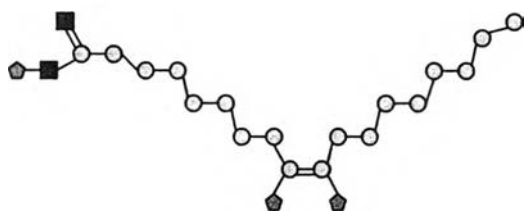
CHAPTER II

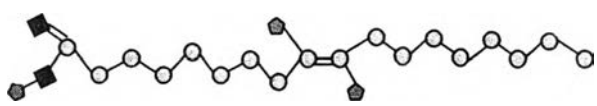
LITERATURE REVIEW

2.1 Physicochemical properties of *trans* fatty acids

Fatty acids are rarely free in nature and almost always are linked to other molecules by their hydrophilic carboxylic acid head group. Fatty acids are classified according to the number of double bonds, and the position of the first double bond in the chain. Fatty acid double-bonds come in two configurations known as *cis* (carbon chains on the same side of a double-bond) and *trans* (carbon chains on the opposite side of a double-bond) (Ettinger, 2000). In natural unsaturated fatty acids have the *cis* configuration. The *cis* configuration caused the fatty acid to crimp, or bend, toward the empty side. The more double bonds per fatty acid, the more bends in the molecule, whereas *trans* unsaturated fatty acid chains are virtually straight that was similar to the structure of saturated fatty acid (Mozaffarian et al, 2006) (Fig.1).



Oleic acid (*cis* configuration)



Elaidic acid (*trans* configuration)

○ Carbon ■ Oxygen ● Hydrogen

Fig. 1. Structures of *cis* and *trans* fatty acids.

The most important *trans* isomeric fatty acids in human biology are monounsaturated fatty acid and diunsaturated fatty acid with 16 and 18 carbon atoms, such as palmitelaidic acid (C16:1 ω-7, *trans*), *trans* octadecenoic acid (C18:1 ω-9 and

C18:1 ω-7, *trans*) and *trans* octadecadiaenoic acid (C18:2 ω-6, *cis-trans*, *trans-cis*, or *trans-trans*). In the different tertiary structures, *trans* isomeric fatty acids have considerably higher melting points than the corresponding *cis* isomers. At room temperature, the predominant dietary *cis* unsaturated fatty acids, palmitoleic acid (C16:1 ω-7), oleic acid (C18:1 ω-9) and linoleic acid (C18:2 ω-6), are fluid whereas their respective *trans* isomers are solid (Koletzko et al, 1997).

2.2 Sources of *trans* fatty acids in food

Two major sources of TFA are partially hydrogenated vegetable fats and ruminant fats. In ruminant animals, TFA are formed in the forestomach by metabolic activity of rumen bacteria so *trans* fats were found in milk fat or tissue fat. Biohydrogenation of linoleic (C18:2 ω-6: LA) and alpha-linolenic (C18:3 ω-3; ALA) acids yield predominantly *trans* isomeric vaccenic acid (C18:1 ω-7, *trans*). The rate of production largely depends on the availability of LA and ALA in the roughage (Koletzko et al, 1997). In dairy fat, mean *trans*-octadecenoic acid content was found 3.42 wt% of total fatty acids, vaccenic acid was found average 48% of all C18:1 *trans* isomers (Precht and Molkentin, 1996), but may be much higher depending on feeding conditions (Kraft et al, 2003).

In industrial, TFA are mainly generated from vegetable oil polyunsaturated fatty acids, either during partial hydrogenation or during refining (Pfeuffer and Schrezenmeir, 2006). These processes convert vegetable oils into semisolid fats for use in margarines, shortening, bakery products, and packaged snacks. Fat hydrogenation process, oil are heated under pressure with hydrogen gas and a metal catalyst (usually nickel). As a consequence, different proportions of *cis* unsaturated fatty acids are transformed to *(1)* saturated fatty acids, *(2)* positional isomers with altered locations of double bonds within the molecules and *(3)* geometric isomers, i.e. *trans* fatty acids. The amount of *trans* isomeric fatty acids produced depends both on the chemical composition of the vegetable oil and on the technical conditions (heat, pressure, catalyst) of the hydrogenation (Koletzko et al, 1997). From the perspective of the food industry, partially hydrogenated vegetable oils are attractive because of their long shelf life, their stability during deep-frying, and their semisolidity, which can be customized to enhance the palatability of baked goods and sweets (Mozaffarian et al, 2006).

Content of TFA in hydrogenated vegetable oils varies in a wide range and may account for up to 60% of all fatty acids, whereas the TFA content of beef and dairy lipids accounts only for up to 5% of the total fatty acids (Stender and Dyerberg, 2003). For partially hydrogenated vegetable oil, it was found that elaidic acid (C18:1 ω-9, *trans*) is the predominant C18:1 *trans* isomer, with a wide range of 15–46% and C18:1 ω-8, *trans* isomer and C18:1 ω-7, *trans* were represented, on average 21% and 13%, respectively (Wolff et al, 2000)

Another industrial food processing which lead to the formation of some TFA is heat-deodorization of vegetable oils. Vegetable oil which was heated to about 190°C may be converted from *cis* to *trans* monounsaturated fatty acids in the order of 1-2% of total fatty acids, but poor deodorization technology with prolonged exposure to higher temperature may yield much higher amounts of TFA (Brtthl, 1995).

