การวิเคราะห์ปริมาณกรดไขมันชนิดทรานส์ในอาหารทอดในน้ำมันท่วม นมและผลิตภัณฑ์นม โดยวิธีแอตเทนนูเอเทดโททัลรีเฟลกชัน - ฟูเรียร์ทรานสฟอร์มอินฟราเรดสเปกโทรสโกปี

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ANALYSIS OF *TRANS* FATTY ACID CONTENT IN DEEP FRIED FOODS, MILK AND DAIRY PRODUCTS BY ATTENUATED TOTAL REFLECTION – FOURIER TRANSFORM INFRARED SPECTROSCOPY

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for the Degree of Master of Science in Pharmacy Program in Food Chemistry and Medical Nutrition
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Thesis Title	ANALYSIS OF *TRANS* FATTY ACID CONTENT IN DEEP FRIED FOODS, MILK AND DAIRY PRODUCTS BY ATTENUATED TOTAL REFLECTION – FOURIER TRANSFORM INFRARED SPECTROSCOPY
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ปฐมาภรณ์ สุ่นปาน : การวิเคราะห์ปริมาณกรดไขมันชนิดทรานส์ในอาหารทอดในน้ำมันท่วม นมและผลิตภัณฑ์นมโดยวิธีแอตเทนนูเอเทดโททัลรีเฟลกชัน – ฟูเรียร์ทรานสฟอร์มอินฟราเรดสเปกโทรสโกปี. (ANALYSIS OF *TRANS* FATTY ACID CONTENT IN DEEP FRIED FOODS, MILK AND DAIRY PRODUCTS BY ATTENUATED TOTAL REFLECTION – FOURIER TRANSFORM INFRARED SPECTROSCOPY) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร.ลินนา ทองยงค์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : ผศ.ดร.ชำนาญ ภัตรพานิช, 96 หน้า.

กรดไขมันไม่อิ่มตัวที่มีพันธะคู่อยู่ในรูปแบบทรานส์เรียกว่ากรดไขมันชนิดทรานส์ ซึ่งส่วนใหญ่จะพบในน้ำมันพืชที่ผ่านกระบวนการไฮโดรจิเนชันบางส่วนซึ่งนิยมนำมาใช้ในการผลิตอาหาร โดยเฉพาะอาหารทอดในน้ำมันท่วมเนื่องจากมีความคงตัวสูงในระหว่างการทอดในน้ำมันท่วม นอกจากนี้ยังพบได้ในธรรมชาติในน้ำนมและเนื้อเยื่อไขมันของสัตว์เคี้ยวเอื้อง จากการศึกษาพบว่าการบริโภคอาหารที่มีกรดไขมันชนิดทรานส์อยู่สูงอาจทำให้เกิดความเสี่ยงต่อการเกิดโรคหลอดเลือดหัวใจได้ ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อวิเคราะห์ปริมาณกรดไขมันชนิดทรานส์ในอาหารทอดในน้ำมันท่วมจำนวน 15 รายการ และในกลุ่มนมและผลิตภัณฑ์นม จำนวน 18 รายการ โดยสุ่มเก็บตัวอย่างอาหารที่จำหน่ายในประเทศไทยในช่วงเดือนกุมภาพันธ์ 2552 ถึง กันยายน 2552 ซึ่งวิธีที่ใช้วิเคราะห์คือ วิธีแอตเทนนูเอเทดโททัลรีเฟลกชัน-ฟูเรียร์ทรานสฟอร์มอินฟราเรดสเปกโทรสโกปี ปริมาณกรดไขมันชนิดทรานส์ทั้งหมดที่วิเคราะห์ได้แสดงเป็นหน่วยกรัมของกรดไขมันชนิดทรานส์ต่ออาหาร 100 กรัม ในอาหารทั้ง 11 กลุ่มดังนี้ ไก่ทอดจากร้านที่เป็นที่รู้จัก 0.08 – 0.14 ไก่ทอดจากร้านค้าข้างทาง 0.01 – 0.02 มันฝรั่งทอด 0.05 – 0.10 ปาท่องโก๋ 0.01 – 0.15 กล้วยแขก ND – 0.02 นมพาสเจอร์ไรซ์ 0.13 – 0.24 นมยูเอชที 0.09 – 0.25 ไอศกรีม 0.04 – 1.00 วิปปิ้งครีม 1.54 – 2.26 เนยแข็ง 1.38 – 2.14 และเนยเหลว 2.06 – 6.99 โดยค่าเฉลี่ยปริมาณกรดไขมันชนิดทรานส์ในอาหารกลุ่มตัวอย่างอยู่ในช่วง 0.01 – 6.99 กรัมต่ออาหาร 100 กรัม จะเห็นได้ว่ากลุ่มอาหารทอดในน้ำมันท่วมในการศึกษานี้มีปริมาณกรดไขมันชนิดทรานส์ต่ำ แต่อย่างไรก็ตามควรจำกัดการบริโภคอาหารกลุ่มนี้เนื่องจากเป็นอาหารที่มีไขมันสูงและอาจมีสารประกอบที่เป็นอันตรายต่อสุขภาพเกิดขึ้นในระหว่างการทอด ส่วนในนมและผลิตภัณฑ์นมพบว่ามีเนยเหลวยี่ห้อหนึ่งที่มีปริมาณกรดไขมันชนิดทรานส์สูงมากคือ 6.99 กรัมต่ออาหาร 100 กรัม

