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Evaluation of an automated hydrolysis and extraction method for quantification of total fat, lipid classes and *trans* fat in cereal products

Jaclyn E. Robinson [a], Rakesh Singh [a], Sandra E. Kays [b,*]

[a] *Department of Food Science and Technology, The University of Georgia, Athens, GA 30602-7610, United States*
[b] *Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 5677, Athens, GA 30604-5677, United States*

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Abstract

The utility of an automated acid hydrolysis–extraction (AHE) system was evaluated for extraction of fat for the quantification of total, saturated, polyunsaturated, monounsaturated, and *trans* fat in cereal products. Oil extracted by the AHE system was assessed for total fat gravimetrically and by capillary gas chromatography (GC) for total fat, lipid classes, and *trans* fat. All AHE system results were compared with parallel determinations using the standard AOAC Method 996.01 or a modified version for *trans* fatty acids. For gravimetric and gas chromatographic evaluations, the AHE system results were equivalent to those using the standard AOAC Method ($\alpha = 0.01$). Thus, the AHE oil extraction system can be used for measurement of total, saturated, polyunsaturated, monounsaturated, and *trans* fat with sufficient accuracy for nutrition labeling purposes, while having the advantages of reduced use of solvent, operator exposure to solvent, operator time, and potential for operator error.
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Keywords: Fat extraction; Acid hydrolysis; Total fat; Saturated fat; Polyunsaturated fat; Monounsaturated fat; *Trans* fat

1. Introduction

To obtain accurate information on the fat content of various foods for manufacturers, consumers, and government agencies responsible for monitoring nutrition labeling information (Code of Federal Regulations, 2006; Federal Register, 2003), accurate and repeatable methods are required for the analysis of total fat and lipid classes. AOAC 996.01 is a universally accepted method for the determination of total, saturated, polyunsaturated, and monounsaturated fat in cereal-based products (AOAC, 2000a) and has sufficient accuracy and repeatability to satisfy current USA nutrition labeling regulations (Ngeh-Ngwainbi, Lin, & Chandler, 1997; Ratnayake, 2004). Modifications of AOAC 996.01 (AOAC, 2000b) and a similar method AOAC 996.06 can be used for measurement of *trans* fatty acids in cereal products (Mossoba, Kramer, Delmonte, Yurawecz, & Rader, 2003). AOAC 996.01 and its modification are identical up to gas chromatographic analysis and involve hydrolysis of the ground sample, extraction of fat into diethyl and petroleum ether solvents, evaporation of the solvents, methylation of the extracted fat, and quantification of fatty acids by gas chromatography (GC) (AOAC, 2000a). The modification for *trans* fat requires a longer GC column and operation of the GC with temperature programming that optimizes separation of *trans* and *cis* isomers. AOAC 996.01 is more accurate than traditional Soxhlet gravimetric methods for crude fat, in that lipid extraction is more complete and quantification of the extract by capillary GC is specific for fatty acids (Zou, Lusk, Messer, & Lane, 1999).

Although accurate and repeatable, AOAC Method 996.01 and similar methods are laborious procedures,

* Corresponding author. Tel.: +1 706 546 3338; fax: +1 706 546 3607.
E-mail address: sandra.kays@ars.usda.gov (S.E. Kays).

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requiring careful attentiveness throughout the duration of analysis. They are also time-consuming, taking 8 h to perform with additional time for capillary GC and its interpretation. The protocol consumes large volumes of diethyl ether and petroleum ether solvents, which are hazardous, flammable, and require specific disposal.