2.3 The transformation of *cis* and *trans* fatty acids in vegetable oils

Heat treatments, such as the frying process, have produced diverse amounts of TFA depending on the oils used. For example, *cis,cis,cis-alpha*-linolenic acid in vegetable oil can change the configuration to *cis,cis,trans* isomer and *trans,cis,cis* isomer immediately at 245°C, and continue in a linear fashion for approximately 8 hours. The *cis,cis,cis-alpha*-linolenic acid converted to *cis,cis,trans-alpha*-linolenic acid forms faster than the *trans,cis,cis-alpha*-linolenic acid, *trans,cis,trans-alpha*-linolenic acid *and cis,trans,cis-alpha*-linolenic acid respectively (Wolff, 1993). In some studies, when hemp seed oil which was heated at 170 - 250°C for 30 minute, *cis* configuration do not isomerize to *trans* configuration but at 200°C and 220°C for 16 hours and at 350°C for 30 minute *trans* configuration can be formed (Mölleken, 1998). From these experiments can demonstrate that heat treatment at high temperature and/or long period induced *trans*-isomerization.

Tsuzuki et al (2008) found that the degree of *trans*-isomerization in the edible oil was relatively low when compared with highly purified unsaturated fatty acid such as triolein, trilinolein and trilinolenin under heated at 180°C for 4 and 8 hour. Because edible oil usually contains the antioxidants tocopherols, which would prevent not only lipid oxidation but also *trans*-isomerization. These results suggest that the geometric isomerization of unsaturated fatty acids during heating accompanies lipid oxidation.

2.4 Health aspects of *trans* fatty acid

Health problems from TFA consumption are an issue of continuing research such as mechanism of *trans* fats to induce cardiovascular disease, type 2 diabetes, cancer, asthma and allergies. The mechanisms through which *trans* fats contribute to coronary heart disease are fairly well understood, while the mechanism for *trans* fat's effect on other disease are not well established.

2.4.1 Coronary heart disease

Coronary heart disease (CHD) results from impeded blood flow to the network of blood vessels surrounding the heart and serving the myocardium. The major underlying cause of CHD is atherosclerosis, which involves structural and composition changes in the innermost or intimal layer of the arteries. These changes produce impaired or inadequate blood flow. Atherosclerosis in the coronary arteries causes myocardial infarction and angina, in the cerebral arteries it causes stroked, and in the peripheral circulation it causes intermittent claudication and gangrene (Krummel, 2000).

The atherosclerosis process begins in childhood and tales decades to advance. It is known that the pathogenesis of atherosclerosis is multifactorial. The lesions that develop are the result of (1) proliferation of smooth-muscle cells, macrophages, and lymphocytes (cells involved in the inflammatory response); (2) formation of smooth-muscle cells into a connective tissue matrix; and (3) accumulation of lipid and cholesterol in the matrix around the cells. The lipid deposits and other materials (cellular waste products, calcium, and fibrin) that build up in the intimal layer are called plaque or atheroma. Plaque forms in response to injuries to the endothelium wall. Endothelial dysfunction occurs early in atherosclerosis and allows lipoproteins to accumulate in the intima. Some of the factors that cause endothelial injury are hypercholesterolemia, oxidized low-density lipoprotein, hypertension, cigarette smoking, diabetes, obesity, homocysteine, and diets high in saturated fat and cholesterol. After injury, platelets adhere to the arterial wall and release growth factors that promote lesion development. Thus, atherosclerosis is an in inflammatory and proliferative response to arterial wall injuries (Krummel, 2000).

In Thailand, cardiovascular diseases (CVD) are one of the major public health probiems. These diseases are rank in 1 of 3 of the main cause of death in Thai people. Data from Bureau of policy and strategy of the Ministry of public health in 2000 and

2001 indicated that Thai people die from CVD about 30.90 and 30.29 people per 100,000 people, respectively or about 5 people per hour (Ekpalakorn, 2003).

Data from World Health Organization (WHO) in 2005 indicated that 30 percent of world population dies from cardiovascular disease (CVD). In 2020, WHO forecast that population would die from CVD about 25 million people that included 19 million or 76 percent in developing countries (Murray, 1996). Of the CVDs, coronary heart disease is the most prevalent cause of death, followed by stroke (Mahan and Stump, 2004).

The major risk factors of coronary heart disease (CHD), known for many decades, include dyslipidemia, hypertension, smoking, and diabetes. Diet has long been known to play a key role in modifying the major risk factors for heart disease, namely, dyslipidemia and hypertension (Root and Anderson, 2004). Saturated fatty acid and TFA have increased cardiovascular risk in several studies. Based on metabolic and prospective cohort studies published in the past 10–15 years, *trans* fats have more adverse effects on the lipid profile and other cardiovascular risk factors and are more strongly associated with incident cardiovascular disease than saturated fatty acid (Erkkila et al, 2008). So, replacement of dietary saturated and *trans* fats with unsaturated fatty acid has been recommended for decades in the prevention of cardiovascular disease.

