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Comparison of fat determination methods depending on fat definition in bakery products

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ABSTRACT

This study aimed to evaluate fat determination depending on fat definition. Three methods were used for the determination of fat classes and content in bread products: the Folch method, the automated Soxhlet method, and the AOAC 996.06 method. The results using these methods were compared. Fat (crude) extracted by the Folch and Soxhlet methods was gravimetrically determined and assessed by fat classes using capillary gas chromatography. In most samples, fat (total) content determined by the AOAC 996.06 method was lower than the fat (crude) content determined by the Folch or automated Soxhlet methods. In addition, the contents of saturated, monounsaturated, polyunsaturated and *trans* fat determined by the AOAC 996.06 method were lower than those obtained by the Folch and automated Soxhlet methods. The analytical fat determination method in food depends on how fat is defined, which produced difference in fat content and content of fat classes.

1. Introduction

Nutritional labeling of food impacts not only on consumers and the food industry, but on international trade as well. There is some variation among different countries in the criteria for nutrition facts labeling. In general, calories, carbohydrates, protein, fat and sodium are listed on nutrition facts labels. In the recent trend, food labels also include sugars, saturated fat, *trans* fat, and cholesterol. Here, attention needs to be paid to fat. Nutritional facts labeling initially listed only the fat content, but now, different types of fats are listed on the label. This is because the high consumption of saturated fatty acids and cholesterol is mainly responsible for hypercholesterolemia, which is in turn responsible for the increase in cardiovascular morbidity and mortality of ischemic origin (Neaton & Wentworth, 1992). Consumption of *trans* fatty acid may increase the risk of coronary heart disease (Ascherio, Katan, Zock, Stampfer, & Willett, 1999; Vijver et al., 2000). Therefore, FDA requires the declaration of the amount of *trans* fatty acid present in foods, including dietary supplements, on the nutrition label (FDA, 2003). This rule took effect on January 2006, and a sharper decline in *trans* fat intake was expected, due to both the reformulation of products and increased consumer awareness (Craig-Schmidt, 2006).

There are significant differences in nutritional facts labels among countries. Because quantitative methods to measure nutrients are differently developed for each country. Some countries (Korea, Japan, and Europe, etc) list crude fat content in the 'Fat' section on food labels (EEC 1990; KFDA 2009; Phamaceutical Society of Japan, 2010), whereas other countries (United States, Canada, and Australia etc.) state total fat on a food label (CFIA, 2011; FSANZ, 2013; Hawkes, 2004). However, 'fat' listed on a food label is not the same as 'total fat'. The definition of fat used in Europe, Korea, and Japan is that of crude fat, so fat stated on a food label is crude fat (interpreted to be food fat). Crude fat is a term used to refer to the crude mixture of fat-soluble material present in foods. The crude fat content of food has traditionally been determined by methodologies that involve extraction with organic solvents, drying of the extract, and a gravimetric determination of fat. These classic methods include the Folch method, the Soxhlet method, as well as the Weibull-Berntrop, and the Rose-Gottlieb methods. The definition of 'total fat', as established by the US Food and Drug Determination (FDA) in 1990 through the Nutritional Labeling and Education Act (NLEA), is 'the sum of all fatty acids obtained in a lipid extract, expressed as triglycerides' (Federal Register, 1993), this change in definition for nutritional labeling purposes requires a methodology change, from extraction and gravimetric analysis to

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GC analysis of fatty acid methyl esters (FAMEs). However, in Hong Kong and Brazil, the definition of total fat corresponds to that of crude fat, so gravimetric methods are accepted to determine total fat in those nations (Brazil, 2003; CFS, 2011).

The Folch (Folch, Lees, & Sloane Stanley, 1957) and Bligh-Dyer (Bligh & Dyer, 1959) methods are considered the classical and most reliable means for quantitatively extracting lipids from various types of animal tissues and bakery products. Due to its mild working conditions (neither high temperature nor pressure required), the Folch method was used as a reference method in studies in relation to the extraction of fats (Pérez-Palacios, Ruiz, Martín, Muriel, & Antequera, 2008; Ruiz-Miménez, Priego-Capote, & Luque de Castro, 2004).

Lipid content has been estimated using Soxhlet, which is the official recommended method. However, the relatively long extraction time (16–24 h) and the high temperatures needed for Soxhlet extraction are its main shortcomings. Automated Soxhlet extractors have several advantages, including shorter extraction time, decreased extractant volume, and simultaneous extraction of several samples (Luque de Castro & Priego-Capote, 2010).

AOAC 996.06 is a universally accepted method for determining total, saturated, polyunsaturated, and monounsaturated fats in food, and has sufficient accuracy and repeatability to satisfy current USA nutrition labeling regulations (Ngeh-Ngwainbi, Lin, & Chandler, 1997; Ratnayake, 2004). In this method, the triacylglycerol (fat) and fatty acids are extracted from food, and are then methylated to fatty acid methyl ester using BF_3 in methanol, and FAMEs are quantitatively measured by capillary gas chromatography. Total fat is calculated as the sum of individual fatty acids expressed as triglyceride equivalents (AOAC, 2005a, b).

