ASEAN Food Journal 14 (3): 197-204 (2007)

Fatty Acid Composition of Five Malaysian Biscuits (Cream Crackers) With Special Reference to *trans*- Fatty Acids

Neo, Y.P., Tan, C.H. and *Ariffin, A.

Department of Food Technology, Faculty of Food Science and Technology
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor

Abstract: The fatty acid composition and *trans* fatty acids (TFA) contents of samples of five Malaysian cream crackers biscuit brands were determined by gas-liquid chromatography, using a 60 m Supelco SP2340 fused silica capillary column and flame ionization detection. The identities of the fatty acids were established by comparing their retention times with authentic standards from Supelco. The results were expressed as relative percentages. The total saturated fatty acids (SFA) in the samples ranged from 48.90% to 54.87% of total fatty acids. As for the polyunsaturated fatty acids (PUFA), the total PUFA in the samples ranged from 9.97% to 11.73% of total fatty acids. Total *trans* fatty acids (TFA) ranged from 0.17% to 0.77% of total fatty acids. The mono-*trans* 18:2 tc or 18:2 ct isomer content ranged from 0.07% to 0.10% of total fatty acids and the di-*trans* 18:2 isomer (9t, 12t) was not detected. The results indicate that all the fat sources of the 5 sample crackers biscuit brands were palm oil based.

Keywords: Cream crackers, composition, SFA, PUFA, TFA, gas chromatography

INTRODUCTION

Crackers are usually defined as biscuits, which are all more or less unsweetened, salty, thin and crisp. Cream crackers biscuits were first introduced around 1885 by the Irish firm called Jacobs. Nowadays, cream crackers have gained popularity among the people in Malaysia, and have maintained a significant place in the sales of biscuits. According to Manley (1996), fats are probably the most important ingredients used in the manufacture of biscuits. They are the third largest component, after flour and sugar. A fat's functionality is determined by its fatty-acid composition.'Fats have received much media attention because a number of ailments are attributed to their presence in modern diet. The principal concern is centered on the levels of saturated and unsaturated fatty acids in the chemical composition. Research shows that certain fatty acids influence the serum levels of lipids and lipoproteins and therefore the incidence of coronary heart disease. Studies have shown that high intake of saturated fat contributes to the development of coronary heart disease. The reduction of saturated fat in the diet will eventually help in lowering blood cholesterol (Grundy, 1986, 1990; Koletzko, 1992a, b). Fatty acids with medium chain lengths have a greater adverse effect (examples: lauric C12:0, myristic C14:0 and palmitic C16:0) than those with longer chain lengths.

Coronary heart disease (CHD) remains the leading cause of death and disability in many countries in the world, including Malaysia. There are several multiple risk factors that act both independently and jointly. Among dietary factors, the type of fat intake and total amount of fat in the diet play

*Corresponding Author
E-mail: *azis@food.upm.edu.my*

important roles in determining risk of coronary heart disease (Hu *et al.*, 2001; Carapelli *et al.*, 2004). More recently, *trans*-fatty acids (TFA) have also been associated with adverse effects. In addition to the negative health effects of saturated fatty acids, an increasing body of evidence implicates *trans*-fats as promoters of heart disease through an increase in the low-density-lipoprotein (LDL)-cholesterol in the same way saturated fatty acids do, and lowering the level of the good high-density-lipoprotein (HDL)-cholesterol (Mensink *et al.*, 1990; Zock *et al.*, 1992; Wood *et al.*, 1993; Judd *et al.*, 1994; San Juan, 1999; Spiller, 2004).

Trans-fatty acids are produced during industrial hydrogenation of edible fats and oils to produce dietary fats with improved texture and other commercially desirable physical properties. During "partial hydrogenation", some double bonds remain but may shift to a different position along a chain and alter their configuration (Spiller, 2004). Small amounts of *trans*-fatty acids are also formed from heat induced isomerization during deodorization under high temperature. The extent of isomerization is more serious in polyunsaturated oils. Depending on the type of unsaturated acids, different *trans*-isomers can be formed from the original *cis*-unsaturated fatty acids (Enig *et al.*, 1984; Van Erp-baart *et al.*, 1998; Tang, 2002).

A study on five types of Malaysian biscuits was done to determine their fatty acids composition and *trans*-fatty acids content.