An automated hydrolysis and extraction (AHE) system that is available commercially, offers an alternative to the manual hydrolysis and extraction required for AOAC 996.01. The method involves a combination of automated acid hydrolysis and rinsing of the sample in a closed system followed by reflux boiling with solvent and automated Soxhlet extraction of the lipid, also in a closed system (Luque de Castro & García-Ayuso, 1998). The percentage of total fat is measured gravimetrically. In addition, the extracted fat can be recovered and total fat and lipid classes measured by capillary GC as in AOAC Method 996.01. Because the AHE system is automated and closed: the operator has less contact with and exposure to solvents and fumes; the operator's time and attention may be directed to other activities during extraction; and the results are less likely to be affected by operator error (Helaleh, Al-Omair, Ahmed, & Gevao, 2005). Furthermore, six samples can be analyzed simultaneously with one unit. Less solvent is consumed per sample using the AHE system and 80% of the solvent can be recovered and reused (Anonymous, 2006). The design of the AHE hydrolyzation unit provides for the rinsing of non-lipid aqueous moieties from the hydrolyzed sample, removing elements that could, otherwise, cause overestimation of gravimetric total lipid. In theory, this should provide for the accurate determination of total fat gravimetrically without use of a gas chromatographic step. Recovery of the lipid, and subsequent saponification and methylation, allow for determination of total, saturated, polyunsaturated, monounsaturated, and *trans* fat by capillary GC. The accuracy of the AHE system for extraction of lipids for the analysis of total fat and lipid components compared to the extraction of lipids by AOAC Method 996.01 has not been reported. Thus, its potential for analysis of lipids for nutrition labeling and monitoring is unknown.

The objective of this study was to evaluate the AHE system for the determination of total fat gravimetrically and for the extraction of fat for the capillary GC determination of total, saturated, polyunsaturated, monounsaturated, and *trans* fat. A diverse range of commercial cereal products with added fat was used for the study, and the results were evaluated against those using AOAC Method 996.01 as the standard.

2. Materials and methods

2.1. Samples and sample preparation

Twelve cereal products with a wide range of grains were purchased from local commercial grocery retailers (Table 1). Based on the Nutrition Facts panel information for each product, total fat content ranged from 4% to 40% and *trans* fat from 0% to 15%. Products also had a wide range in sugar (0–50%), fiber (0–6%), and protein (2–10%) content. To ensure that a variety of cereal products were represented, products were selected from four categories: snacks, cookies and crackers, baking mixes, and breakfast products. A high fat (total fat $\geqslant$25%), medium fat (25% < total fat $\geqslant$ 13%), and low fat (<13% total fat) cereal product was selected for each category, except for the breakfast product category, which contained one medium fat product, and two low fat products. Overall, the products had a wide variety of additives including fruits, nuts, flavors, spices, sweeteners, fats, flavor enhancers, gums, emulsifiers, leavening agents, and preservatives. Frying, baking, extruding, milling, and malting processes were all represented by products included in the study.

Table 1
Cereal products and their composition[a]

Product group	Product	Grains[b]	%[a]				
			Total fat	Carbohydrate	Sugars	Protein	Dietary fiber
Snack products	Corn chips[c]	Corn	37.9	51.7	0.0	6.9	3.4
	Snack mix[c]	Wheat, barley, rye	20.0	66.7	3.3	10.0	3.3
	Pretzels	Wheat, barley	3.6	82.1	10.7	7.1	3.6
Cookies and crackers	Crackers with peanut butter[c]	Wheat, barley	25.6	59.0	10.3	10.3	2.6
	Oatmeal cookies with raisins[c]	Wheat, oats	21.4	64.3	28.6	7.1	3.6
	Chocolate wafer snacks	Wheat	8.7	87.0	39.1	4.3	4.3
Baking mixes	Pie crust mix[c]	Wheat	35.0	65.0	0.0	5.0	0.0
	All-purpose baking mix[c]	Wheat	15.0	65.0	2.5	7.5	0.0
	White cake mix	Wheat	8.1	81.4	48.8	2.3	2.3
Breakfast products	Granola	Oats, wheat	12.5	72.9	25.0	10.4	6.3
	Toaster pastries[c]	Wheat, corn	9.6	71.2	30.8	3.8	1.9
	Corn crunch	Corn, oats	5.5	85.2	44.4	3.7	3.7

[a] % composition is based on nutrition label declarations and serving size.
[b] Grains are listed in order of predominance in the products.
[c] Denotes products used for *trans* fat analysis.

Products were ground using a household coffee grinder manufactured by Kitchen Aid (St. Josephs, MI, USA) to reduce the particle size and obtain a homogeneous sample. The ground products were transferred to polyethylene bags, stored at −20 °C, and analyzed for total, saturated, polyunsaturated, monounsaturated, and *trans* fat within three to four days. It was established previously in a medium fat (10% fat) and a high fat (28% fat) cereal product sample in duplicate that total fat, lipid classes, and *trans* fat were stable over seven days at −20 °C.