2.4.1.1 Mechanism of *trans* fatty acid to increase risk of coronary heart disease

(a) Effect on lipid profile

Several reports clearly demonstrated that modest intake of TFA can deleteriously affect lipoproteins by increasing low density lipoprotein cholesterol (LDL-C) and triglycerides but decreasing high density lipoprotein cholesterol (HDL-C) in blood level (Hargreaves et al, 1991; Stampfer et al, 1991; Castelli et al, 1992; Mensink et al, 2003; Lichtenstein et al, 2001; Dyerberg et al, 2006; Mozaffarian et al, 2006). Dietary TFA can affect plasma lipoproteins negatively in humans than saturated fatty acid. The difference between the effects of saturated and TFA on human lipoprotein metabolism was TFA depress HDL-C whereas saturated fatty acid typically increase HDL-C but both generally in conjunction with an LDL-C increase (Sundram et al, 1997).

(b) Effect on cholesteryl ester transfer protein

Cholesteryl ester transfer protein (CETP) is a hydrophobic glycoprotein that is secreted from the liver and that circulates in plasma, bound mainly to HDL-C. Cholesteryl ester transfer protein is antiatherogenic by virtue of its ability to increase the rate of reverse cholesterol transport, the pathway in which cholesterol in peripheral tissues is transported to the liver for elimination in bile. This pathway involves an initial uptake of cell cholesterol by HDL-C, where it is esterified before being transferred by CETP to LDL-C and VLDL-C. The cholesteryl esters in the VLDL-C/LDL-C pool are subsequently delivered to the liver and ultimately eliminated from the body as a component of bile. Thus, to the extent that CETP enhances the rate of reverse cholesterol transport, it may be an antiatherogenic factor. However, the fact that CETP redistributes cholesteryl esters from the nonatherogenic HDL-C to the potentially atherogenic VLDL-C/LDL-C implies that it may also be proatherogenic. Because CETP decreases the concentration of HDL-C, it may decrease the anti-inflammatory impact of this lipoprotein fraction in a process that is ultimately proatherogenic. Thus, on theoretical grounds, CETP may be either proatherogenic or antiatherogenic, depending on which of the HDL-C functions is dominant (Barter, 2000).

Some researcher investigated the acute effects of meals high in either *trans* meal or oleic acid (*cis* meal) on CETP and the apo(a) content of triacylglycerol rich lipoproteins. The result showed that ingestion of *trans* meal induces a greater postprandial increase in CETP activity and triacylglycerol-apo(a) concentrations than do meals in which TFA are replaced with oleic acid (Gatto et al, 2003). Furthermore, Tol, et al (1995) concluded that the higher CETP activity contributed to the higher LDL-C and lower HDL-C levels observed after consumption of the TFA diet. In addition to, the CETP activity was significantly inhibited by the *cis* (oleic acid) and increased by the *trans* (elaidic acid) monounsaturated isomers (Lagrost, 1992). In contrast, the study of Aro et al (1997) showed that TFA do not effect on CETP activity.

(c) Effect on systemic inflammation

Systemic inflammatory activation is an emerging risk factor for coronary artery disease, insulin resistance, diabetes, dyslipidemia, and heart failure. Elevated interleukin-6 (IL-6) concentrations are associated with insulin resistance, lipid abnormalities, coronary artery disease risk, and heart failure mortality.

C-reactive protein (CRP) and IL-6 concentrations also predict incident diabetes. Soluble tumor necrosis factor-alpha-receptors 1 and 2 (sTNF-R1 and sTNF-R2) are independently associated with insulin resistance, lipid abnormalities, coronary artery disease risk, diabetes, and heart failure mortality (Mozaffarian et al, 2004).

Tumor necrosis factor (TNF) is cytokine that produced by immune cells such as monocytes and macrophages that were activated by antigens. TNF helps regulate the inflammation by stimulate the other cytokines and inflammatory mediator production such as interleukin-1, interleukin-6, interleukin-8. Furthermore, TNF has function to stimulate fibroblast to produce adhesive molecules for induced lymphocyte move to inflammatory site (Abbas et al, 2003). C-reactive protein is plasma protein that produced by liver. This protein has gained considerable currency as a new risk factor for heart disease in the last few years and has led to a renewed interest in the role of systemic inflammation in heart disease (Root, 2004).

Intake of TFA has been positively associated with systemic inflammatory markers in healthy women such as it was positively associated with concentrations of both sTNF-R1 and sTNF-R2 by 10% and 12% higher, respectively, in the highest intake 3.9 g/day than in the lowest intake 1.8 g/day. Moreover, *trans* fats intake was positively associated with IL-6 and CRP concentrations in women with higher body mass index (Mozaffarian et al, 2004).

The risk of cardiovascular events was lowest among women with low total cholesterol levels. However, even among the women with low total cholesterol levels, the risk of cardiovascular events was significantly higher among those with high levels of CRP and serum amyloid A (cytokines such as IL-6) than among those with low levels of these markers. CRP, plasma protein produced by liver, proved to be the strongest and most significant predictor of the risk of future cardiovascular events (Ridker et al, 2000).