MATERIALS AND METHODS

Sampling

Five brands of cream crackers biscuits were purchased from hypermarkets in Serdang between December 2004 and February 2005. Each brand was coded with a letter (A, B, C, D and E). Samples were selected to include the major manufacturers of biscuits in Malaysia. The analyses were carried out in triplicate.

Chemicals

Petroleum spirit 40 - 60°C, n-hexane, sodium methoxide, 1M methanolic solution, diethly ether and acetic acid were purchased from Merck (Darmstadt. Germany). All the solvents and chemicals used for analyses were of laboratory or analytical grade as required by the method used.

Preparation of Samples for Analyses

The biscuit samples were ground into crumbs. Biscuit moisture content was determined by weight loss techniques involving heating crumb from crushed or ground biscuits. Total lipids were extracted using Soxhlet Method (AOAC Methods 920.39 C for cereal fat). Petroleum spirit 40 - 60°C (AOAC, 1995) was the solvent used for the determination of fat content. Ten grams of ground crackers were weighed into a pre-dried extraction thimble and the oil was extracted using the solvents mentioned above. The time of each extraction was 16 hours. The oil was recovered after stripping off the solvent in a rotary evaporator.

Fatty Acids Methyl Esters (FAME)

Fatty acids methyl esters were prepared by methylation of fatty acids, as described by the MPOB test methods (Ainie *et al.*, 2005).

Gas Liquid Chromatography

Determination of fatty acids methyl esters by gas liquid chromatography was done was the MPOB test methods (Ainie *et al.*, 2005) by using Hewlett Packard 5980 Gas Chromatograph fitted with polar SP-2340 (supelco USA) capillary column (0.25 mm id x 60m x 0.2 μm). The identification of fatty acids was based on authentic reference standards and retention time for *trans*-fatty acids in Malaysia Palm Oil Board (MPOB). The results were expressed as relative percentages.

Thin Layer Chromatography

Determination of the presence of the various lipid fractions in the lipid extract by thin-layer-chromatography was done using a procedure published by Nielsen (1998). TLC was

performed using silica gel G (60 F_{254} of Merck) as the adsorbent and hexane-diethyl ether-glacial acetic acid (70:30:1, by vol) as the eluting solvent system. Plates were sprayed with sulphuric acid to show the separation. The identification of various lipid fractions was based on reference standards published by Nielsen (1998).

Statistical Analyses

Each sample of the replicate treatments was analyzed and the results were expressed as mean values ± standard deviation (SD). The results were compared using analysis of variance (ANOVA) at 5% significance level using the Statistical Analysis System Software (SAS). The means were tested for significant differences using Tukey's test.

RESULTS AND DISCUSSION

The TLC plate was coated with Silica Gel F_{254} and placed in hexane-diethyl ether-glacial acetic acid (70:30:1, by vol) which was the eluting solvent system. A small amount of the lipid sample to be analyzed was spotted onto the TLC plate. With time, the solvent moved up the plate due to capillary forces and separates different lipid fractions on the basis of their affinity for the absorbing material. The bands were visualized by spraying with 50% sulphuric acid in ethanol followed by heating for 1 hour at 105°C.

Based on reference standards published by Nielsen (1998), various lipid fractions were identified. The migration may vary somewhat in absolute value but the relative order of mobility was unchanged. TLC separates acylglycerols like triacylglycerols, diacylglycerols, monoacylglycerols, cholesterol and free fatty acids from the majority of the other neutral lipids. The Rf value of various lipid fractions are summarized in Table 1. The Rf value of the monoacylglycerols of lipid extracted from five different brands of crackers was either 0.049 or 0.055. The Rf values of the 1,3-diacylglycerols of five samples ranged between 0.331 to 0.344. The Rf values of 1,3-diacylglycerols ranged from 0.448 to 0.472 and the Rf values of the triacylglycerols of 5 samples ranged from 0.896 to 0.908. Free fatty acids were formed during the process of lipolysis (the breakdown of fat stored in fat cells). The process of triglyceride breakdown or lipolysis results in the release of fatty acids and glycerol. Free Fatty acids were only present in both brands C and E. This might be due to the storage condition of brands C and E.

