CHAPTER IV

RESULTS

4.1 Optimization of fat extraction conditions for bakery products

In this study, sonication method was used for isolation of fat from bakery products. In order to get the higher lipid yields, the appropriate conditions for lipid extraction including ultrasonic intensity levels, extraction time and extracting solvent parameter were investigated. In this study, butter cookie and/or butter cake were chosen as a representative of bakery products for investigates the optimum fat extract condition.

4.1.1 Effect of ultrasonic intensity levels on lipid yields

The effect of ultrasonic intensity levels on lipid yield by *n*-hexane as a solvent is shown in Table 5. It was found that lipid yield at 80% intensity was higher than those at 40% intensity level but not significant. Some study showed that low-intensity ultrasound does not alter the physical or chemical properties of the material through which the ultrasonic wave propagates. In the other hand, high ultrasonic intensity will induce the bubble of cavitations that generate intense pressures, shear and temperature which can produce physical, chemical and mechanical effects (Ma et al, 2008). Thus, 40% ultrasonic intensity level was chosen for lipid extraction.

Table 5. Effect of ultrasonic intensity levels on lipid yields.

Extraction time (min)	Average lipid yield [a] (mg/g sample)	
	40% ultrasonic intensity	80% ultrasonic intensity
60	245.6	246.6
90	243.8	244.1
120	250.0	251.0

[a] lipid yield of butter cookie by *n*-hexane as solvent

4.1.2 Effect of extraction times on lipid yields

The effect of extraction times on lipid yields is given in Table 6. Butter cookie and butter cake were chose as a representative of bakery products that low moisture content and medium moisture content, respectively. The data show that the lipid yield increased with increasing extraction times and reached the maximum value at 120 minute. Thus, 120 minute at 40% ultrasonic intensity was favorable for fat extraction from bakery products.

Table 6. Effect of extraction time on lipid yields.

Extraction time (min)	Average lipid yield [a] (mg/g sample)	
	Butter cookie	Butter cake
60	245.6	174.1
90	243.8	177.4
120	250.0	179.7
150	245.0	113.6

[a] at 40% ultrasonic intensity by *n*-hexane as solvent

4.1.3 Effect of different kinds of solvents on lipid yields

Table 7 exhibits the efficiencies of *n*-hexane and the others four different solvent mixtures including, (a) *n*-hexane (b) *n*-hexane : acetone (4:1 v/v) (c) *n*-hexane : acetone : petroleum ether (4:1:1 v/v) (d) *n*-hexane : acetone : methanol (4:1:1 v/v) and (e) *n*-hexane : acetone : methanol (4:1:1.5 v/v) on lipid yields. When the food sample was extracted at 40% ultrasonic intensity for 120 minute. The data show that when *n*-hexane was used as a solvent it obtained fat from butter cookie higher than other solvent mixtures. Therefore, *n*-hexane was selected as the extraction solvent.

Table 7. Efficiency of different solvent mixtures of fat extraction form butter cookie at 40% ultrasonic intensities for 120 minute.

Type of solvent [a]	Average lipid yield (mg/g sample)	% of value labeled [b]
Hex	250.0	93.76
Hex:Acet (4:1)	200.7	75.28
Hex:Acet:Pet (4:1:1)	176.4	66.15
Hex:Acet:Met (4:1:1)	76.7	28.75
Hex:Acet:Met (4:1:1.5)	53.6	20.1

[a] Hex = hexane, Acet = acetone, Pet = petroleum ether and Met = methanol

[b] Total fat content of butter cookie that declared on label was 266.7 mg/g cookie

It is indicated from the present results that the optimal conditions for fat extraction from bakery products were using *n*-hexane as a solvent, ultrasonic intensity level at 40% and 120 minute for extraction time.

4.2 Determination of total fat contents in selected foods

Total fat contents of bakery products and partially hydrogenated vegetable oils were determined by sonication method and modified Bligh and Dyer method, respectively (Smedes et al., 1996). Extracted fat was dried in a vacuum desiccator until constant weight was achieved. Value for total fat content of each type of food was expressed as range and mean ± standard deviations of duplicate (Table 8). Different brands had the different amount of fat due to difference fats and oil source to make their products. In group of bakery products, the highest amount of average total fat content was found in butter cookie (255.15 ± 28.40 mg/g food) and the lowest was found in rich butter bun (144.50 ± 56.79 mg/g food). And the other group, average total fat content of shortening (985.75 ± 6.93 mg/g food) was higher than margarine (837.03 ± 11.03 mg/g food).