Fat extractions have been carried out in many studies. However, only few studies (Ali, Angyal, Weaver & Rader, 1997; Rader et al., 1995; Zou, Lusk, Messer, & Lane, 1999) have compared different definitions of extracted fats, which had been performed in the 1990s when the definition of "total fat" was emerging. However, these studies did not include comparison of other fats such as saturated, polyunsaturated, or *trans* fat. Our previous study (Shin et al., 2013) was performed with cookies and biscuits, and this study is a continuation with bread products. These bakery products usually contain butter, cream, shortening and other processed fat, thus determination of fat content and fat classes in these bakery products are important.

Therefore, the aim of this study was to investigate the difference between these parameters by obtaining crude fat content (by gravimetric methods) and total fat content (by GC analysis) present in bread products, and by comparing these results. The crude fat was extracted from bread products using both the Folch method and automated Soxhlet method, and the total fat was extracted using the AOAC 996.06 method. The fatty acid profile of the extracted crude fat was also determined by gas chromatography. The results were compared to saturated, monounsaturated, polyunsaturated and *trans* fat values obtained by the AOAC 996.06 method.

2. Materials and methods

2.1. Samples

Twelve bread products purchased from four bakeries were used. The products were categorized as croissants, pastries, pies, others. Reference values for these bakery products came from the food labels provided by the manufacturers. All food labels contained fat, saturated fat, and *trans* fat content, but only fat content and saturated fat content were used as reference values in this study, because *trans* fat content <0.2 g per serving size can be expressed as 0 (for some products the *trans* fat value on the nutrition facts was 0) in Korea (KFDA, 2011). Products were ground with a grinder (OMNI International, Kennesaw, GA, USA) to achieve a homogenate. Homogenized samples were subjected to fat extraction. The ground samples were then stored at −20 °C.

2.2. Reagents and standards

Chloroform and methanol, iso-octane, and diethyl ether were HPLC grade. All other reagents were ACS grade. The internal standard for GC analysis was triundecanoin (C11:0) purchased from Sigma–Aldrich (St. Louis, MO, USA), and the fatty acid standard was the Supelco 37 Component FAME mix (Bellefonte, PA, USA).

2.3. Folch method

Fifteen grams of sample were mixed with 300 mL chloroform/methanol (2:1, v/v) and were shaken for 20 min, and 60 mL of distilled water was added and shaken for 5 min. After phase separation, the chloroform phase was filtered through sodium sulfate then collected, evaporated in a rotary evaporator at 45 °C, and the residue was further dried in an oven at 104 °C for 1 h and then cooled in a desiccator. The extracted crude fat was recorded gravimetrically. Approximately 25 mg of fat was transferred to a test tube to determine saturated, polyunsaturated, monounsaturated, and *trans* fat. The fat was methylated and then analyzed by capillary GC-flame ionization detection (FID).

2.4. Automated Soxhlet method

One gram of homogenized sample was placed in a thimble (22 mm × 28 mm, i. d.; Foss North America, Eden Prairie, MN, USA), fitted with metal adaptors, which was then loaded into an automated Soxtherm fat extraction system (Gerhardt, Germany). Briefly, beakers that had been dried in an oven at 104 °C and weighed were placed beneath each extraction thimble in the extraction unit, and diethyl ether was added to each of the six extraction chambers. After extraction, the extract was dried in an oven at 104 °C and was then cooled in a desiccator prior to gravimetric fat determination. The beakers were weighed, and the percent crude fat was calculated. To determine saturated, polyunsaturated, monounsaturated fat and *trans* fat content, approximately 25 mg fat from the beakers was transferred to a test tube. The fat was methylated and then analyzed by capillary GC-FID.

2.5. AOAC 996.06 method

A ground and homogenized sample (containing 100–200 mg fat) was accurately weighed and placed in a Mojonnier flask. Approximately 100 mg of pyrogallic acid was added, followed by 2 mL of triundecanoin (5 mg/mL, C11:0) internal standard solution, 2 ml of ethanol, and 10 mL of 8.3 M HCl. The flask was placed in a shaking water bath at 70–80 °C, set at moderate agitation speed, and maintained for 40 min. After cooling to room temperature (20–25 °C), diethyl ether and petroleum ether were added and shaken. The solutions were allowed to stand for more than 1 h until and the top and bottom layers were clearly separated. The ether (top) layer was collected and evaporated. The remaining residue contained the extracted fat. To determine total, saturated, polyunsaturated, monounsaturated fat and *trans* fat content, extracted fat from the beakers was transferred to a test tube. The fat was methylated and then analyzed by capillary GC-FID. Each fatty acids was converted to its triglyceride equivalents summed to obtain total fat. Saturated, polyunsaturated, monounsaturated fat and *trans* fat were calculated as the sum of respective fatty acids.