High humidity and temperature of storage of brands C and E biscuits might lead to the hydrolysis of fat due to the reaction of fats with water. Hydrolysis of fat would cause the breakdown of triglyceride and release of the free fatty acids. Oven drying method was carried out to determine the moisture content of the crackers. The moisture content of various sample crackers are summarized in Table 2. The samples contained 2.084 – 5.072% moisture. The moisture content of the manufactured cream crackers should be 3 – 4%, thus, brand C had significantly higher value of moisture content (Table 2). The high moisture content of both brands C and E cause them to be prone to hydrolysis, hence the release of free fatty acids.

On the other hand, the moisture content of brand D was relatively high (4.420%), but free fatty acid were not detected during the separation of various lipid components using TLC. For that reason, the storage condition might not be the only reason, which led to the formation of free fatty acids. It can also be attributed to the free fatty acids content of the oil used for making of the crackers. Both brands C and E might have had oil, which contains relatively higher content of free fatty acids compared to the other samples. The free fatty acids content of the oil used might actually exceed 0.5%. Higher moisture content and higher FFA value would accelerate the rate of lipolysis.

The concentration of different volatile fatty acid methyl esters (FAME) present in the sample was then analyzed using Gas Chromatography (GC). The FAME were dissolved in hexane, methylated with sodium

Table 1: Rf value of various lipid fractions of five Malaysian cracker biscuits (A, B, C, D, E) using Thin Layer Chromatography

Fraction Collector	Brand														
	A			B			C			D			E		
	x	y	Rf	x	y	Rf	x	y	Rf	x	y	Rf	x	y	Rf
Monoacylglycerols	0.8	16.3	0.049	0.7	16.3	0.043	0.8	16.3	0.049	0.9	16.3	0.055	0.9	16.3	0.055
1.2-Diacylglycerols	5.5	16.3	0.337	5.5	16.3	0.337	5.4	16.3	0.331	5.4	16.3	0.331	5.6	16.3	0.344
1,3-Diacylglycerols	7.7	16.3	0.472	7.5	16.3	0.460	7.4	16.3	0.454	7.3	16.3	0.448	7.7	16.3	0.472
Free Fatty Acids	-	-	-	-	-	-	10.4	16.3	0.638	-	-	-	10.9	16.3	0.669
Triacylglycerols	14.6	16.3	0.896	14.7	16.3	0.902	14.6	16.3	0.896	14.7	16.3	0.902	14.8	16.3	0.908

Table 2: Moisture content of 5 Malaysian cracker biscuits (A, B, C, D and E)

	Brand				
	A	B	C	D	E
Moisture Content	2.084 ±0.05^{d}	3.944 ±0.10^{c}	5.072 ±0.12^{a}	4.420 ±0.20^{b}	4.148 ±0.03^{c}

Results expressed as moisture content. Values are means ± SD for five samples triplicate analyses. Values with different letters are significantly different (P<0.05) using Tukey's test.

Table 3: Fatty acid composition of five Malaysian cracker biscuits (A, B, C, D and E)

Fatty acid	Brand				
	A	B	C	D	E
12:0	0.30 ± 0.0^{b}	0.40 ± 0.0^{a}	0.27 ± 0.06^{b}	0.20 ± 0.0^{c}	0.37 ± 0.06^{a}
14:0	1.30 ± 0.10^{a}	1.17 ± 0.06^{b}	1.20 ± 0.0^{ab}	1.17 ± 0.06^{b}	1.17 ± 0.06^{b}
16:0	41.77 ± 1.59^{d}	45.77 ± 0.50^{b}	43.40 ± 0.56^{c}	48.70 ± 0.10^{a}	43.07 ± 0.15^{cd}
16:1***c***	0.33 ± 0.06^{a}	0.20 ± 0.0^{b}	0.20 ± 0.0^{b}	0.20 ± 0.0^{b}	0.20 ± 0.0^{b}
18:0	5.30 ± 0.26^{a}	4.47 ± 0.06^{b}	4.10 ± 0.0^{c}	4.40 ± 0.10^{b}	4.23 ± 0.06^{bc}
18:1***t***	0.23 ± 0.21^{b}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.57 ± 0.06^{a}	0.0 ± 0.0^{c}
18:1***c***	39.07 ± 0.76^{a}	36.37 ± 0.35**c**	37.97 ± 0.32^{b}	33.43 ± 0.06^{d}	37.73 ± 0.25^{b}
18:1***i***	0.63 ± 0.57^{a}	0.83 ± 0.12^{a}	0.73 ± 0.06^{a}	0.70 ± 0.0^{a}	0.73 ± 0.06^{a}
18:2***tc***	0.07 ± 0.06^{a}	0.10 ± 0.0^{a}	0.07 ± 0.06^{a}	0.10 ± 0.0^{a}	0.10 ± 0.0^{a}
18:2***ct***	0.07 ± 0.06^{a}	0.10 ± 0.0^{a}	0.10 ± 0.0^{a}	0.10 ± 0.0^{a}	0.10 ± 0.0^{a}
18:2***cc***	10.17 ± 0.46^{c}	9.73 ± 0.06^{d}	10.93 ± 0.12^{b}	9.60 ± 0.0^{d}	11.40 ± 0.0^{a}
18:3***ccc***	0.30 ± 0.10^{a}	0.23 ± 0.06^{a}	0.30 ± 0.0^{a}	0.30 ± 0.0^{a}	0.33 ± 0.06^{a}
20:0	0.23 ± 0.21^{a}	0.30 ± 0.10^{a}	0.40 ± 0.0^{a}	0.40 ± 0.0^{a}	0.40 ± 0.0^{a}
others	0.23 ± 0.12^{a}	0.33 ± 0.12^{a}	0.33 ± 0.06^{a}	0.13 ± 0.15^{a}	0.17 ± 0.12^{a}