(d) Effect on endothelial cell function

Increasing evidence indicated the important role of endothelial dysfunction in the development of cardiovascular disease. Histological evidence confirms that vascular plaque rupture is characterised by infiltration of leucocytes. The binding of leucocytes to vascular endothelium is mediated by a variety of cell surface adhesion receptors principally the selectins (such as E-selectin) and integrins. The main endothelial integrin receptors are vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). They are induced by

endothelial dysfunction, suggesting that they may represent biological markers of atherosclerosis and might predict an increased risk of its acute manifestations. When the endothelium encounters inflammatory stimuli, E-selectin, soluble vascular cell adhesion molecule 1 (sVCAM-1) and soluble intercellular adhesion molecule 1 (sICAM-1) are over expressed. Long term consumption of *trans* fats, even at lower levels, could be detrimental to endothelial function. The biological mechanisms underlying the adverse effects of TFA on endothelial function are not clear. *Trans* fatty acids are incorporated into endothelial cell membranes and thus could alter cellular and macromolecular components acting at the interface of the blood vessel wall. This could result in changes in the antihemostatic properties, altered vascular tone, hyperadhesiveness to blood leukocytes, and increased cytokine and growth factor production, all of which are characteristics of endothelial dysfunction (Caterina et al, 2000).

In healthy women who intake high *trans* fats 3.7± 0.6 g/day compared with the lowest intake (1.5± 0.3 g/day), E-selectin, sICAM-1, and sVCAM-1 levels were 20%, 10% and 10%, respectively, higher. *trans* Fats intake was positively related to plasma concentration of sVCAM-1 and sICAM-1 (Lopez-Garcia et al, 2005).

2.4.1.2 Epidemiological studies of *trans* fatty acid and cardiovascular disease

The strongest epidemiological evidence relating levels of TFA in the diet to the risk of heart disease comes from three major prospective studies [The Health Professionals Follow-up study (USA 1996), the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (Finland 1997), and the Nurses' Health Study (USA 1997)) covering about 150,000 subjects monitored for 6-14 years and one study from the Zutphen Elderly Study (Holland 2001), which covers 667 men over an observation period of 10 years. These studies assessed the intake of TFA with the aid of a detailed questionnaire on the composition of the diet. The validity of the self-reported dietary composition was supported by random comparison between the fatty acid composition calculated on the basis of the completed questionnaire and the fatty acid composition measured in adipose tissue. These four studies all find a positive association between the intake of TFA and the risk of heart disease. The relative risk of heart disease, associated with an absolute increase of 2 per cent energy in the intake of TFA, was, following statistical correction for a large number of known risk factors

for heart disease, 1.36 (95% confidence interval 1.03-1.81) in the Health Professionals Follow-up Study; 1.14 (0.96-1.35) in the Alpha Tocopherol Beta-Carotene Cancer Prevention Study; 1.93 (1.43-2.61) in the Nurses' Health Study and 1.28 (1.01-1.61) in the Zutphen Elderly Study. All in all, the relative risk of heart disease associated with an increase in the *trans* fatty intake of 2 per cent energy in the 4 studies referred to above is 1.25 (1.11-1.40) (Stender and Dyerberg, 2003).

2.4.2 Other effects of *trans* fatty acid on health problem

2.4.2.1 Effect on human development

The recent investigations suggest that TFA may affect human fetal growth and infant development. Several clinical investigations demonstrated that TFA were inversely correlated to the level of blood plasma arachidonic acid (C20:4 ω6), docosahexaenoic acid (C22:6 ω3), the product/ substrate ratios of arachidonic acid:linoleic acid and docosahexaenoic acid:alpha-linolenic acid (Larque' et al, 2001 ; Kummerow et al, 2004 ; Decsi et al, 2001). Arachidonic acid and Docosahexaenoic acid (DHA) are important for growth and neutal development. Arachidonic acid is important in second messenger and cell signaling pathways, in cell division, and as a precursor of the thromboxane A2, interleukins, cytokines, chemokines, clotting factors, growth factors. Deficiencies of arachidonic acid also have clinical implications, including growth retardation, reproductive failure and dry skin that is characterized by augmented transdermal water loss. DHA is found in large amounts in the retina of the eye and the brain so if deficiency in DHA the central nervous system was dysfunction and the vision was impaired (Mahan and Stump, 2004). *Trans* fatty acid in the maternal diet can transfer to fetus by placenta and can cross through breast milk, so if maternal consumes a lot of foods that containing high *trans* fats its will effect to the development of fetus (Elias et al, 2001; Mosley et al, 2005).

2.4.2.2 Effect on cancer

A study across 11 European countries in 1997 showed that TFA were a strong positive significant correlation with the incidence of colorectal cancer [r = 0.93; 95% CI = 0.74 – 0.98] (Bakker et al, 1997). Women with higher TFA levels were more likely to have developed breast cancer, although the effect appeared to be confined to those individuals with the lowest levels of polyunsaturated fat (Kohlmeier et al, 1997). In contrast, the association between colorectal adenomatous polyps and the consumption of foods containing partially hydrogenated oils was examined in a case–control study no significant association was found between total dietary *trans*

fatty acids and adenomas (OR = 0.90; 95% CI = 0.40–2.0) (McKelvey et al, 1999). The evidence relating high intake of TFA to the risk of colorectal cancer is unconvincing (Nkondjock et al, 2003).