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507 65761 33 : MAJOR FOOD CHEMISTRY AND MEDICAL NUTRITION
KEYWORDS: *TRANS* FATTY ACID/ FOURIER TRANSFORM INFRARED SPECTROSCOPY/ DEEP FRIED FOODS/ MILK AND DAIRY PRODUCTS

PATAMAPORN SOONPAN : ANALYSIS OF *TRANS* FATTY ACID CONTENT IN DEEP FRIED FOODS, MILK AND DAIRY PRODUCTS BY ATTENUATED TOTAL REFLECTION – FOURIER TRANSFORM INFRARED SPECTROSCOPY. ADVISOR : THESIS ASST. PROF. LINNA TONGYONK, D.Sc, THESIS CO-ADVISOR : ASST. PROF. CHAMNAN PATARAPANICH, Ph.D., 96 pp.

The unsaturated fatty acid which has the double bond in the *trans* configuration is called *trans* fatty acid (TFA). TFAs are mostly found in partially hydrogenated vegetable oil which are commonly used in food process, especially in deep fried foods. It is because of high stability during deep-frying. Moreover, naturally occurring TFAs are found in milk and lipid tissues of ruminant. Several researchers showed that high consumption of diet high in TFAs was associated with greater risk of cardiovascular disease. Therefore, this study was conducted to investigate the TFA content in fifteen samples of deep fried foods and eighteen samples of milk and dairy products which were available in Bangkok area between February 2009 and September 2009, using attenuated total reflection fourier transform infrared spectroscopy. The total TFA content in this study expressed as grams of TFA content per 100 grams of food sample in eleven groups of foods were as follows: fried chicken from well-known fast food 0.08 – 0.14, fried chicken from street vender 0.01 – 0.02, french fries 0.05 – 0.10, deep fried dough stick (pa-tong-koh) 0.01 - 0.15, deep fried banana (kluay-kag) ND – 0.02, pasteurized milk 0.13 – 0.24, UHT milk 0.09 – 0.25, ice-cream 0.04 – 1.00, whipping cream 1.54 – 2.26, cheese 1.38 – 2.14 and butter 2.06 – 6.99. The mean TFA values in all selected foods ranged from 0.01 – 6.99 g/100 g food. From the present study, deep fried food category exhibited low TFA content. However, consumers should avoid and limit consuming this food because it contains high fat and may have some harmful compounds, which occur during frying. In milk and dairy products, the highest content of TFA was found in the selected brand of butter (6.99 g/100 g food).