Results expressed as percentage of total fatty acid methyl esters. Values are means ± SD for five samples triplicate analyses. Values with different letters within a row are significantly different (P<0.05) using Tukey's test.
c = cis
i = isomer
t = trans

methoxide, and then injected into a GC injection chamber. The samples were then heated in the injection chamber to volatilize the FAME and then carried into the separating column by a heated carrier gas. As the FAME was carried through the column by the mobile gaseous phase, the esters were separated into a number of peaks based on differences in their molecular weights and polarities, which were detected using a flame ionization detector.

Determination of the total fatty acid profile allows one to calculate the type and concentration of fatty acids present in the original lipid sample (Carapelli *et al., 20*04). The fatty acid composition of various samples are summarized in Table 3. By comparing results obtained in Table 3 with results of Malaysian Palm Oil Board (MPOB) done by Tang (2002), the source of fat of the five samples was confirmed to be palm oil.

Palm oil should be classified as both a saturated fat and unsaturated fat. It contains equal proportions of saturated fatty acids and unsaturated acids. The saturated acids are made up of 44% palmitic acid and 5% stearic

Table 4: Fatty acid composition of five Malaysian cracker biscuits (A, B, C, D and E)

Fatty acid	Brand				
	A	B	C	D	E
[a]SFA	48.900 ± 1.23[c]	52.100 ± 0.46[b]	49.367 ± 0.57[c]	54.867 ± 0.15[a]	49.233 ± 0.29[c]
[b]PUFA	10.333 ± 0.32[c]	9.967 ± 0.12[d]	11.233 ± 0.12[b]	9.900 ± 0.0[d]	11.733 ± 0.06[a]
[c]TFA	0.367 ± 0.32[b]	0.200 ± 0.0[b]	0.167 ± 0.06[b]	0.767 ± 0.06[a]	0.200 ± 0.0[b]

Results expressed as percentage of fatty acids composition. Values are means ± SD for five samples triplicate analyses. Values with different letters within a row are significantly different (P<0.05) using Tukey's test.
[a]SFA = saturated fatty acids
[b]PUFA = polyunsaturated fatty acids
[c]TFA = *trans* fatty acids

Table 5: *Trans* fatty acid content of five Malaysian cracker biscuits (A, B, C, D and E)

Trans Fatty acid	Brand				
	A	B	C	D	E
18:1***t***	0.23 ± 0.21[b]	0.0 ± 0.0[c]	0.0 ± 0.0[c]	0.57 ± 0.06[a]	0.0 ± 0.0[c]
18:2***tc***	0.07 ± 0.06[a]	0.10 ± 0.0[a]	0.07 ± 0.06[a]	0.10 ± 0.0[a]	0.10 ± 0.0[a]
18:2***ct***	0.07 ± 0.06[a]	0.10 ± 0.0[a]	0.10 ± 0.0[a]	0.10 ± 0.0[a]	0.10 ± 0.0[a]

Results expressed as percentage of *trans* fatty acids composition. Values are means ± SD for five samples triplicate analyses. Values with different letters within a row are significantly different (P<0.05) uisng Tukey's test.
c = cis
t = trans

acid. The unsaturated fatty acids consist of 39% oleic acid (monounsaturates) and 11% linoleic acid (polyunsaturates). The results obtained for both total saturated fatty acids (SFA) and polyunsaturated (PUFA) of five samples could be categorized in the average range of a palm oil product. From Table 4, the total SFA in the samples ranged from 48.90% to 54.87% of total fatty acids. The total saturated fatty acids content was significantly higher (P<0.05) in brand D and was significantly lower (P<0.05) in brand A.