2.2. Reagents and standards

Chloroform and methanol were HPLC grade and heptane was capillary GC grade. All other reagents were ACS grade. The Standard Reference Material (SRM) 1846, infant formula, was purchased from the U.S. Department of Commerce National Institute of Standards and Technology (NIST) (Gaithersburg, MD, USA). SRM 1846 contains 27.1 ± 0.59% total fat calculated on an as delivered basis. All AOAC 996.01 and modified AOAC 996.01 analyses were verified using SRM 1846 in parallel with samples.

Standards for GC were as follows. The internal standard for GC analysis was tritridecanoin (C13:0), purchased from Sigma–Aldrich (St. Louis, MO, USA), and was prepared to a concentration of 20 mg/mL in chloroform. KEL-FIM-FAME-5 mix used in AOAC Method 996.01 for the identification and quantification of fatty acids was obtained from Matreya (St. Pleasant Gap, PA, USA) and is a 19 component fatty acid methyl esters (FAME) mixed standard. Supelco 37 Component FAME mix used for identification of fatty acids in the modified AOAC Method 996.01 was purchased from Supelco (Bellafonte, PA, USA) and is a 37 component FAME mixed standard.

2.3. AOAC Method 996.01 for total, saturated, polyunsaturated, and monounsaturated fat

Total, saturated, polyunsaturated, and monounsaturated fat in cereal products and in the standard reference material (SRM 1846) were determined by AOAC Method 996.01 (AOAC, 2000a). Briefly, ground cereal products were weighed in triplicate into Mojonnier tubes using 2 g of sample for products with 13% fat or less (based on nutrition label values). Sample size was reduced for samples with >13% fat. The samples were wet with 2 mL of ethanol. One mL of internal standard (20 mg/mL tritridecanoin, C13:0) was added to each sample, and samples were hydrolyzed with 10 mL of 8 N HCl at 80 ± 2 °C for 40 min. Lipids were extracted from the hydrolyzed sample matrix three times with equal quantities of diethyl ether and petroleum ether. The organic ether phase, containing the extracted fat, was decanted into flasks and the solvents evaporated on a steam bath. The ether extract was methylated by refluxing with 0.5 N NaOH in methanol followed by 14% boron trifluoride in methanol. The FAME obtained were suspended in *n*-heptane and transferred to two vials.

One vial of FAME was immediately analyzed for total fat and fatty acids (AOAC Method 996.01) using a flame ionization detector Agilent Technologies 6890N gas chromatograph fitted with an Agilent Technologies 7683B series injector, Agilent Technologies 7683 series autosampler, a split/splitless injection liner and an Rtx®-2330, 10% cyanopropylphenyl–90% biscyanopropyl polysiloxane capillary column (30 m × 0.25 mm i.d., 0.2 μm film thickness) purchased from Restek Corp. (Bellefonte, PA, USA). Helium was the carrier gas, with a gas flow velocity of 24 cm/s. The split ratio was 50:1. A single injection of 1 μL was made per sample replicate. Injector temperature was 250 °C, and detector temperature was 275 °C. Hydrogen and air flows were set to 34 mL/min and 300 mL/min, respectively. Oven temperature programming consisted of an initial temperature of 120 °C held for 4 min, followed by an increase in temperature of 5 °C/min until 230 °C, with a hold time of 5 min. Helium was the make-up gas with a flow rate of 45 mL/min. FAME were measured against the C13:0 internal standard; the KEL-FIM-FAME-5 mix was run in parallel with the samples and used in the identification and quantification of individual fatty acids. Each fatty acid was converted to its triglyceride equivalent weight and triglycerides summed to obtain total fat. The sum of individual fatty acids was used directly to obtain saturated, polyunsaturated, and monounsaturated fat. Total fat and lipid classes were reported on a dry weight basis. Dry weight was determined on individual samples independently at 105 °C in a forced air oven (AOAC Method 935.29, AOAC, 2000c).