Table 8. Total fat contents of bakery products and partially hydrogenated vegetable oils.

Products	**Total fat content (mg/g food)**	
	Range	**Mean ± SD**
Butter cookie (n=3)	226.3 - 289.1	255.1 ± 28.40
Cracker (n=3)	183.2 - 199.1	191.4 ± 7.16
Croissant (n=3)	62.9 - 316.7	196.4 ± 144.00
Brownie (n=3)	62.5 - 192.3	146.5 ± 65.14
Crispy pie (n=3)	205.3 - 236.2	221.4 ± 13.92
Sandwich chocolate cookie (n=3)	186.5 - 265.7	213.8 ± 40.27
Cake cream roll (n=3)	160.1 - 183.7	173.9 ± 11.04
Rich butter bun (n=3)	93.5 - 215.6	144.5 ± 56.79
Margarine (n=3)	826.9 - 850.8	837.0 ± 11.03
Shortening (n=3)	978.2 - 993.7	985.7 ± 6.93

4.3 Determination of *trans* fatty acid content of the bakery product and partially hydrogenated vegetable oil

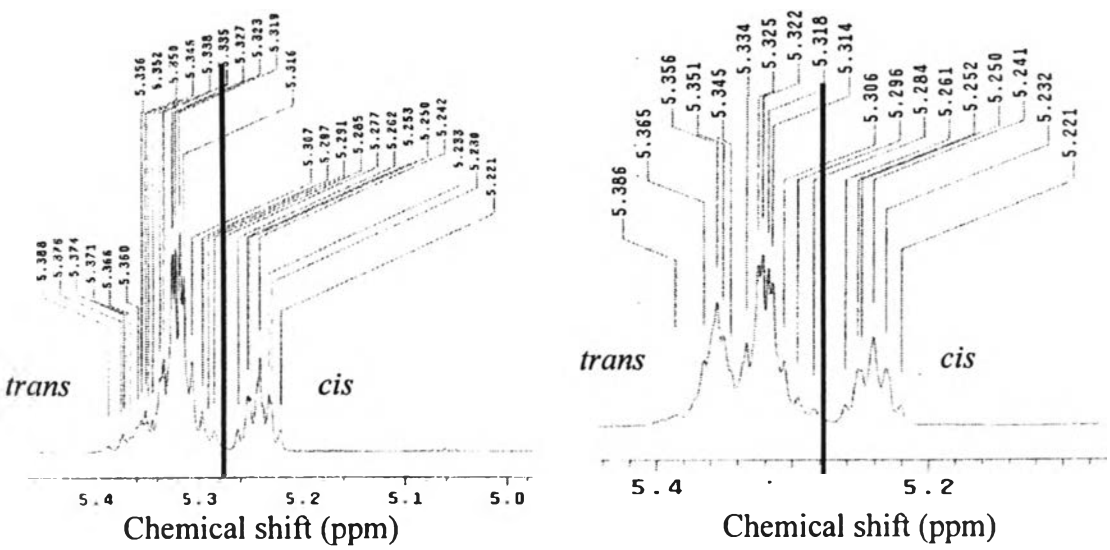
4.3.1 Fatty acid spectrum of selected foods

The main fourier transform infrared bands that related to fats of all samples are summarized in Table 9. The fingerprint range (1500-900 cm^{-1}) containing the C-H out of plane deformation band (966 cm^{-1}) that is uniquely characteristic of isolated double bonds with *trans* configuration.

Table 9. Characteristics infrared.bands of bakery products and partially hydrogenated vegetable oils