The significantly higher value of total SFA in brand D might be due to using of RBD hard stearin for the making of biscuits, as the harder the fat, the higher the degree of saturation. Hard stearin could be obtained through double fractionation process or combination of hydrogenated fat with non-hydrogenated fat (Ariffin, 2004). Among the SFA, palmitic acid (16:0) presented the highest value ranging from 41.8% to 48.7%, followed by stearic acid (18:0) which varied from 4.1% to 5.3%. As for the PUFA, the total PUFA in the samples ranged from 9.97% to 11.73% of total fatty acids. The total polyunsaturated fatty acids content was significantly higher (P<0.05) in brand E and was significantly lower (P<0.05) in brand D (Table 4). The results showed that the amount of SFA and PUFA varied considerably among the analyzed brands because the biscuits manufacturers might use RBD olein with RBD soft stearin or RBD hard stearin to achieve a final product with the desired characteristics. The values of total *trans*-fatty acids (TFA) for all the brands analyzed are summarized in Table 4. The amount of total TFA in the samples ranged

from 0.17% to 0.77% of total fatty acids. Total *trans* content was significantly higher ($P<0.05$) in brand D.

From Table 5, it can be seen that the TFA comprised isomers of 18:1 and 18:2 acids. *Trans* 18:1 isomers were the major group of TFA present in both brands A and D. The amount of *trans* 18:1 isomers for both brands A and D were significantly higher ($P<0.05$) than the other brands. The amount of *trans* 18:1 isomers for brand D was significantly higher which might be due to the use of hard stearin as one of the fat sources. As mentioned earlier, hard stearin could be obtained through the combination of hydrogenated fat and the non-hydrogenated fat. Thus, the higher amount of *trans* 18:1 isomers in brand D might be due to the use of hydrogenated fat. There was no significant difference in the amount of *trans* 18:2 tc and *trans* 18:2ct isomers for brands A to D. The mono-*trans* 18:2 isomer content ranged from 0.07% to 0.10% of total fatty acids (Table 5). The di-*trans* 18:2 isomer (9t, 12t) was not detected.

With reference to the results of Malaysian Palm Oil Board (MPOB) researched by Tang (2002), *trans* fatty acids were not detected in crude palm oil. The presence of *trans*-fatty acids in all the brands analyzed might be due to isomerization during deodorization which is normally carried out at 250°C - 260°C under vacuum. This is supported by the observation of Kochhar *et al.* (1982) that in the refining of crude soyabean oil (a highly unsaturated oil), *trans*-fatty acids were not detected in the neutralized and bleached oil, but only in the final product after deodorization. Small amounts of *trans*-fatty acids would form from heat induced isomerization during deodorization under high temperature (Kovari *et al.,* 1997; Bertoli *et al.,* 1997). The extent of isomerization is more serious in polyunsaturated oils.

The results show that the amount of *trans* monounsaturated and polyunsaturated fatty acids in all the brands studied were very low or even undetectable. Thus palm oil was the only fat sources for making biscuits in all the studied brands. For similar biscuits, the *trans* content found was 12.7% for US (Enig *et al.,* 1984), 11.1% for Argentina (Tavella *et al.,* 1998), 9.1% for Greece (van Erp-baart *et al.,* 1998) and 2.0% for New Zealand (Lake *et al.,* 1996). The high level of *trans* content could be due to the presence of hydrogenated polyunsaturated fat for making of biscuits.

CONCLUSION

The results of this study indicate that the five Malaysian cream crackers biscuits contain low or no *trans*-fatty acids, both monosaturated and polyunsaturated. The fat analyzed was mainly palm oil based, which contain equal proportions of saturated fatty acids and unsaturated acids. The saturated acids are made up of 44% palmitic acid and 5% stearic acid. The unsaturated fatty acids consist of 39% oleic acid (monounsaturates) and 11% linoleic acid (polyunsaturates). This survey provides further evidence that palm oil products are excellent hard-stocks for *trans*-free formulation of texturized fatty products such as margarines, shortenings, confectionery fats and vanaspati. These products can advantageously replace hydrogenated fats, which contain not only trans-fatty acids, but also possibly a host of other unnatural, and polymerized fatty acids formed during hydrogenation to reduce their unsaturation (Hoffman, 1989).