Department:….Food and Pharmaceutical Chemistry….Student's signature.....
Field of Study:.Food Chemistry and Medical Nutrition.Advisor's signature.....
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LIST OF ABBREVIATIONS

ºC	degree celsius
-2D	negative second derivative
Ag	silver-ion
ALA	alpha linolenic acid
AOAC	Association of Official Analytical Chemists
AOCS	the American Oil Chemists Society
ATR	attenuated total reflection cell
CETP	cholesteryl ester transfer protein
CHD	coronary heart disease
CLA	conjugated linoleic acid
CVD	cardiovascular disease
CRP	C-reactive protein
DHA	docosahexaenoic acid
DUAE	dynamic ultrasound-assisted extraction
FAME	fatty acid methyl esters
FDA	Food and Drug Administration
FMASE	focused microwave assisted soxhlet extraction
FTIR	fourier transform infrared spectroscopy
GC	gas chromatography
HDL-C	high density lipoprotein cholesterol
HPLC	high performance liquid chromatography
ICAM-1	intercellular adhesion molecule-1
IL-6	interleukin-6
IR	infrared spectroscopy
LA	linoleic acid
LDL-C	low density lipoprotein cholesterol
Lp[a]	lipoprotein a
MTFA	monounsaturated *trans* fatty acids
ND	non detectable at the level of traces
NMR	nuclear magnetic resonance
PHVO	partially hydrogenated vegetable oil
PTFA	polyunsaturated *trans* fatty acids
SD	standard deviation
SFE	supercritical fluid extraction
SPE	solid phase extraction
TFA	*trans* fatty acid
TLC	thin layer chromatography
TNF	tumor necrosis factor
TNF-R	tumor necrosis factor receptor
VLDL-C	very low density lipoprotein cholesterol
VCAM-1	vascular cell adhesion molecule-1
WHO	World Health Organization
dp	depth of penetration
wt%	percent by weight

no.	number
ml	milliliter
min	minute
v/v	volume by volume
rpm	rounds per minute
mg	milligram
g	gram
ppm	part per million
et al	*et alia* (and others)

CHAPTER I

INTRODUCTION

In recent years, *trans* fatty acids (TFAs), more commonly known as *trans* fats, have been gaining a lot of interest from the scientific and health professional communities primarily because of the potential role of *trans* fatty acids on cardiovascular risk. Due to the increasing evidences of the effects of TFA on public health, many counties have mandated the declaration of the TFA content on the nutrition labels for all conventional foods and supplements.

Fatty acids constitute the main class of lipids in the human diet, being found in nature mainly as glycerol esters that originate triacylglycerols. In the vegetal and animal kingdoms, fatty acids generally have *cis* unsaturations. In this form, the hydrogens bound to the double bond carbons are on the same side. In another possible configuration, called *trans*, the hydrogens are bound to unsaturation carbons on opposite sides (Martin et al, 2007). Monounsaturated *trans* fatty acids (MTFA), a group of fatty acids that have only one unsaturation, which is necessarily in the *trans* form. Polyunsaturated *trans* fatty acids (PTFA), similarly to their *cis* counterparts, have two or more unsaturations, either all *trans* or not (Dutton ,1979 ; Wolff, 1992).

These fatty acids are found in two major sources, natural and industrial sources. In natural source, *trans* fats originate from milk fat and tissue fat of ruminants such as cows, goat and sheep. Bacteria in their stomaches can produce TFA by biological hydrogenation process. Industrial TFA are mainly generated from vegetable oil polyunsaturated fatty acids, either intentionally during partial hydrogenation or unintentionally during refining (Pfeuffer and Schrezenmeir, 2006).

The concern with the ratio of intake of foods containing high TFA amounts has grown in the recent years mainly due to the hazardous effects of these lipids on plasma lipoproteins that increase low density lipoprotein (LDL-C) and lipoprotein a(Lp[a]) levels and decrease the levels of high density lipoprotein (HDL-C). This condition contributes to increase the LDL-C/HDL-C ratio, which is considered an important indicator of the risk of development of cardiovascular diseases (Ascherio et

al., 1999 ; Hunter, 2005 ; Mozaffarian et al., 2006; Lichtenstein et al., 2001 ; Dyerberg et al., 2006). In addition, TFA also promote systemic inflammatory responses in healthy persons (Mozaffarian et al., 2004). Moreover, TFA may affect human fetal growth and infant development (Larque´ et al., 2001 ; Kummerow et al., 2004 ; Decsi et al., 2001).