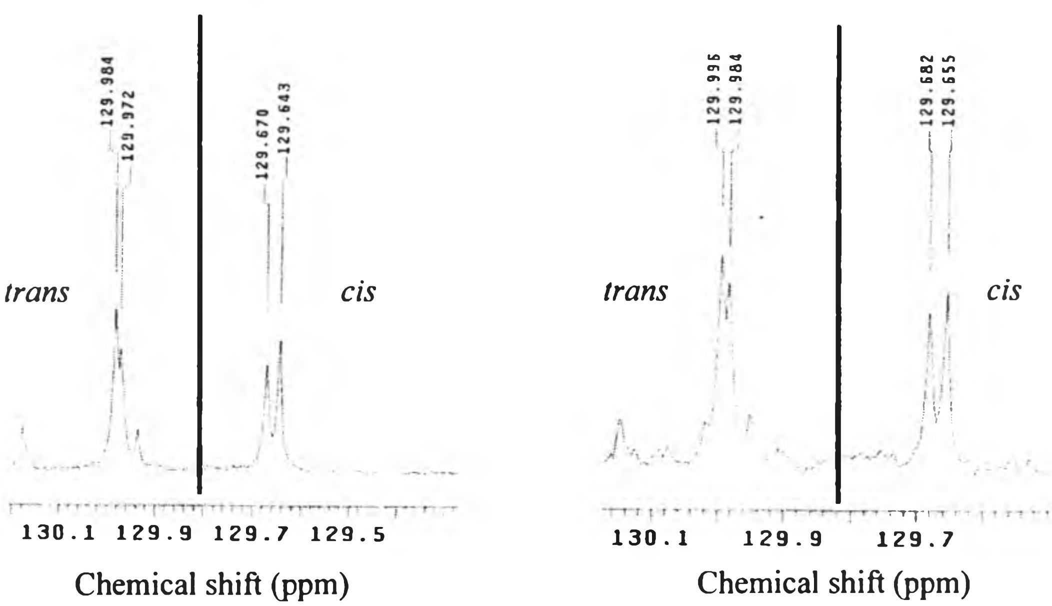
Wavenumber (cm^{-1})	Assignment
3356 - 3474	O-H stretching of alcohol
2852 - 2853	C-H stretching of $-CH_2-$
2323 - 2681	O-H stretching of -COOH
1743 - 1744	C=O stretching of fatty acid glycerol ester linkage
1652 - 1656	C=C stretching (*cis*)
1228 - 1235	C-O stretching of -COOH
1159 - 1096	C-O stretching of alcohol
966	=C-H bending out of plane (*trans*)
912 - 915	O-H bend out of plane of COOH
721	C-H rocking of $C-(CH_2)_n-C$ when n>4

One sample of each group of sample (butter cookie and shortening) was chosen for identification of *trans* fatty acid by using Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR and ^{13}C- NMR) for confirm the results of FTIR analysis. The *cis* and *trans* isomers can be distinguished by the coupling constants as well as by some chemical shifts. ^{1}H-NMR spectrum of the olefinic proton was observed at 5.22-5.26 ppm (*cis*) and 5.28-5.39 ppm (*trans*) for shortening and at 5.22-5.26 ppm (*cis*) and 5.28-5.39 ppm (*trans*) for butter cookie (Fig. 4). ^{13}C -NMR spectrum of shortening, the olefinic signals (Fig. 5) and allylic signals (Fig. 6) were observed at 129.64, 129.67 ppm (*cis*) and 129.97,129.98 ppm (*trans*) and at 27.14, 27.17, 27.19 ppm (*cis*) and 31.88, 31.90 ppm (*trans*), respectively. In addition, the olefinic and allylic signals of butter cookie were observed at 129.65, 129.68 ppm (*cis*) and 129.98,129.99 ppm (*trans*) and at 27.15, 27.18, 27.20 ppm (*cis*) and 31.89, 31.91 ppm (*trans*), respectively. The apparent of allylic group position of *cis* and *trans* isomers were very difference.



(A) (B)

Figure 4. [1]H NMR spectral region relative to olefinic protons (A) shortening A; (B) butter cookie A.



(A) (B)

Figure 5. [13]C NMR spectral region relative to olefinic group (A) shortening A; (B) butter cookie A.