REFERENCES

Ainie, K., Siew, W. L., Tan, Y. A., Noraini, I., Mohtar, Y., Tang, T. S. and Nuzul, A. I. 2005. MPOB test methods, a compendium of test on palm oil products, palm kernel products, fatty acids, food related products and others, pp. 308-317. Malaysia: Malaysia Palm Oil Board (MPOB).

AOAC. 1995. Official methods of analysis. 16th edn. Gaithersburg, M.: AOAC International.

Carapelli, R., Martin, C. A., Matsushita, M., Souza, N. E. and Visantainer, J. V. 2004. Trans fatty acid content of Brazilian biscuits. Journal of Maringa State University, Food Chemistry, 10.022.

Enig, M. G., Pallansch, L. A., Sampugna, J. and Keeney, M. 1984. Fatty acids composition of the fat in selected food items with emphasis on trans components. Journal of American Oil Chemist Society, 60: 1788-1795.

Grundy, S. M. 1990. Trans monounsaturated fatty acids and serum cholesterol levels. New England Journal of Medicine, 323: 480 - 487.

Grundy, S. M. 1986. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. New England Journal Medicine, 323: 789-795.

Hu, F. B., Manson, J. E. and Willet, W. C. 2001. Types of dietary fat and risk of coronary heart disease: A critical review. Journal of American College Nutrition, 20: 5-19.

Judd, J. T., Clevidence, B. A., Muesing, R. A., Wittes, J., Sunkin, M. E. and Podczasy, J. J. 1994. Dietary tran fatty acids: Effect on plasma lipids and lipoproteins of healthy men and women. American Journal of Clinical Nutrition, 59: 861-868.

Koletzko, B. 1992b. Fats for brains. European Journal of Clinical Nutrition, 46: S51- S62.

Koletzko, B. 1992a. Trans fatty acids may impair biosynthesis of long-chain polyunsaturates and growth in man. Acta Paediatric Scandinavia, 81: 302-306.

Manley, D. 1996. Technology of biscuits, crackers and cookies. 2nd edn. pp. 133-152. Cambridge England: Woodhead Publishing Limited.

Mensink, R. P. and Katan, M. B. 1990. Effect of dietary trans-fatty acids on high density and low-density lipoprotein cholesterol levels in health subjects. New England Journal of Medicine, 323: 439-445.

Mensink, R. P., Zock. P. L., Katan, M. B. and Hornstra, G. 1992. Effect of dietary cis and trans-fatty acids on serum lipoprotein (a) levels in human. Journal of Lipid Research, 33: 1493-1501.

Nielsen, S. S. 1998. Food analysis. 2nd edn. pp. 203-233. Gaithersburg, Maryland: Aspen Publishers.

San Juan, P. M. F. 1999. Fatty acid composition of commercial Spanish fast food and snack food. Journal of Food Composition and Analysis, 13: 275-281.

Spiller, G. A. 2004. The trans fats dilemma and natural palm oil. pp. 27-49. Rocklin, California: Pascoe Publishing Inc.

Tang, T. S. 2002. Fatty acids composition of edible oils in the Malaysian market, with special reference to trans-fatty acids. Journal of Oil Palm Research, 14: 1-8.

Van Erp-baart, M. A., Couet, C., Cuadrodo, C., Kafatos, A., Stanley, J. and Van Poppel, G., 1998. Trans fatty acids in bakery products from 14 European countries: the trans fair study. Journal of Food Composition and Analysis, 11: 161-169.

Wood, R., Kubena, K., Brien, B., Tseng, S. and Martin,G. 1993. Effect of butter, mono- and polyunsaturated fatty acid-enriched butter, trans-fatty acid margarine, and zero trans-fatty acid margarine on serum lipids and lipoproteins in healthy men. Journal of Lipid Research, 34: 1-11.

Zock, P. L. and Katan, M. B. 1992. Hydrogenation alternatives: effect of trans fatty acids and stearic acid versus linoleic acid on serum lipid and lipoproteins in human. Journal of Lipid Research, 33: 399-410.