2.4.2.3 Effect on diabetes

Since TFA might interfere with cell membrane functions, there are reasons to believe that high *trans* fat intakes could affect insulin sensitivity and consequently diabetes risk. Data from controlled intervention studies investigating the effects of TFA on insulin sensitivity are reviewed. The results show no difference in the acute insulin response between the TFA diet and monounsaturated fatty acid (MUFA) diet in young healthy women (Louheranta et al, 1999). In addition, some studies showed that no significant effect of TFA on insulin sensitivity in lean healthy subjects. However, an elevated insulin response after high TFA intake versus monounsaturated fatty acid in subjects with type 2 diabetes could indicate increased insulin resistance (Ris´erus et al, 2006).

2.5 Legislation relating to the level of *trans* fatty acids in commercial food

In recent years authority agencies of many countries do concern about the potential health hazards of TFA in food. The US Department of Agriculture set up a limited intake of TFA which is 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). Unfortunately, the estimation of world wide consumption of dietary TFA in 1998 – 1999 reviewed that TFA content of the diet range from less than 1 g/person/day in Asian/Pacific countries, such as Japan and Korea, to 4–20 g/person/day in subpopulations of some Western countries such as United state, Canada, Iceland, Netherlands, Belgium and Norway (Craig-Schmidt, 2006).

From above survey, some countries announce legislation to control TFA in foods. Denmark became the first country to introduce laws strictly regulating the sale of many foods containing *trans* fats in March 2003 with a transition period until January 1, 2004. The Order has been notified to the European Union (EU) and World Trade Organization. The Danish rules impose a maximum of 2% TFA in oils and fats destined for human consumption. This means that the limit applies only to industrial product TFA but not to oils and fats of animal origin. The measure covers all oils and fats used in foodstuffs placed on the Danish market or exported from Denmark (Leth

et al, 2006). An alternative approach has been taken in the USA where the Food and Drug Administration (FDA) ruled that effective 1 January 2006 nutrition labels for all conventional foods and supplements must indicate the content of TFA. (Mozaffarian et al, 2006). For a food to be labeled *"trans* free" or "o gram *trans* fats" it must contain less than 0.2 g per serving in Canada whereas in USA was 0.5 g per serving (Mossoba et al, 2007). Currently in Thailand there is no regulation of either *trans* fats content or inclusion of *trans* fats content on food labels.

2.6 A situation of *trans* fatty acids levels in some foods in other countries

2.6.1 Denmark

The content of TFA in Danish food has been monitored since the last 30 years. For margarines and shortenings the content of TFA has steadily declined from about 10 g/100 g margarine in the 1970s to practically free TFA margarines in 1999. A broader range of food was monitored with 253 samples in 2003 and 148 samples in 2005 after the Danish regulation has been implemented. The investigations revealed that the TFA content has been reduced or removed from the products which formorly show high TFA content such as french fries, microwave oven popcorn and various bakery products. Furthermore, all *trans* fats were already removed from margarine and shortenings in Denmark market (Leth et al, 2006).

2.6.2 United State of America

In July of 2006, a survey was conducted to assess current levels of *trans* fat in three food categories: margarines and butters; cookies and snack cakes; and savory snacks. Most margarines, butters (21 of 29), cookies and snack cakes (34 of 44) were labeled as containing 0 g *trans* fat per serving. All of the products sampled in these categories were labeled as contained < 3 g *trans* fat per serving. In contrast, although most savory snacks (31 of 40) were labeled as containing 0 g *trans* fats per serving, some (n=3) were labeled as containing ≥ 3 g per serving (Albers et al, 2008).

2.6.3 Other countries

Trans fatty acid content in several foods of New Zealand, Argentina, Austria, Turkey, Pakistan, Costa Rica, Brazil, and Thailand are given in Table 1-4. The major TFA observed in all margarine brands of Pakistan was elaidic acid in the range of 2.2–34.7%. Other *trans* fats determined in the margarine samples were C18:2, *trans,trans*-9,12 and C20:3 *trans, trans, trans*-1,4,8 in the ranges of 0.1–1.5 and 0.1%, respectively. The higher amount of TFA demonstrated the poor quality of margarine

in the Pakistan's market which is harmful to the health of consumers (Kandhro et al, 2008). Most margarines in Costa Rica are soybean and palm oil base. Hard margarines were in general higher in 18:1 *trans* fatty acids, while soft margarines were higher in 18:2 *trans* fatty acids. Moreover, ruminant fat (beef) has a similar composition of 18:1 *trans* fatty acids than dairy products, and their content is higher than that for chicken and pork. The most abundant 18:1 *trans* in both beef and dairy is C18:1 ω-7,*trans* (Baylin et al, 2007).

In addition, milk fat samples from Brazil, Europe and Indonesia, collected during different seasons showed a variation of total TFA isomers of C18:2 in the range of 0.20–0.80 g/100 g total fatty acid. On the other hand, total conjugated C18:2 (*c*9,*t*11 or CLA) ranged from 0.33 to 1.37 g/100 g total fatty acid in all the analysed samples (Dionisi et al, 2002).

Table 1. *Trans* fatty acids content in bakery and snack products in various countries.