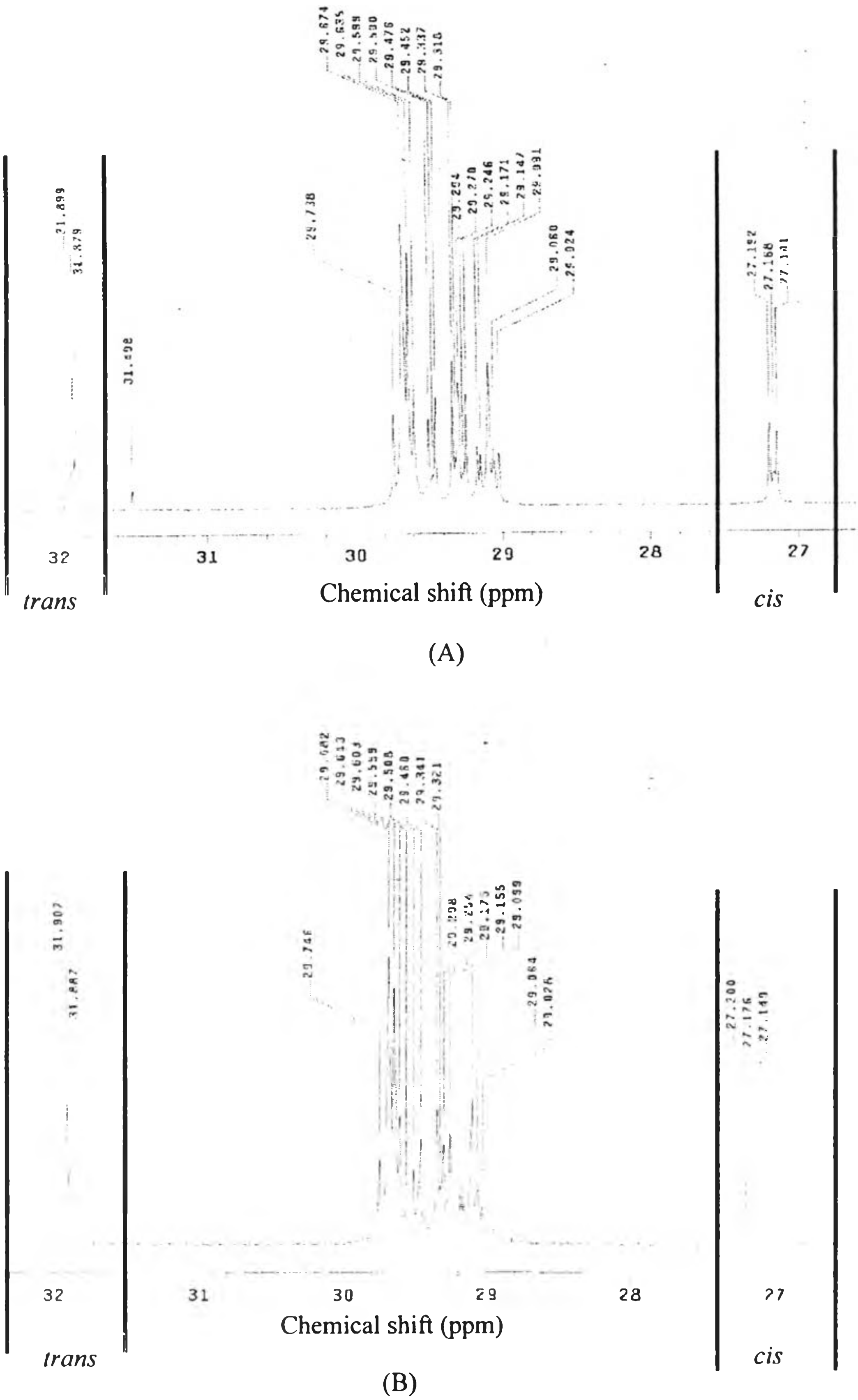


Figure 6. ^{13}C NMR spectral region relative to allylic carbon (A) shortening A; (B) butter cookie A.

4.3.2 Total *trans* isomers contents in selected foods

The total *trans* isomers contents of selected foods were determined by Attenuated total reflection Fourier transform infrared spectrometer (ATR-FTIR), AOAC Official Method 2000.10. The new ATR-FTIR procedure that measures the height of the negative second derivative of the *trans* absorption band relative to air was used to improve sensitivity and accuracy.

The height of the negative second derivative of the *trans* absorption band at 966 cm^{-1} was integrated between the fixed limits 990 and 945 cm^{-1} by using the appropriate software (Table 10).

Table 10. The height of the negative second derivative of *trans* absorption band of selected foods.

PRODUCT	Peak Height (-)	Average peak height (-)
Shortening (brand 1)_a	0.0017	0.0017
Shortening (brand 1)_b	0.0016	
Shortening (brand 2)_a	0.0015	0.0015
Shortening (brand 2)_b	0.0015	
Shortening (brand 3)_a	0.0023	0.0024
Shortening (brand 3)_b	0.0024	
Margarine (brand 1)_a	0.0014	0.0015
Margarine (brand 1)_b	0.0016	
Margarine (brand 2)_a	0.0016	0.0017
Margarine (brand 2)_b	0.0017	
Margarine (brand 3)_a	0.0017	0.0017
Margarine (brand 3)_b	0.0017	
Butter cookie (brand 1)_a	0.0099	0.0100
Butter cookie (brand 1)_b	0.0101	
Butter cookie (brand 2)_a	0.0029	0.0029
Butter cookie (brand 2)_b	0.0028	
Butter cookie (brand 3)_a	0.001	0.0011
Butter cookie (brand 3)_b	0.0011	
Brownie (brand 1)_a	0.002	0.0021
Brownie (brand 1)_b	0.0021	

Note : a = flask 1   b = flask 2

Table 10. The height of the negative second derivative of *trans* absorption band of selected foods (continued).

