CHAPTER III

MATERIALS AND METHODS

3.1 Instruments

An ultrasonic bath (Sonorex Digital 10P, Bandelin electric, Germany), vortex (Vortex-genie 2, Scientific industries, USA) and centrifuge (Rotoflx 32, Hettich, Germany) were used for fat extraction. A rotary evaporator (CH-9230, Buchi labortechnik AG, Switzerland) and vacuum desiccator (Heraeus, Germany) were used to evaporate the solvent from sample extracts.

Trans fatty acid was determined by Fourier transform infrared spectrometer (Perkin Elmer Spectrum One FTIR, USA) and Zinc selenide crystal (ZnSe trough plate 45°, Perkin-Elmer, USA) attenuated total reflection infrared cell.

3.2 Reagents

Fatty acid standards; Trielaidin [1,2,3, tris(*trans*-9-octadecanoate)] and Triolein [1,2,3, tris(*cis*-9-octadecanoate)] with purity of ≥ 99% were purchased from Sigma-Aldrich (St. Louis, MO, USA). *n*-Hexane was supplied by Carlo Erba (Rodano, Italy). Petroleum ether was purchased from J. T. Baker Chemicals Co. (Phillipsburg, NJ, USA) and anhydrous Sodium sulfate was obtained from Merck (Darmstadt, Germany). Acetone was purchased from Chromanorm (Paris, France) and Methanol was obtained from BDH (Poole, England).

3.3 Methods

3.3.1 Sampling

Some kinds of bakery products including butter cookie, sandwich chocolate cookie, cracker, brownie, cake cream roll, rich butter bun, crispy pie, croissant and partially hydrogenated vegetable oils; margarine and shortening were selected in order to investigate *trans* fatty acid contents. Three different brands of each type of food were purchased in Thailand during September 2007 and February 2008.

Sample was crushed into small pieces and then homogenized again and stored in polyethylene bag at 4°C until use. After extraction, the fat was weighted and frozen at -10°C until analysis (within four days after extraction)

3.3.2 Experimental design

The overall experiment to determine total fat and *trans* fatty acids content in selected foods is shown in Figure 3.

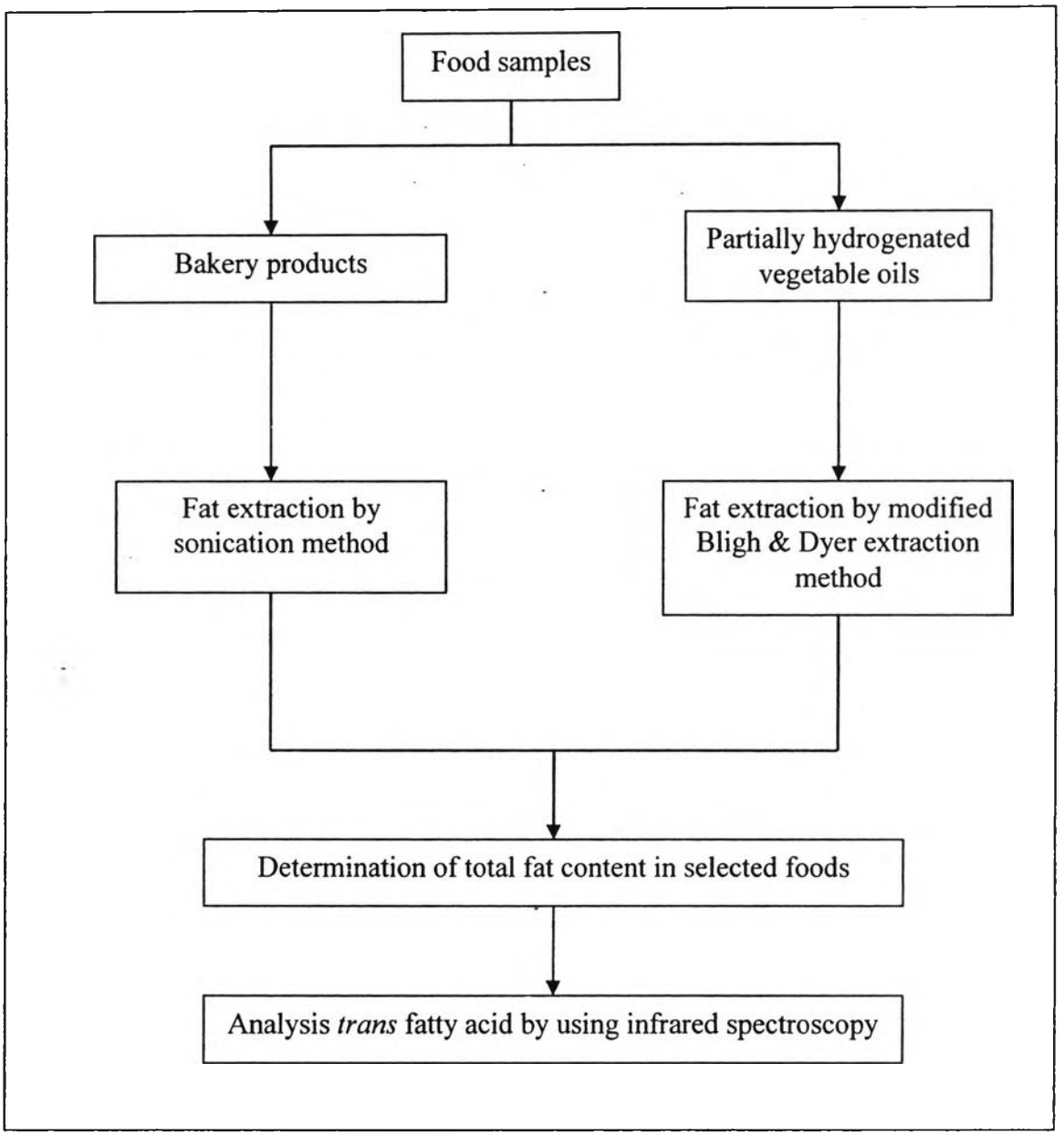

Figure 3. Overall experiment to determine total fat and *trans* fatty acids content in selected foods.

