% power for 20 s; E = 80% power for 80 s; F = 80% power for 60 s; G = 80% power for 40 s; H = 80% power for 20 s; I = 60% power for 80 s; J = 60% power for 60 s; K = 60% power for 40 s; L = 60% power for 20 s; M = 40% power for 80 s; N = 40% power for 60 s; O = 40% power for 40 s; P= 40% power for 20 s; Q = control (the conventionally treated sample).

analysis is appropriate for samples containing a high moisture content that may interfere with the lipid extraction process.

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REFERENCES

1. Innis, S.M., Essential Fatty Acids in Growth and Development, *Progr. Lipid Res. 30*:39–103 (1991).
2. Bligh, E.G., and W.J. Dyer, A Rapid Method of Total Lipid Extraction and Purification, *Can. J. Biochem. Physiol. 37*: 911–917 (1959).
3. Ichihara, K., A. Shibahara, K. Yamamoto, and T. Nakayama, An Improved Method for Rapid Analysis of the Fatty Acids of Glycerolipids, *Lipids 31*:535–539 (1996).
4. Shimasaki, H., F.C. Phillips, and O.S. Privett, Direct Transesterification of Lipids in Mammalian Tissue for Fatty Acid Analysis *via* Dehydration with 2,2′-Dimethoxypropane, *J. Lipid Res. 18*:540–543 (1977).
5. Lepage, G., and C.C. Roy, Improved Recovery of Fatty Acid Through Direct Transesterification Without Prior Extraction or Purification, *Ibid. 25*:1391–1396 (1984).
6. Lepage, G., and C.C. Roy, Direct Transesterification of All Classes of Lipids in a One-Step Reaction, *Ibid. 27*:114–120 (1986).
7. Lepage, G., and C.C. Roy, Specific Methylation of Plasma Nonesterified Fatty Acids in a One-Step Reaction, *Ibid. 29*:227–235 (1988).
8. Sukhija, P.S., and D.L. Palmquist, Rapid Method for Determination of Total Fatty Acid Content and Composition of Feedstuffs and Feces, *J. Agric. Food Chem. 36*:1202–1206 (1988).
9. Ohta, A., M.C. Mayo, N. Kramer, and W.E.M. Lands, Rapid Analysis of Fatty Acids in Plasma Lipids, *Lipids. 25*:742–747 (1990).

10. Folch, J., M. Lees, and G.H. Sloane Stanley, A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues, *J. Biol. Chem. 226*:497–509 (1957).
11. Pare, J., R. Jocelyn, G. Matni, J.M.R. Belanger, K. Li, C. Rule, B. Thibert, V. Yaylayan, Z. Liu, P. Jacquault, and D. Mathe, Use of the Microwave-Assisted Process in Extraction of Fat from Meat, Dairy, and Egg Products Under Atmospheric Pressure Conditions, the 5th Symposium of the AOAC International Europe Section, *J. Assoc. Off. Anal. Chem. 80*:928–933 (1997).
12. Leray, C., T. Grcic, G. Gutbier, and M. Bnouham, Microwave Oven Extraction Procedure for Lipid Analysis in Biological Samples, *Analusis 23*:65–67 (1995).
13. Batista, A., W. Vetter, and B. Luckas, Use of Focused Open Vessel Microwave-Assisted Extraction as Prelude for the Determination of the Fatty Acid Profile of Fish—A Comparison with Results Obtained After Liquid–Liquid Extraction According to Bligh and Dyer, *Eur. Food Res. Technol. 212*:377–384 (2001).
14. AOAC, *Official Methods of Analysis of the Association of Official Analytical Chemists*, 14th edn., Arlington, VA, 1990.
15. Statistical Analysis System, *SAS User's Guide: Statistics*, SAS Institute, Cary, NC, 2001.
16. Huberty, C.J., *Applied Discriminant Analysis*, John Wiley & Sons, New York, 1994.
17. Varnam, A.H., and J.P. Sutherland, *Meat and Meat Products, Technology, Chemistry and Microbiology*, Vol. 3, Food Product Series, Chapman & Hall, London, 1995.

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