Since *trans* fats consumption produces specific health problems especially in elevating risk of coronary heart disease. So, this consumption of such TFA in population was considerably concerned by dietitians. Many kind of foods were collected and determined the level of TFA content in some countries such as Argentina, USA, Costa Rica, Austria, New Zealand, etc (Tavella et al., 2000; Baylin et al., 2007; Albers et al., 2008; Saunders et al., 2008; Wagner et al., 2008). The two common identification methods for total TFA detection in food are based on gas chromatography (GC) and infrared spectroscopy (IR) (AOAC, 2005).

During gas chromatographic (GC) analysis fatty acid in samples need to be converted into its corresponding volatile methyl esters prior to separation in a very long capillary columns (100 m) which coated with highly polar stationary phases. However GC analysis time can be taken up to 1.5 hours injection. Another major drawback of the GC analysis is the overlapping of sample peaks, such problem can be solved by prior fractionation of sample of *cis* and *trans* isomers with silver-ion thin layer chromatography (Ag-TLC), silver-ion solid phase extraction (Ag-SPE) or reversed phase high performance liquid chromatography (HPLC). In addition, lack of standards chemical of all *trans* fatty acid isomers is also problematic to GC analysis, while the excessive processing during partial hydrogenation, heating and oxidation may leads to the formation of many *trans* containing fatty acid isomers (Milosevic et al., 2004 ; Destaillats et al., 2007). Therefore, GC method may not be the most appropriate choice for routine determination of TFA in labeling purposes.

Another alternative method, infrared spectroscopic method (IR) is specific and rapid analytical method for the determination of total TFA. The quantitative of total TFA by infrared spectroscopic method is base on the C-H out-of-plane deformation band observed at wave number 966 cm^{-1}, which is uniquely characteristic of isolated *trans* double bonds, regardless of the chain length or the position of the isolated *trans* double bond. (Mossoba et al., 2007) The new attenuated total reflection – fourier

transform infrared (ATR-FTIR) spectroscopy official method can be conveniently applied to determination of the total *trans* configuration content of fats in the vast majority of food products which containing more than 5 % *trans* fat, as percent of total fat (AOAC, 2005). In addition, it was recently reported that by using the negative second derivative (-2D) instead of the absorption spectrum itself, spectral features were enhanced such that TFA levels as low as 0.5 % could be readily measured (Milosevic et al., 2004).

The purpose of this study was to determine the TFA content in deep fried foods, milk and dairy products which distributed in Thailand during February 2009 and September 2009 by ATR-FTIR. The results can be the basic for setting upthe limit of TFA content in these kinds of foods in Thailand. Additionally, for labeling purposes, the study intends to propose the IR method as fast in routine TFA determination instead of slow the GC technique which currently use in Thailand.

Operational Definition of Terms

Deep fried dough stick: Thai common name is pa-tong-koh. It is one of Chinese desserts which is prepared by wheat flour and cut into short stick and pressed two sticks together. Deep-frying in oil is done until golden brown.

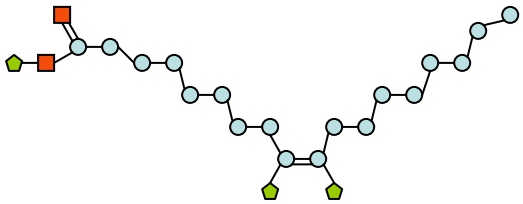
Deep fried banana: Thai common name is gluay-kag or gluay-tod. It is one of Thai desserts, which is prepared by slicing bananas and dipping them in flour and deep-frying in oil until golden brown.

CHAPTER II

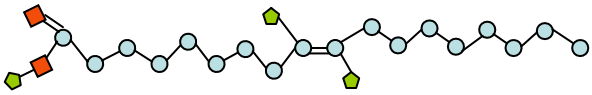
LITERATURE REVIEW

2.1 Physicochemical properties of *trans* fatty acids

Fatty acid isomers are classified into two types, positional and geometric isomers. Positional isomers are formed when double bonds of the fatty acid molecule shift from their original position to other positions in the molecule. Geometric isomers of unsaturated fatty acids are categorized into two forms 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) (Figure 1). The *cis* fatty acids, which are commonly found in natural oils and fats, are relatively more reactive and require lower activation energy to be transformed to the *trans* isomer. On the other hand, TFA have a linear structural conformation that permits tighter stacking of molecules, hence, allowing them to have melting points similar to those of saturated fatty acids (Ettinger, 2000; Lai and Lo, 2006). The melting points of the two forms are very different with the *trans* isomers having the higher melting points. It was this higher melting behavior which made the TFA so valuable in commerce (Dijkstra et al, 2007).