PRODUCT	Peak Height (-)	Average peak height (-)
Brownie (brand 2)_a	0.0023	
Brownie (brand 2)_b	0.0025	0.0024
Brownie (brand 3)_a	0.0023	
Brownie (brand 3)_b	0.0023	0.0023
Sandwich chocolate cookie (brand1)_a	0.0009	
Sandwich chocolate cookie (brand 1)_b	0.0009	0.0009
Sandwich chocolate cookie (brand 2)_a	0.0005	
Sandwich chocolate cookie (brand 2)_b	0.0005	0.0005
Sandwich chocolate cookie (brand 3)_a	0.0005	
Sandwich chocolate cookie (brand 3)_b	0.0005	0.0005
Cake cream roll (brand 1)_a	0.001	
Cake cream roll (brand 1)_b	0.001	0.0010
Cake cream roll (brand 2)_a	0.0026	
Cake cream roll (brand 2)_b	0.0027	0.0027
Cake cream roll (brand 3)_a	0.0011	
Cake cream roll (brand 3)_b	0.0012	0.0012
Croissant (brand 1)_a	0.0017	
Croissant (brand 1)_b	0.0017	0.0017
Croissant (brand 2)_a	0.0026	
Croissant (brand 2)_b	0.0027	0.0027
Croissant (brand 3)_a	0.001	
Croissant (brand 3)_b	0.001	0.0010
Rich butter bun (brand 1)_a	0.0041	
Rich butter bun (brand 1)_b	0.0041	0.0041
Rich butter bun (brand 2)_a	0.0027	
Rich butter bun (brand 2)_b	0.0027	0.0027
Rich butter bun (brand 3)_a	0.0017	
Rich butter bun (brand 3)_b	0.0017	0.0017
Crispy pie (brand 1)_a	0.0019	
Crispy pie (brand 1)_b	0.0019	0.0019
Crispy pie (brand 2)_a	0.0019	
Crispy pie (brand 2)_b	0.0019	0.0019
Crispy pie (brand 3)_a	0.0014	
Crispy pie (brand 3)_b	0.0015	0.0015

Note : a = flask 1   b = flask 2

Table 10. The height of the negative second derivative of *trans* absorption band of selected foods (continued).

PRODUCT	Peak Height (-)	Average peak height (-)
Cracker (brand 1)_a	0.0009	
Cracker (brand 1)_b	0.0009	0.0009
Cracker (brand 2)_a	0.0007	
Cracker (brand 2)_b	0.0007	0.0007
Cracker (brand 3)_a	0.0007	
Cracker (brand 3)_b	0.0007	0.0007

Note : a = flask 1 b = flask 2

The calibration standards that prepared from Trielaidin and Triolein are shown in Table 11. Graph of the negative second derivative of the *trans* absorption band relative to air of standard solution are shown in Figure 7.

Table 11. Composition of elaidic acid and oleic acid in standard mixture.

Triolein (g)	Trielaidin (g)	Total (g)	% *trans* fat of total fat
0.3000	0.0016	0.3016	0.53
0.2888	0.0150	0.3038	4.94
0.2712	0.0301	0.3013	9.99
0.2103	0.0901	0.3004	29.99
0.1503	0.1500	0.3009	49.95