3.3.3 Fat Extraction

(I) Extraction of bakery products

To find out the optimum extraction conditions required for obtain high lipid yield, three parameters (extraction time, extraction solvent and ultrasonic intensity levels) were investigated. Extractions were carried out according to the following procedure:

(a) Ultrasonic intensity levels

Four grams of each sample and beads were placed in 250 ml round bottom flask with 60 ml of *n*-hexane. The sample-solvent suspension was immersed into the ultrasonic bath using at 40% and 80% ultrasonic intensity levels for 60, 90 and 120 minute. After extraction, the mixture was filtered through filter paper, Whatman no.42. If the filtrate was cloudy it should be centrifuge for 10 minute at 2000 rpm. The filtrate was evaporated by rotary evaporator and the extract was dried in vacuum desiccator for 90 minute. Finally, chose the best intensity that obtained high lipid yield.

(b) Extraction time

The extraction was carried out in the same manner as described in section (a) using ultrasonic intensity level that obtained high lipid yield for 60, 90, 120 and 150 minute. The appropriate time which gave the highest lipid yield was selected.

(c) Extraction solvents

Each sample was extracted with different kinds of solvents and solvent mixtures namely: *n*-hexane, acetone : *n*-hexane, acetone : *n*-hexane : petroleum ether and acetone : *n*-hexane : methanol. Using the extraction time and ultrasonic intensity levels that gave the highest lipid yield. The results obtained were compared with the values declared in the nutrition fact label of the products (90 – 110 % of label amount). The appropriate conditions were further utilized to extract fat from other bakery products.

(II) Extraction of partially hydrogenated vegetable oil

All samples were extracted with petroleum ether by modifying the procedure of Bligh and Dyer (1959). Briefly, 2.5 g of sample was placed in 50 ml centrifuge glass tube and mixed with 15 ml of petroleum ether for 2 minute on vortex. Then 10 ml of petroleum ether was added and the mixture was shaken vigorously for 2 minute. Nine milliliters of distilled water were added and the mixture was vortexed again for 2 minute. The layers were separated by centrifugation for 10 minute at 2000

rpm. The upper layer was transferred to a 125 ml pear-shaped flask. The lower layer was extracted two times with 25 ml of petroleum ether by centrifugation for 10 minute at 2000 rpm. The petroleum ether phase was added to the first extract. The filtrate was evaporated by rotary evaporator and the residue was dried in vacuum desiccator for 90 minute.

3.4 Determination of *trans* fatty acids content of the bakery product and partially hydrogenated vegetable oil

3.4.1 Principle

Attenuated total reflection - Fourier transform infrared spectrometer (AOAC official method 2000.10) is applicable to the accurate determination of total isolated *trans* unsaturated fatty acid in fats and oils. A unique absorption band with a maximum at 966 cm^{-1}, arising from a C-H deformation vibration of a *trans* double bond, is exhibited in the spectra of all compounds containing an isolated trans group; this band is not observed in the spectra of the corresponding saturated and *cis* unsaturated fatty acids.

3.4.2 Preparation of the standard solution

Trielaidin (TE) and Triolein (TO) primary standards were used to prepare the calibration curve. TE 0.0015, 0.0150, 0.0300, 0.0900, 0.1500 g were mixed with TO 0.2985, 0.2850, 0.2700, 0.2100, and 0.1500 g respectively to prepare 0.5, 1, 5, 10, 30 and 50% *trans* calibration standards.

3.4.3 Preparation of test samples

The fat sample was melt gently on stream bath. If it was still cloudy because of the presence of water, it would be treated with anhydrous sodium sulfate until it was clear.

3.4.4 Attenuated total reflection - Fourier transform infrared spectrometer (ATR-FTIR) determination

The operation parameters of FTIR was set up according to the manufacturer's for using a zinc selenide ATR cell with following parameters: resolution of 4 cm^{-1} in the spectral range of 1050 – 900 cm^{-1}, 64 scan. The test portion was filled to cover the horizontal surface of the crystal. The ATR cell must be maintained a constant temperature of 65 ± 2 °C to ensure that the sample was fully melted. Single beam spectrum collected of air was used as reference (background). The single-beam spectrum of the test portion was collected against that of the reference background and convert into absorbance. To improve sensitive and

accuracy, a new ATR-FTIR procedure that measures the height of the negative second derivative of the *trans* absorption band relative to air was used. After each analysis, ATR cell was clean with acetone.

3.4.5 Calculations

The absorbance spectrum wavenumber scale expanded in the region from 1050 to 900 cm^{-1} was integrate the height under the 966 cm^{-1} band between the limits 990 and 945 cm^{-1}. The linear regression equation was calculate from the height versus % *trans* fat plot of *trans* calibration standards curve.

The % *trans* fat for test samples was calculate by substitution the value of the integrated height of the negative second derivative of *trans* band in the following equation:

$$\textit{Trans}\text{ fat as trielaidin, \%} = \frac{\text{height} - \text{intercept}}{\text{slope}}$$