Oleic acid (*cis* configuration)



Elaidic acid (*trans* configuration)

Carbon Oxygen Hydrogen

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

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 are found in milk fat or tissue fat. Biohydrogenation of linoleic (C18:2 ω-6: LA) and alpha-linolenic acids (C18:3 ω-3*;* ALA) yield predominantly *trans* isomeric vaccenic acid (C18:1 ω-11, *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, while vaccenic acid was found 40-70% of all C18:1 *trans* isomers (Precht and Molkentin, 1996), and may be much higher depending on feeding conditions (Kraft et al, 2003). The concentration of TFA in dairy and meat fats is usually low: from 3-8% of total fat (Kodali and List, 2005).

In food industry, TFA are mainly generated from vegetable oil polyunsaturated fatty acids, either during partial hydrogenation or during refining (Pfeuffer and Schrezenmeir, 2006). During 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 parametress (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 (Mozaffarian et al, 2006). Partial hydrogenation of vegetable oils brings about desirable physical and chemical characteristics to foods cooked in these oils, giving them distinctive flavor, crispness, creaminess and plasticity (Dijkstra et al, 2007). Wolff et al. (2000) found that elaidic acid (C18:1 ω-9, *trans*) is the predominant C18:1 *trans* isomer in partially hydrogenated vegetable oil, with wide range of 15-46%, and the C18:1 (ω-9, *trans*) isomer is the second (mean, 21%) and vaccinic acid represented on average 13%.

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 deodorizing 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, can produce diverse amounts of TFA depending on the oils used. Deep-fat frying is probably one of the most dynamic processes in all of food processing. Essentially, the processes involve immersing a food item in a large quantity of heated oil or fat, which is normally replenished and reused several times before being disposed. Deep-fat frying produces a product with desired sensory characteristics, including fried food flavor, golden brown color, and a crisp texture (Warner, 2004). For example, French fries were fried over seven consecutive days at 185±5^{o}C or 215±5^{o}C. The amount of TFA formed during frying increased when temperature and time increased. When the low frying temperature (185±5^{o}C) applied, the amount of *trans* isomers increased from 2.4% to 3.3%. The increase in frying temperature to 215^{o}C caused extensive *trans* isomerisation of fatty acids. The total contribution of *trans* isomers in oil increased 2.5-fold, from 2.4-5.9% (Aladedunye and Przybylski, 2009) Furthermore,Wolff (1993) found that *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. 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 and/or reusing oil several times induced *trans*-isomerization.

Tsuzuki et al (2008) showed 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 Influence of *trans* fatty acids on health

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 effect of TFA 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 internal 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 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 developed the results 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 disease (CVD) is one of the major public health problems. This disease is 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 died 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 died from 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

Randomized controlled trials consistently showed that TFA increased LDL-cholesterol (LDL-C) similar to SFA, and decreased HDL-cholesterol (HDL-C) compared with SFA. The results showed a dose-dependent relationship between TFA intake and the ratio of LDL-C:HDL-C and TC:HDL-C that was stronger than that for SFA since SFA also increased HDL-C (Ascherio, 2006). Results from a meta-analysis of clinical studies demonstrate that the replacement of carbohydrate with TFA caused