2.4. Modified AOAC Method 996.01 for trans fatty acids

The second vial of FAME in heptane was immediately stored at −20 °C and analyzed by GC within 24 h for *trans* fatty acids using a modification of the GC portion of AOAC Method 996.01. The modification entailed use of an Agilent Technologies 6890N Gas Chromatograph, operating with flame ionization detector and Supelco 2560 fused-silica capillary column (100 m × 0.25 mm i.d., 0.2 μm film thickness) purchased from Supelco (Bellefonte, PA, USA). Helium was the carrier gas, with a gas flow velocity of 18 cm/s. The split ratio was 50:1. A single injection of 1 μL was made per sample replicate. Injector temperature was 200 °C, and detector temperature was 250 °C. Hydrogen and air flows were set to 40 mL/min and 450 mL/min, respectively. Oven temperature programming consisted of an initial temperature of 120 °C held for 5 min, an increase in temperature of 3 °C/min until 240 °C, and a hold time of 20 min at 240 °C. Helium was the make-up gas with a flow rate of 45 mL/min. FAME were measured against the C13:0 internal standard; Supelco 37 component FAME mix was used in the identification and quantification of individual fatty acids. The sum of all fatty acids containing *trans* isomers was used directly to obtain *trans* fat, which was reported on a dry weight basis. The modified AOAC 996.01 Method was used to measure total,

saturated, polyunsaturated, monounsaturated, and *trans* fat for investigation of the stability of ground products and FAME at −20 °C. It was established in a medium fat (10% fat) and a high fat (28% fat) cereal product sample in duplicate that FAME of *trans* fatty acids were stable at −20 °C for seven days.

2.5. Automated hydrolysis and extraction (AHE) method for total, saturated, polyunsaturated, and monounsaturated fat

Ground cereal samples were weighed in triplicate into glass Soxcap capsules (Foss North America, Eden Prairie, MI, USA) and fitted with corresponding disposable polyester filters (Foss North America). The quantity of sample used was as described for AOAC Method 996.01. For samples to be analyzed by capillary GC, 1 mL tritridecanoin (C13:0) internal standard (20 mg/mL) was added. Samples were then hydrolyzed with the Soxcap™ 2047 Hydrolysis Unit (Foss North America) as follows. The glass capsules were loaded into a tray that holds six capsules, the tray lowered into the hydrolysis unit and the samples hydrolyzed for 1 h in boiling 4 N HCl. After hydrolysis, the samples were rinsed with water sufficiently to increase the pH of the rinse water to that of the tap water. The tops of the capsules were fitted with a cellulose thimble (22 mm × 28 mm i.d.; Foss North America), inverted, transferred to freeze-drying jars and freeze-dried to constant weight (20 h) using a Virtis 25EL Freezemobile (Gardiner, NY, USA). The capsules with cellulose thimbles intact and containing the dried, hydrolyzed samples, were fitted with metal adaptors, and loaded into a Soxtec® 2050 Auto Fat Extraction System (Foss North America) for solvent extraction. Briefly, aluminum cups that had been dried in a vacuum oven (30 °C) and weighed were placed beneath each extraction thimble in the extraction unit, and petroleum ether, as solvent, was added to each of six extraction chambers. The automated extraction programming consisted of the sample being in contact with boiling petroleum ether for 40 min, a sample rinsing stage of 40 min, a recovery stage of 10 min, and an evaporation/drying stage of 5 min. After extraction, to determine total fat gravimetrically (AHE-G method), excess petroleum ether was evaporated from the aluminum cups in a vacuum oven (30 °C), the cups weighed, and % total fat calculated.

To determine total, saturated, polyunsaturated, and monounsaturated fat (AHE-GC method), the lipids from the aluminum cups were transferred to a boiling flask by rinsing multiple times with petroleum ether. Following this, the solvent was evaporated, lipids methylated, and then analyzed by capillary GC-FID, as per AOAC 996.01. *Trans* fat was determined, using the capillary GC conditions described for the modified AOAC 996.01 Method.

2.6. Statistical analysis

Values for total, saturated, polyunsaturated, monounsaturated, and *trans* fat were expressed as means ± standard deviations of three replicates. Significant differences between methods were tested using the paired *t*-test ($\alpha = 0.01$) within parameters.