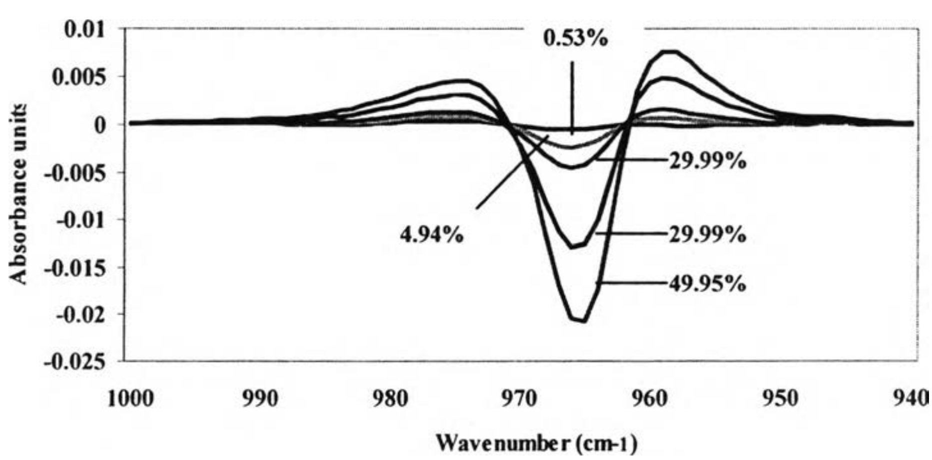


Figure 7. Negative second derivative ATR-FTIR spectrum of standard mixtures.

The graph of standard calibration was obtained by plotting the % *trans* fatty acid vs the peak height at 966 cm^{-1} band, with a correlation coefficient (r^2) of 0.9996 (Figure 8).

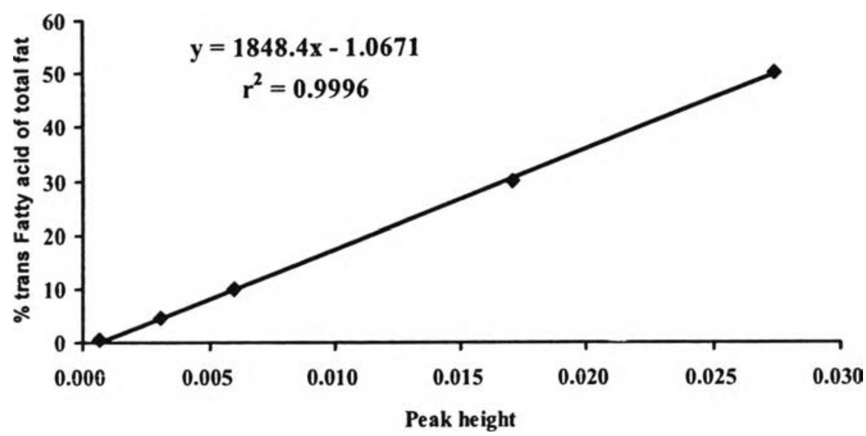


Figure 8. The graph of standard calibration of *trans* fatty acid.

The percentage of *trans* isomers was calculated by using the regression equation of *trans* standards mixtures calibration. Table 12 shows the results of *trans* fatty acid contents in selected foods. The total *trans* fatty acids contents of the selected products ranged from 0.74 to 17.53% total fat or 0.14 – 5.07 g/100 g food. The *trans* isomers content of the same product that produced by different manufacturers showed different values of *trans* fat level.

Table 12. *Trans* fatty acid contents in selected foods.

Products	***Trans* fatty acid content**			
	% of total fat		**g/ 100 g food**	
	Range	**Mean ± SD**	**Range**	**Mean ± SD**
Shortening (n=3)	1.85 - 3.42	2.46 ± 0.84	1.84 - 3.37	2.43 ± 0.82
Margarine (n=3)	1.85 - 2.22	2.07 ± 0.19	1.54 - 1.89	1.73 ± 0.17
Butter cookie (n=3)	1.02 - 17.53	7.63 ± 8.73	0.26 - 5.07	2.10 ± 2.59
Brownie (n=3)	2.86 - 3.51	3.23 ± 0.33	0.18 - 0.68	0.49 ± 0.27
Sandwich chocolate cookie (n=3)	ND - 0.74	0.74	ND - 0.14	0.14
Cake cream roll (n=3)	0.93 - 3.97	2.03 ± 1.68	0.16 - 0.73	0.36 ± 0.32
Croissant (n=3)	0.93 - 3.97	2.37 ± 1.53	0.14 - 0.83	0.42 ± 0.36
Rich butter bun (n=3)	2.22 - 6.65	4.31 ± 2.22	0.21 - 0.88	0.64 ± 0.37
Crispy pie (n=3)	1.76 - 2.59	2.31 ± 0.48	0.42 - 0.58	0.51 ± 0.08
Cracker (n=3)	ND - 0.74	0.74	ND - 0.14	0.14

ND = Non quantified at the level of traces (TFA <0.5% of total fat)

The highest average amount of total *trans* fat was found in shortening and followed by butter cookies, margarine, rich butter bun, crispy pie, brownie, croissant, cake cream roll, cracker and sandwich chocolate cookie, respectively.