an increasing in the ratio of TC:HDL-C and this effect was almost twice the magnitude of replacing a mixture of SFA for carbohydrate (Mensink et al, 2003). In the Nurses' Health Study, higher levels of total TFA in red blood cells were associated with increased LDL-C and ratios of LDL-C:HDL-C. Furthermore, individuals who consumed the most TFA had a 3.3 times higher risk for developing CHD compared to those who consumed the least amount of TFA (Sun, 2006). Lichtenstein et al (1999) demonstrated that TFA consumption resulted in a dose-dependent increasing in LDL-C and decreasing in HDL-C. Six experimental diets that provided 30% energy from total fat were evaluated. Two-thirds of the fat was provided by either soybean oil (<0.5 g TFA per 100 g of fat), semi-liquid margarine (<0.5 g per 100 g), soft margarine (7.4 g per 100 g), shortening (9.9 g per 100 g), stick margarine (20.1 g per 100 g), or butter (1.25 g per 100 g). Compared with the butter diet, the vegetable fat diets elicited the following reductions in TC, LDL-C, and HDL-C: (1) soybean oil diet: 10, 12, and 3%, respectively; (2) the semi-liquid margarine diet: 10, 11, and 4%, respectively; and (3) the stick margarine diet: 3, 5, and 6%, respectively. The stick margarine diet increased the TC:HDL-C ratio by 40%; the other vegetable fats decreased it. The diets containing the fats with the least TFA, the soybean oil and semi-liquid margarine diets, had the most beneficial effects on lipids/lipoproteins. In addition to the effects on LDL-C and HDL-C, TFA increased triglycerides and Lp(a), when substituted for SFA. It has been estimated that reducing TFA intake by 2% of energy would result in a decreasing in triglyceride levels of approximately 3 mg/dl (Ascherio et al, 1999). Furthermore, increasing amounts of TFA resulted in an increasing in small dense LDL particles in a dose-dependent manner, when compared with a diet rich in SFA (Mauger et al, 2003). As reviewed by Ascherio et al (1999), an estimated reduction in TFA intake of 2% of calories would result in a 7% reduction in CHD mortality due to the effects on the ratio of LDL-C:HDL-C. Intervention studies that demonstrate the specific effects of individual TFA isomers on blood lipids are lacking.

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;

Lichtenstein et al, 2001; Mensink et al, 2003; 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 circulates in plasma and 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 currently 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).

Evidence from both randomized, controlled trials and observational studies have suggested that TFA may have an adverse effect on inflammatory markers. In a randomized, controlled trial in 50 healthy men, consumption of TFA 8% of total energy for 5 weeks increased plasma levels of IL-6 and CRP compared with consumption of equivalent amounts of oleic acid (*cis*-18:1), and also increased plasma levels of CRP compared with equivalent carbohydrate consumption (Baer et al, 2004). Among 19 individuals with hypercholesterolemia, consumption of TFA 6.7% of total energy compared with consumption of TFA 0.6% of total energy for 1 month increased production of IL-6 and TNF by macrophages (Han et al, 2002). In the Nurses' Health Study, TFA intake, assessed by semiquantitative food-frequency questionnaires, was positively associated with TNF receptor levels in healthy women,

and was also associated with levels of CRP and IL-6 in women with overweight (Mozaffarian et al, 2004).

Proinflammatory effects of TFAs could account, at least partly, for adverse effects on clinical cardiovascular end points. For instance, increased levels of CRP associated with median dietary TFA intake of 2.1% of total energy as opposed to the levels associated with 0.9% of total energy would predict an approximate 30% increase in the risk of coronary heart disease (Lopez-Garcia et al, 2005).

(d) Effect on endothelial cell function

Endothelial dysfunction is an early sign of both metabolic dysfunction and atherosclerotic disease. Histological evidence confirms that vascular plaque rupture is characterized 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 (Caterina et al, 2000).

In addition to proinflammatory effects, evidence suggests that TFA could also impair endothelial function and such effects could be more substantial than those of saturated fatty acid. A randomized, controlled trial in 50 men revealed that increased TFA consumption resulted in elevated levels of E-selectin (Baer et al, 2004). This result is consistent with the findings of an observational study which a habitually high intake of TFA is associated with increased levels of E-selectin and soluble cell adhesion molecules (Lopez-Garcia et al, 2005). In a randomized, controlled trial, TFA intake 9% of total energy for 4 weeks reduced flow-mediated vasodilation in the brachine artery by nearly one-third, compared with values seen with saturated fatty acid intake (de Roos et al, 2001). Conversely, in a second trial, TFA intake 9% of total energy for 8 weeks produced no different effect on similar measures from those of omega-3 fatty acid or saturated fatty acid intake (Dyerberg et

al, 2004). Thus, on the basis of changes in circulating biomarkers and functional measures, TFA consumption might adversely affect endothelial health; further investigation is needed to confirm the presence, magnitude, and dose response of such effects (Micha and Mozaffarian, 2009).