3. Results and discussion

Results showed that there was no significant difference between the AOAC-GC method and the AHE-G method for determination of total fat in cereal products (Table 2; paired *t*-test, $\alpha = 0.01$). Thus, with the AHE system total fat can be measured gravimetrically, eliminating the need for the labor intensive manual extraction of lipid required for the AOAC Method and eliminating the need for GC. The AHE gravimetric method is a significant improvement over long established gravimetric methods. Traditional Soxhlet crude fat gravimetric measurements, lacking hydrolysis, extract bound lipids incompletely causing the underestimation of total fat (Ali, Angyal, Weaver, & Rader, 1997; Ranhotra, Gelroth, & Vetter, 1996; Zou et al., 1999). Alternatively, gravimetric measurements of fat that include a hydrolysis step often include some non-lipid moieties that are freed during hydrolysis and extracted along with the lipid, causing the overestimation of total fat in foods (Rader et al., 1995; Ranhotra et al., 1996; Zou et al., 1999). The AHE gravimetric method has an advantage over these methods in that it includes hydrolysis and rinsing of the sample to remove water soluble, non-lipid substances after acid hydrolysis. The removal of these non-lipid materials from the sample allows for a gravimetric measurement that is a more accurate estimation of total fat. Because of the automatic nature of the AHE system and the fact that it is a closed system, the

Table 2
Measurement of total fat (%) in cereal products by the standard GC method and by an automated gravimetric method[a]

Product group	Product	AOAC-GC[b]	AHE-G[c]
Snack products	Corn chips	29.59 ± 0.21	30.28 ± 0.19
	Snack mix	20.48 ± 0.16	20.32 ± 0.15
	Pretzels	4.82 ± 0.05	4.62 ± 0.08
Cookies and crackers	Crackers with peanut butter	24.75 ± 0.26	25.00 ± 0.37
	Oatmeal cookies with raisins	22.12 ± 0.94	21.80 ± 0.70
	Chocolate wafer snacks	10.24 ± 0.11	9.90 ± 0.22
Baking mixes	Pie crust mix	45.35 ± 0.89	42.89 ± 0.55
	All-purpose baking mix	16.36 ± 0.20	16.70 ± 0.17
	White cake mix	8.56 ± 0.14	8.52 ± 0.14
Breakfast products	Granola	13.26 ± 0.14	12.64 ± 0.26
	Toaster pastries	10.49 ± 0.13	10.53 ± 0.12
	Corn crunch	6.20 ± 0.02	6.02 ± 0.06

[a] Values are means ± SD, for triplicate analyses performed on the same day. No significant difference between methods, paired *t*-test, $\alpha = 0.01$.
[b] AOAC-GC, oil extracted from products by AOAC 996.01 prior to GC analysis.
[c] AHE-G, oil extracted by automated hydrolysis and extraction system prior to gravimetric analysis.

analysis for total fat can be accomplished with less solvent than the AOAC Method, with minimal operator exposure to solvent and methylation reagents, with reduced potential for operator error and without a GC step. However, determinations for lipid classes involve fatty acid analysis and, thus, require GC.

Differences between the AHE-G and AOAC-GC methods for total fat ranged from −0.69% to 0.62% with the exception of the pie crust mix where the difference was 2.46% with large variation in the replicates. Although the difference between methods for the pie crust mix was large it was not significant (Student's *t*-test, $\alpha = 0.01$, $n = 3$ repli-

Table 3
Determination of total, saturated, polyunsaturated, and monounsaturated fat (%) extracted from cereal products by the standard method and an automated method[a]