Trans fatty acid content	New Zealand (Saunders et al, 2008)	Argentina (Tavella et al, 2000)	Austria (Wagner et al, 2008)	Brazil (Martin et al, 2005)	Thailand (Pinkaew 2002)
	Mean ± SD (% of total fatty acid)	Mean (18:1n 9t) (% of total fatty acid)	Mean ± SD (g /100 g food)	Range (% of total fatty acid)	Range (% of total fatty acid
Bakery products					
Biscuits and cakes	1.1 ± 1.2				
Pies and pastry	4.3 ± 1.7				
Sliced bread		8.20			
Cookies and crackers		10.50			
Butter cookie					0.4-1.8
Butter cake					0.5-2.3
Puff pastry					0.3-5.2
Sandwich bread					0-3.7
Sausage bun					0.1-1.6
Yeast doughnut					0.1-26.2
Cake doughnut					0.2-21.2
Biscuits				12.2-31.2	
Snack products					
Snack bars	0.5 ± 0.3				
Potato chips		0.00	0.18 ± 0.31		
Cheetos and cheese-flavored sticks		6.00			

Table 2. *Trans* fatty acids content in oil and partially hydrogenated vegetable oil in various countries.

Trans fatty acids content	New Zealand (Saunders et al, 2008)	Argentina (Tavella et al, 2000)	Turkey (Karabulata et al, 2006)	Pakistan (Kandhro et al, 2008)	Costa Rica (Baylin et al, 2007)	Thailand (Pinkaew 2002)
	Mean ± SD (% of total fatty acid)	Mean (18:1n 9t) (% of total fatty acid)	Range (18:1 t) (% of total fat)	Range (% of total fatty acid)	Mean ± SD (% of total fatty acid)	% of total fatty acid
Oil						
Corn oil					1.41 ± 1.65	
Canola oil					0.79 ± 0.34	
Soybean oil					1.48 ± 0.57	
Sunflower oil					2.11± 0.96	
Sesame oil					1.24 ± 0.13	
Olive oil					0.31 ± 0.23	
Doughnut oil						13.4-28.7
Partially hydrogenated vegetable oil						
Margarines/ spreads	5.3 ± 1.6					
Margarines		27.50	0.4-39.4	2.2-34.8		0-2.1
Stick margarine					13.25 ± 2.26	
Stick light margarine					14.30 ± 2.34	
Tub margarine					10.83 ± 5.12	
Tub light margarine					11.32 ± 1.20	
Shortening			2.0 - 16.5		1.30 ± 0.52	1-2.4
Partially hydrogenated soybean oil					5.32 ± 2.39	
Non dairy coffee creamer					33.72	

Table 3. *Trans* fatty acids content in meat and dairy products in various countries.

Trans fatty acids content	Argentina (Tavella et al, 2000)	Costa Rica (Baylin et al, 2007)	Thailand (Pinkaew 2002)
	Mean (18:1 n 9t) (% of total fatty acid)	Mean ± SD (% of total fatty acid)	% of total fatty acid
Meat products			
Chicken fat		2.99 ± 2.67	
Beef fat		8.48 ± 1.18	
Pork fat		1.76 ± 0.37	
Beef			0.8-1.0
Canned tuna in oil		2.95 ± 0.92	
Canned tuna in water		1.59	
Dairy products			
Butter	4.63	6.47 ± 1.16	
Cheese		7.06 ± 1.07	
Milk		7.48 ± 1.38	0.5-0.8

Table 4. *Trans* fatty acids content in other products in various countries.

Trans fatty acids content	New Zealand (Saunders et al, 2008)	Austria (Wagner et al, 2008)	Costa Rica (Baylin et al, 2007)	Thailand (Pinkaew 2002)
	Mean ± SD (% of total fatty acid)	Mean ± SD (g /100 g food)	Mean ± SD (% of total fatty acid)	% of total fatty acid
Instant soups		2.41 ± 2.00		
Breakfast cereals		0.02 ± 0.01		
Chocolate	1.3 ± 1.5			
Mayonnaise			1.74 ± 0.69	
Pasta dishes		0.11 ± 0.10		
Pizzas		0.17 ± 0.08		
Cooled ready to eat products		0.33 ± 0.66		

Table 4. *Trans* fatty acids content in other products in various countries (continued).

***Trans* fatty acids content**	**New Zealand (Saunders et al, 2008)**	**Austria (Wagner et al, 2008)**	**Costa Rica (Baylin et al, 2007)**	**Thailand (Pinkaew 2002)**
	Mean ± SD (% of total fatty acid)	**Mean ± SD (g /100 g food)**	**Mean ± SD (% of total fatty acid)**	**% of total fatty acid**
Doughs		0.87 ± 0.98		
Ice cream			5.47 ± 0.94	
Sour cream			6.37 ± 0.32	
Mixed nuts			0.27 ± 0.08	
Eggs			0.53 ± 0.12	
Fried chicken				0-0.4
Toast with butter and sugar				0.1-2.1
Rotee				0.1-0.6
Patonggo				0-3.9

2.7 Fat extraction methods

Lipid extraction is carried out in several different ways depending on the sample matrix. Thus, some extraction methods namely, Roese-Gottlieb, Mojonnier, Folch, Werner-Schmid, Bligh-Dyer methods, etc. are based on hydrolysis (either acid, alkaline or enzymatic) before solvent extraction but some others involve only the solvent extraction step such as soxhlet. A high temperature and long extraction times with a second re-extraction step to ensure complete removal are needed for classical digestion or extraction (Priego-Capote et al, 2005). At present, methods based on supercritical fluid extraction (SFE), closed systems at high temperature and pressure, focused microwave assisted soxhlet extraction (FMASE) and dynamic ultrasound-assisted extraction (DUAE) have been proposed (Garcia-Olmo et al, 2004).