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 come 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. All of four studies confirmed the 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 percent energy in the 4 studies referred to above was 1.25 (1.11-1.40) (Stender and Dyerberg, 2003).

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

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 which is characterized by augmented transdermal water loss. DHA is found in large amounts in the retina of eye and brain, therefore the deficiency in DHA may cause the central nervous system dysfunction and the vision impairment (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 mother 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

The relationship between dietary fat and risk of cancer has been the subject of copious research studies worldwide. In epidemiological studies, cancer of the breast, colon, rectum and prostate are the common types of cancers that have been extensively investigated in relation to fat intake (Dijkstra et al, 2007).

The EURAMIC Study in several European countries and Israel showed that cancers of the breast and colon were associated with *cis* monounsaturated fatty acids and positively with TFA, based on mean fatty acid composition of adipose tissue samples (Bakker et al, 1997; Kohlmeier et al, 1997). In relation to colorectal cancer, the overall evidence seems to indicate a lack of consistent positive association between intake of TFA and risk of colorectal cancer. Among cohort studies, the Women's Health Study on about 40,000 US-women showed that total fat intake was not related to colorectal cancer risk, neither were intakes of individual fat types nor major fatty acids including TFA (Lin et al, 2004) However, they found a positive association for fried foods (e.g. French fried, fried chicken and fried fish; RR = 1.86 between fifth and first quintile intake).

As for risk of prostate cancer, there is some evidence indicated that dietary fat was associated with increasing of prostate cancer risk and that fatty acids may had unique effects on prostate cancer risk. For TFA intake, epidemiological

evidence from a study on about 15,000 US physicians reported that plasma levels of TFA was associated with increased risk of developing prostate cancer, which was specific to non-aggressive tumors (Chavarro et al, 2008). The β-Carotene and Retinol Efficacy Trial (CARET), a randomized trial of supplemental β-carotene and retinol for the prevention of lung cancer among 18,314 heavy smokers and asbestos-exposed workers, began in 1985 and ended prematurely in 1996 when it was determined that the supplements increased risk of lung cancer, cardiovascular disease and total mortality but had no effects on prostate cancer incidence or mortality. However, the authors found consistent trends for increasing prostate cancer risk with higher serum levels of C18 but not C16 TFA (King et al, 2005).

2.4.2.3 Effect on diabetes

Since TFA might interfere with cell membrane functions, studies have been conducted to examine the effects of TFA on insulin sensitivity and consequently diabetes risk. In the large prospective Nurses Health Study on about 85,000 US female nurses who were followed for 16 years, Salmeron et al (2001) found that while polyunsaturated fatty acid intake was associated with a substantial reduction in diabetes risk, TFA and dietary cholesterol were associated with increased risk. The authors estimated that replacing 5% of energy from saturated fatty acid with energy from polyunsaturated fatty acid was associated with a 35% lower risk and that replacing 2% of energy from TFA with polyunsaturated fatty acid was associated with a 40% lower risk. In contrast, others did not find consumption of TFA significantly associated with diabetes risk. These include the prospective studies on male health professionals (Meyer et al, 2001; Van Dam et al, 2002) and randomised crossover studied (Louheranta et al 1999; Lovejoy et al, 2002). As pointed out by Ris´erus (2006), the literature suggests that TFA has no significant effect on insulin sensitivity in lean healthy subjects.

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

In recent years authority agency of many countries consider the potential health hazards of TFA in food. The Department of Agriculture of America 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 worldwide 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 States, Canada, Iceland, Netherlands, Belgium and Norway (Craig-Schmidt, 2006).

From above survey, some countries declared the 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 to indicate *trans* fats on nutrition labeling in food packaging.