Component	Product group	Product	AOAC-GC[b]	AHE-GC[c]
Total fat	Snack products	Corn chips	29.59 ± 0.21	29.81 ± 0.47
		Snack mix	20.48 ± 0.16	20.21 ± 0.26
		Pretzels	4.82 ± 0.05	4.66 ± 0.11
	Cookies and crackers	Crackers with peanut butter	24.75 ± 0.26	24.15 ± 0.13
		Oatmeal cookies with raisins	22.12 ± 0.94	21.36 ± 0.05
		Chocolate wafer snacks	10.24 ± 0.11	9.66 ± 0.02
	Baking mixes	Pie crust mix	45.35 ± 0.89	45.45 ± 0.57
		All-purpose baking mix	16.36 ± 0.20	15.85 ± 0.26
		White cake mix	8.56 ± 0.14	8.42 ± 0.09
	Breakfast products	Granola	13.26 ± 0.14	13.03 ± 0.06
		Toaster pastries	10.49 ± 0.13	10.60 ± 0.15
		Corn crunch	6.20 ± 0.02	5.91 ± 0.08
Saturated fat	Snack products	Corn chips	4.78 ± 0.11	5.02 ± 0.14
		Snack mix	3.74 ± 0.02	3.84 ± 0.07
		Pretzels	0.86 ± 0.03	0.85 ± 0.12
	Cookies and crackers	Crackers with peanut butter	4.46 ± 0.05	4.29 ± 0.03
		Oatmeal cookies with raisins	4.64 ± 0.20	4.49 ± 0.02
		Chocolate wafer snacks	1.25 ± 0.08	1.12 ± 0.002
	Baking mixes	Pie crust mix	11.03 ± 0.33	11.32 ± 0.10
		All-purpose baking mix	4.03 ± 0.05	3.84 ± 0.10
		White cake mix	3.36 ± 0.08	3.23 ± 0.10
	Breakfast products	Granola	7.30 ± 0.03	7.12 ± 0.04
		Toaster pastries	2.34 ± 0.02	2.31 ± 0.03
		Corn crunch	3.93 ± 0.03	3.84 ± 0.06
Polyunsaturated fat	Snack products	Corn chips	3.09 ± 0.01	3.06 ± 0.04
		Snack mix	4.93 ± 0.03	4.83 ± 0.08
		Pretzels	2.63 ± 0.05	2.51 ± 0.07
	Cookies and crackers	Crackers with peanut butter	5.05 ± 0.05	4.91 ± 0.01
		Oatmeal cookies with raisins	1.91 ± 0.07	1.83 ± 0.01
		Chocolate wafer snacks	3.00 ± 0.02	2.84 ± 0.01
	Baking mixes	Pie crust mix	5.27 ± 0.13	5.44 ± 0.08
		All-purpose baking mix	2.56 ± 0.27	2.31 ± 0.02
		White cake mix	3.22 ± 0.04	3.22 ± 0.02
	Breakfast products	Granola	2.07 ± 0.03	2.05 ± 0.01
		Toaster pastries	1.11 ± 0.02	1.09 ± 0.02
		Corn crunch	1.07 ± 0.01	0.95 ± 0.01
Monounsaturated fat	Snack products	Corn chips	20.46 ± 0.14	20.43 ± 0.31
		Snack mix	10.91 ± 0.11	10.65 ± 0.10
		Pretzels	1.11 ± 0.02	1.09 ± 0.03
	Cookies and crackers	Crackers with peanut butter	14.15 ± 0.15	13.89 ± 0.10
		Oatmeal cookies with raisins	14.59 ± 0.63	14.10 ± 0.04
		Chocolate wafer snacks	5.54 ± 0.07	5.27 ± 0.01
	Baking mixes	Pie crust mix	25.97 ± 0.73	26.70 ± 0.38
		All-purpose baking mix	9.22 ± 0.12	9.01 ± 0.13
		White cake mix	1.59 ± 0.02	1.59 ± 0.02
	Breakfast products	Granola	3.20 ± 0.08	3.19 ± 0.02
		Toaster pastries	6.58 ± 0.12	6.74 ± 0.10
		Corn crunch	0.87 ± 0.01	0.81 ± 0.004

[a] Values are means ± SD, for triplicate analyses performed on the same day. No significant difference between methods within a parameter, paired *t*-test, $\alpha = 0.01$.
[b] AOAC-GC, oil extracted from products by AOAC 996.01 prior to GC analysis.
[c] AHE-GC, oil extracted by automated hydrolysis and extraction system prior to GC analysis.

cates). The sample was observed to be inhomogeneous in that there were relatively large pieces of solid fat in the flour mixture, which presented problems with grinding, mixing, and sampling. The poor homogeneity of the sample contributed significantly to the large variation in the triplicate gravimetric determinations for total fat. Relatively large standard deviations were observed for this sample for total fat and monounsaturated fatty acids measured by the AHE-GC and AOAC-GC methods. In the future such problem samples could be handled by increased replication.

