CHAPTER V

DISCUSSION

This study focused on some food categories which contain high TFA such as partially hydrogenated vegetable oil and bakery products. Two groups of these samples were chosen because major source of TFA found in partially hydrogenated vegetable oil and this kind of vegetable oil is attractive in food industry for enhance the palatability of bakery products. Several methods are available for determining total TFA content such as gas chromatography (GC) or infrared (IR) absorption spectroscopy. In this study, IR procedure based on attenuated total reflection (ATR) fourier transform infrared (FTIR) spectroscopy was selected to determine total TFA content in selected foods. The ATR-FTIR procedure offers several advantages over the GC procedure. The analysis time is shorter than GC (about 5 minute per analysis) and calculates TFA content from a linear regression equation. Small quantities of test samples are required. The need for weighing and quantitatively diluting test samples with solvent is eliminated with ATR-FTIR (Ali et al, 1996).

The contents of TFA in some foods that determined by GC and ATR-FTIR were compared. In study of Ali et al (1996) showed that products with TFA content greater than 5% of total fat, results obtained by ATR-FTIR were higher than those obtained by GC but products with TFA content less than 5% the GC procedure were significantly higher than those obtained by the ATR-FTIR procedure. Fritsche et al (1998) found that the TFA levels determined by ATR-FTIR were usually higher than those determined by GC, partly because the GC method underestimates *trans* C18:1 isomers in favor of *cis* C18:1 isomers. And another reason for such a difference may be due to the fact that the weak abosorbance near 985 and 948 cm^{-1} of conjugated dienes overlap with the 966 cm^{-1}. At lower levels of TFA (< 1%), GC may be more accurate than ATR-FTIR for quantitation of total TFA (Mossoba et al, 1996).

The ATR-FTIR method works well for fats that contain more than 5% *trans* fat (Milosevic et al, 2004). However, to improve sensitivity and accuracy, a new ATR-FTIR procedure that measures the height of the negative second derivative of the *trans* absorption band at 966 cm^{-1} relative to air was recently proposed. The second derivative of an absorbance spectrum enhanced the resolution of IR bands, and

made it possible small shifts in IR band position and the presence of low interferences (Mossoba et al, 2007). Miolsevic et al (2004) compared the GC method with the new ATR-FTIR method that employs the negative second derivative to determination of low levels (0.5 – 5%) of *trans* fats. It was found that negative second derivative ATR-FTIR method is capable to determine low level of TFA content. Therefore, negative second derivative ATR-FTIR method was used for determining total TFA content in this study.

Total fat contents of bakery products were isolated by ultrasonic extraction with *n*-hexane as solvent. The results obtained from some foods were compared with the total fat contents that declared on the labels of those products. In this study, fats that extracted from cracker brand 2 was 119.06% of the labeled amount. Normally, fat content shown on the labels are ± 10% of total fat content of the product. The higher fat content may cause by adding excess fat ingredients into the formula during the process of manufacturing.

Generally margarine contains appropriate ratios of hard vegetable fats derived from coconut, palm kernel, interesterified vegetable oils and/or hydrogenated vegetable oils. During the industrial catalytic hydrogenation process, some *cis* fatty acids are reduced and isomerized to *trans* isomers which exhibit physical properties similar to saturated fats (Kandhro et al, 2008). In Thailand most margarine are derived from soybean and palm oil base. Margarine and shortening are major sources of TFA in the diet (Karabuluta et al, 2006). In this study, average total TFA content of shortening was higher than margarine because shortening consists of 100% fat that obtain from partially hydrogenated vegetable oil, without any flavor but margarine made from partially hydrogenated vegetable oil that often mixed with skimmed milk, salt, antioxidant, emulsifiers and other flavors. Some studies have shown that TFA elevated levels of serum LDL-C and lower HDL-C which may be harmful to consumers's health (Mcdonale and Mossoba, 1998). *Trans* fatty acid levels of margarine and shortening in this study ranged from 1.85-2.22% and 1.85-3.42% of total fat, respectively. These results were lower than those of products available in some other countries such as 0.4-39.4% of total fat in margarines available in Turkey (Karabulut et al, 2006); 18.2-31.8% of total fat in these products from Argentina (Tavella et al., 2000) and 2.0-16.5% of total fat in shortening from Turkey.