Ultrasonic irradiation of aqueous solutions induces acoustic cavitation into liquid media: when an ultrasonic wave passes though a liquid, the wave's oscillating pressure can cause a cavitation phenomenon which involves the generation, grown, oscillations, splitting and implosions of numerous tiny gas bubbles called cavitation bubbles. As a result of cavitational bubble implosion, extreme temperatures and pressures are generated at the centre of the collapsed bubble, which results in solute

thermolysis as well as the formation of hydroxyl radical and hydrogen peroxide. When a cavitating bubble collapses near the surface of a solid sample particle, microjets of solvent, propagated toward the surface at velocities greater than 100 ms^{-1}, cause pitting and mechanical erosion of the surface which leads to particle rupture and consequently, to smaller particle size. As consequence of the cavitation phenomena, when slurry is subjected to ultrasonic irradiation, the analyte present in the solid may be extracted into the liquid media (Capelo et al, 2005).

The ultrasonic irradiation allows extraction of the total fat contents in a time shorter than that required by the soxhlet extraction method. The time was shortened more than five times, from 16 to 3 hour, in the case of cookies and more than eight times, from 8 to 1 h, in the case of snacks as compared with conventional soxhlet extraction (Ruiz-Jiménez et al, 2004).

Furthermore, the ultrasound irradiation has been compared with the Folch extraction method; the proposed method could be suitable for the extraction of TFA prior to their quantification. The results of Luque de Castro et al, (2004) study showed that ultrasound irradiation accelerates neither the isolation of the target analytes without degradation nor alteration of the double bonds position. The advantages of the proposed extraction method as faster alternative to the Folch method for routine analysis have been thus demonstrated (Luque de Castro et al, 2004). Thus, ultrasound irradiation method is proposed for the determination of the total fat content in bakery products.

2.8 Solvents for extraction of fat from food

Type of solvent for lipid extraction depends on both the chemical nature of the sample and the type of lipid extract. The solvents for lipid isolation are ethers (diethyl ether, petroleum ether, isopropyl ether), hydrocarbons (hexane, benzene, cyclohexane), hydrocarbon (chloroform, dichloromethane), alcohols (methanol, ethanol, isopropanol), acetone, and acetonitrile, or their mixtures (Shahidi and Wannasundara, 1998).

Lipids are usually classified into two groups: the neutral or non-polar lipids (triglycerides, diglycerides, monoglycerides, sterols, etc.) and the more polar lipids (free fatty acids, phospholipids, sphingolipids, etc.)(Smedes et al, 1996). Neutral lipid non-polar lipids can easily be extracted by nonpolar solvents such as petroleum ether, hexane. On the other hand, if the sample contains polar lipid compounds polar

solvents such as methanol must be used for quantitative determination. A quantitative extraction of the nonpolar and polar lipids is ensured by solvent mixture (Sempore and Bezard, 1996). Folch et al. (1956) developed an extraction method using a solvent mixture of chloroform/methanol, followed by purification of the extracts with a KCl solution. Bligh and Dyer (1959) modified the existing Folch's method and obtained a rapid method for total lipid extraction and purification (Smedes et al, 1996). The non polar character of n-hexane provided a more effective extraction of the fat contents than the mixture of a polar (methanol) and a medium polar solvent (chloroform) (Garcia-Olmo et al, 2004).

Therefore, solvent used for lipid extraction from foodstuffs should have a relatively low boiling point and should be evaporated readily without leaving any residues when recovering lipids. The solvent should less toxicity and readily penetrate sample particles (Shahidi and Wannasundara, 1998).

After extraction, the fat content has traditionally been determined by gravimetry, chromatography, gas chromatography, infrared spectroscopy or nuclear magnetic resonance (NMR) (Garcia-Olmo et al, 2004). Gas chromatography (GC) and infrared spectroscopy (IR) are the two most common methods used to determine total TFA in foods (Mossoba et al, 2007). However, GC does not allow direct individual separation, and the formation of more volatile products from the analytes makes mandatory a derivatisation step, usually to fatty acid methyl esters (FAMEs); so the analysis time is considerably increased as compared with IR spectroscopy (Luque de Castro, et al, 2004).

2.9 *Trans* fatty acids determination

The quantitation and identification of TFA are complicated by the present of wide range of positional isomeric monoene, diene, and triene fatty acid in hydrogenated oils. The official methods from the Association of Official Analytical Chemists (AOAC) and the American Oil Chemists Society (AOCS) for determine TFA contents in foods are based on either gas chromatography (GC) or infrared (IR) absorption spectroscopy.

2.9.1 Gas chromatography (GC) method

The GC method involves acid digestion of the sample, extraction of the lipids with organic solvents, addition of an internal standard, and methylation to prepare fatty acid methyl esters (FAME) for GC analysis. The method is modified by use of a

100 m highly polar fused silica capillary column and tailored GC conditions to give optimum separation of *trans* isomers (Kim et al, 2007). However, even if peak co-elutions are reduced using optimal phases in columns as long as 100 m, all peaks are not perfectly resolved. This fact was a great limitation of direct GC for studies which needed individual identification and quantitation of each isomer but it may provide relatively quickly a good idea of the C 18 unsaturated fatty acid content and a rough isomer distribution, In order to obtain better separation with the same kind of columns, a pre-fractionation of *cis/trans* geometrical isomers is needed by argentation liquid chromatography (Ledoux et al, 2000). It is a time-consuming separation technology and is solvent-based.

