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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

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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,

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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.