Total fat is defined by the Nutrition Labeling and Education Act (Code of Federal Regulations, 2006) as "total lipid fatty acids...expressed as triglycerides" and this definition is met by AOAC Method 996.01. It is not known to what extent the mono-, di- and tri-glycerides in cereal product samples are hydrolyzed by the 4 N HCl in the AHE-G method, thus, to use the AHE-G method for determination of total fat for nutrition labeling purposes an assumption is needed that the lipid is present predominantly as glycerides. The extent of breakdown of the mono-, di- and tri-glycerides during hydrolysis in 4 N HCl could depend on the sample composition and be determined by future research.

There was no significant difference between the AOAC-GC method and AHE-GC method for determination of total, saturated, polyunsaturated, and monounsaturated fat (Table 3; $P > 0.01$). The modified AOAC 996.01 and AHE-GC results for *trans* fat were also equivalent, supporting the conclusion that the AHE method of lipid extraction performs comparably to the standard AOAC Method (Table 4; $P > 0.01$). Although the GC part of the analysis is identical for both AHE-GC and AOAC-GC methods, the AHE system of lipid extraction has the advantages of operator safety because of the closed system and reduced exposure to solvents, and the advantages of lower labor intensity and reduced solvent use with the ability to regenerate the solvent.

The difference between the AHE-GC and AOAC-GC methods for total fat in individual samples is small and ranges from −0.22% to 0.66%. The standard error of the laboratory (SEL) or pooled standard deviation of the repeatability (ASTM, 1995) of the AOAC Method for total fat in previous work in our laboratory is 0.33% (Vines, Kays, & Koehler, 2005) thus the differences are within or very close to the expected error of the laboratory. The SEL for saturated fat is 0.25%, for polyunsaturated fat is 0.14%, for monounsaturated fat is 0.33% and for *trans* fat is 0.19% (Kays, unpublished data). Thus, for all the parameters the differences between the values for each method within samples can be considered small and, as for total fat measured by the AHE-GC method, within or close to the accuracy usually encountered for AOAC 996.01 and modified AOAC 996.01 in our laboratory.

Table 4
Determination of *trans* fat (%) in oil extracted from cereal products by the standard method and an automated method[a]

Product group	Product	Lipid extraction method	
		AOAC-modified GC[b]	AHE-modified GC[c]
Snack products	Corn chips	12.56 ± 0.08	12.56 ± 0.22
	Snack mix	5.15 ± 0.08	5.00 ± 0.17
Cookies and crackers	Crackers with peanut butter	2.87 ± 0.04	2.82 ± 0.04
	Oatmeal cookies with raisins	8.18 ± 0.35	7.97 ± 0.11
Baking mixes	Pie crust mix	15.47 ± 0.33	15.58 ± 0.14
	All-purpose baking mix	4.41 ± 0.09	4.85 ± 0.06
Breakfast products	Toaster pastries	3.12 ± 0.06	3.18 ± 0.07

[a] Values are means ± SD, for triplicate analyses performed on the same day. No significant difference between methods, paired *t*-test, $\alpha = 0.01$.
[b] AOAC-modified GC, oil extracted from products by AOAC 996.01 prior to GC analysis optimized for *trans* fat.
[c] AHE-GC-modified GC, oil extracted by automated hydrolysis and extraction system prior to GC analysis optimized for *trans* fat.

4. Conclusions

An automated AHE lipid extraction system has been demonstrated to allow determination of total fat in cereal products gravimetrically and total, saturated, polyunsaturated, monounsaturated, and *trans* fatty acids by GC with comparable accuracy to AOAC Method 996.01. The AHE method of lipid extraction has distinct advantages over AOAC 996.01 due to its increased safety for the operator, the closed automatic system, the decreased amount of solvent required, and the decreased potential for operator error. For the determination of total fat, the AHE gravimetric method gives equivalent results to AOAC 996.01, thus, enabling the determination of total fat with the same advantages as the AHE-GC method but having the added advantage of eliminating the need for gas chromatography.

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