The present study demonstrates that TFA content in butter cookie group varied widely (table 9). Each 100 g of butter cookie contain 0.26 - 5.07 g of TFA, depending

on the brand consumed. There are several explanations for the variability of TFA content of foods within a food category. First, the manufacture conditions of hydrogenated oils which can result in variable content of TFA. Such conditions including temperature, hydrogenation pressure, type and amount of catalyst and agitation. Second, food processing may use single hydrogenated or non-hydrogenated fats or oils or many possible combinations of them to achieve the desired final product characteristics. *Trans* fatty acids content in each food may vary because food producers can change type and content of oil depending on current supplier and cost. (Innis et al, 1999).

In this study, NMR was used to confirm the presence of TFA signal that detected by FTIR analysis. For ^{1}H-NMR spectrum, signals of the olefinic protons of elaidic acid (C18:1 *trans*) are slightly downfield (by about 0.03 ppm) compared to oleic acid (C18:1 *cis*). Thus, the shift difference between the peaks of the olefinic proton is appropriate for characterize *cis* and *trans* isomers (Frost and Gunstone, 1975). For this study olefinic protons of *trans* fatty acid of shortening and butter cookie were slightly downfield about 0.05 ppm compared to olefinic protons of *cis* fats (Fig.5). For ^{13}C-NMR spectrum, the olefin carbon atoms and their associated allylic groups have chemical shifts which depend on double bond position and configuration and, for glycerol esters, on whether the unsaturated centre is in the α or β chain (Bus et al, 1977). As a preliminary to the investigation of partially hydrogenated oils a mixture of glycerol trioleate (*cis*) and glycerol trielaidate (*trans*) was examined. Well resolved signals were observed for the olefinic and allylic signals at 130.48, 130.17, 130.00 and 129.70 ppm (C10, C9; *trans* and C10, C9; *cis* respectively) and at 32.64, 32.59, 27.24 and 27.19 ppm (C11, C8; *trans* and C11, C8; *cis* respectively) (Gunstone, 1993). ^{13}C NMR spectrum of this study can distinguished *cis* and *trans* isomers (Fig.5,6). Figure 4 show a peak of *trans* double bond of butter cookie A that is broader than the shortening A. It means that TFA content of butter cookie A is more than that of shortening A which is correlated to the ATR-FTIR analytical result. The TFA content in butter cookie A and shortening A were 5.07 g/ 100 g sample and 2.08 g/ 100 g sample, respectively.

Although, the average TFA content of bakery products and partially hydrogenated vegetable oil of Thailand in this study were less than 2.5 g/100 g food. The consumers should aware and avoid consuming products containing TFA, since intake of TFA have an adverse effect on the development of heart disease that is more

than 10 times greater than those who intake saturated fat (Stender and Dyerberg, 2003). For minimize the risk of coronary heart disease, the Department of Agriculture (USA) made a limited intake of TFA; a key recommendation of the new food-pyramid guidelines, subsequent to the recommendations of the Dietary Guidelines Advisory Committee that the consumption of TFA be kept below 1 percent of total energy intake (Mozaffarian, 2006).

From above results, total fat content did not correlate with total TFA content such as rich butter bun which contained the lowest level of total fat content, but showed the fourth highest TFA content. Food manufacture may use non-hydrogenated oils higher than partially hydrogenated oils to attain the final product. The TFA levels in foods can vary widely depending on the content of partially hydrogenated oils. So, the way to identify total TFA content in foods is to read the TFA content on the nutrition labels of foods or watch out for partially hydrogenated oils in the ingredients list.

Nowadays, the food industries try to reduce TFA in their products and simultaneously preserve the structural and palatable characteristics of the food product. Developed technologies are currently in use by the food and edible oil industries in products with minimal to zero TFA content. First, modification of the chemical hydrogenation process to produce partially hydrogenated fats with low TFA content such as electrocatalytic hydrogenation, precious catalyst hydrogenation and supercritical fluid state hydrogenation (Jang et al, 2005). Second, modification fatty acid composition of oil seed through plant breeding and genetic engineering techniques. And the other methods are interesterification of mixed fats, use of tropical oils (palm kernel oil, and coconut oil) and fractionated tropical oils (Tarrago-trani et al, 2006). Some of these technologies have been available to the food industry for some time; however, an alternate method to hydrogenation alternatives cost more expensive than conventional hydrogenation. Therefore, it could not easily replace the conventional hydrogenated vegetable oils. So consumers should avoid or limit foods that contain partially hydrogenated vegetable oils.

At present in Thailand, there is no legal regulation to enforce the manufacture to label or declare TFA content on the product produced. With such label, consumers can be awared and avoid risk of intake of TFA. Therefore, we strongly urge to health authority to implement such awareness to consumer in different means of regulation.