2.9.2 Infrared spectroscopy (IR) method

The quantitation of fatty acids with isolated *trans* double bonds by IR spectroscopy has been widely used. IR procedure was recently proposed for hydrogenated fats and applied to commercial food products. By using an attenuated total reflection (ATR) cell the infrared measurement became rapid because it could then be carried out on neat (without solvent) analytes. Hence, the requirement to weigh and quantitatively dilute *trans* analytes in carbon disulfide was eliminated (Fritsche et al, 1998). IR procedure based on ATR Fourier transform infrared (FTIR) spectroscopy was voted as Official Method AOAC 2000.10 by the AOAC and Official Method AOCS Cd 14d-99 by the AOCS. Infrared spectroscopy is a technique base on the vibrations of any specific bonding of a molecule. An infrared spectrum is commonly obtained by passing infrared radiation through a sample and determining what fraction of the incident radiation is absorbed at a particular energy. The energy at which any peak in an absorption spectrum appears corresponds to the frequency of a vibration of a part of a sample molecule. Fourier transform infrared spectroscopy has dramatically improved the quality of infrared spectra and minimized the time required to obtain data. FTIR spectroscopy is based on the idea of the interference of radiation between two beams to yield and interferogram. The latter is a signal produced as a function of the change of pathlength between the two beams. The two domains of distance and frequency are interconvertible by the mathematical method of Fourier-transformation. The radiation emerging from the source is passed through an interferometer to the sample before reaching a detector. Upon amplification of the signal, in which high-frequency contributions have been eliminated by a filter, the

data are converted to digital form by an analog-to-digital converter and transferred to the computer for Fourier transformation (Stuart, 2004)

The ATR-FTIR method is based on the measurement of the intensity of an absorption band at 966 cm^{-1} unique to unsaturated fatty acids containing an isolated *trans* double bond. Spectra of saturated and *cis* unsaturated fatty acids do not exhibit this band. The procedure is extremely fast and simple. ATR utilizes the phenomenon of total internal reflection, which occurs when a beam of infrared electromagnetic radiation propagating through a crystal of very high refractive index reflects off the boundary of the crystal with a sample of lesser refractive index at an angle of incidence that is higher than the critical angle. The beam of radiation is totally reflected at the boundary, and an evanescent wave whose amplitude decays exponentially with the distance from the interface is formed in the sample (Fig. 2).

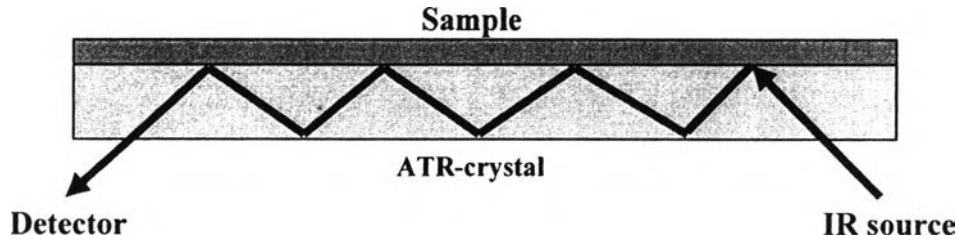


Fig. 2. The path of infrared light that reflect inside of ATR crystal.

The sample interacts with the evanescent wave, resulting in the absorption of infrared radiation by the sample. Since the penetration of the evanescent wave into the sample is on the order of a wavelength, ATR provides a short effective pathlength, enabling a minute amount of sample to be analyzed in its neat form (Milosevic, V., et al, 2004). The depth of penetration (dp) of the IR light into the *trans* fat is extremely small, and depends on angle of incidence (θ), lower refractive index (η_2), high refractive index (η_1), and the wavelength (λ), as shown in the equation, $dp = \lambda/2\pi\eta_1[\sin^2(\theta)-(\eta_2/\eta_1)^{1/2}$. As a result, the effective pathlength of the IR beam into the fat increases as λ increase or as the frequency decreases (Mossoba et al, 2007). Approximately 50 μL of melted sample is spread onto the surface of a preheated ATR crystal and the spectrum is recorded. No sample preparation other than the melting of solid fats is needed. Since only enough of the sample to cover the

surface of the crystal (as little as 0.77 μL) is needed, this method is ideally suited for the analysis of oils and fats extracted from food samples (Milosevic, V., et al, 2004).

However, the precision of this official ATR-FTIR method was limited to more than 5% *trans* fats of total fats. But a new procedure, so called negative second derivative (-2D), was used to enhance spectral features since it strongly favors higher frequency components in the Fourier decomposition of the spectrum. The precision of this method is 0.1% *trans* fat as a percentage of total fat (Milosevic, M., et al, 2004). This negative second derivative IR procedure was successfully used to eliminate interferences from both conjugated and saturated fats. Therefore, the negative second derivative procedure suitable for the rapid determination of total *trans* fats at low levels for food labeling purposes too (Mossoba et al, 2007).