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

2.6.1 Thailand

Two studies analyzed TFA content in various food categories including bakery products, oil, partially hydrogenated oil, dairy products and fried foods. They revealed that there were TFA contents during not detectable to 3.42 % of

total fat in shortenings and margarines. TFA contents ranged of 0 – 17.53% of total fat in bakery products, 0 – 3.9 % of total fat in fried foods. These results showed varying TFA contents in Thai foods (Pinkaew, 2002; Narkwichian, 2008). TFA contents of some products in Thailand show in Table 1.

Table 1. *Trans* fatty acids content in various products in Thailand.

Products	***Trans* fatty acids content (% of total fatty acid)**	
	Pinkaew, 2002	**Narkwichian, 2008**
Partially hydrogenated vegetable oil		
Shortening	1.0 - 2.4	1.85 - 3.42
Margarines	0 - 2.1	1.85 - 2.22
Bakery products		
Butter cookie	0.4 - 1.8	1.02 - 17.53
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	
Brownie		2.86 - 3.51
Sandwich chocolate cookie		ND - 0.74
Croissant		0.93 - 3.97
Rich butter bun		2.22 - 6.65
Crispy pie		1.76 - 2.59
Cracker		ND - 0.74
Dairy products		
Milk	0.5 - 0.8	
Butter	0.8 - 2.0	
Fried foods		
Fried chicken	0 - 0.4	
Deep freid dough stick	0 - 3.9	

ND = Non detectable at the level of traces

2.6.2 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 10g/ 100g 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 decreased or diminished from the products which formally 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).

2.6.3 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 , cookies and snack cakes 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 foods in this group were labeled as containing ≥ 3 g per serving (Albers et al, 2008).

2.6.4 Other countries

TFA content in several foods of New Zealand, Argentina, Austria, Turkey, Pakistan, Costa Rica, Brazil. 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. In Pakistan's margarine market, higher TFA content of the product indicated poor quality and believed to be harmful to consumer's health (Kandhro et al, 2008). Baylin et al (2007) observed *trans* fatty acids in meat and dairy products in Costa Rica. They showed that these products had TFA content between 1.59 to 8.48 % of total fat.

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 acids. 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 acids in all the analysed samples (Dionisi et al, 2002).

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 and Luque de Castro, 2004).

Moreover, the ultrasound irradiation, by using hexane as solvent, has been compared with the Folch extraction method in the study of Ruiz-Jimenez et al. (2004) indicated that both methods had similar efficiencies, as well as percent of TFA content and the ultrasound irradiation did not alter the double bond position. Moreover, this method is faster than the Folch method. These good results demonstrated the ability of the ultrasound irradiation for extracting fat for TFA determination which could substitute the Folch method in routine analysis. Thus, ultrasound irradiation method is proposed for the determination of the total fat content in deep fried food in this study.

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), chlorinated 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 and Thomased, 1996). Neutral lipid non-polar lipids can easily be extracted by non-polar solvents such as petroleum ether, hexane. On the other hand, if sample contains polar lipid compounds polar solvents such as methanol must be used for quantitative determination. A quantitative extraction of the non-polar 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 and Thomased, 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 gravimetric, chromatographic, gas chromatographic, infrared spectroscopic or nuclear magnetic resonance (NMR) methods (Garcia-Olmo et al, 2004). Gas chromatographic (GC) and infrared spectroscopic (IR) methods 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 current official methods from for determinating TFA content in foods are based on methods from the Association of Official Analytical Chemists (AOAC) and the American Oil Chemists Society (AOCS) which consist of gas chromatography (GC) or infrared (IR) absorption spectroscopy.

2.9.1 Gas chromatographic (GC) method

The GC method has been the most widely used analytical method to analytical method to analyze fatty acid from foods by hydrolytic methods which involves acid digestion of the samples, extraction of the lipids with organic solvents, addition of an internal standard, and methylation to prepare fatty acid methyl esters (FAME) (Kim et al, 2007). The most recent GC methods to determine TFA describe separations that require long capillary columns with highly polar stationary phases. Under these conditions, a separation is based on the chain length of the fatty acid, degree of unsaturation, and the geometry and position of double bonds. *Trans* positional isomers are followed by *cis* positional isomers, but there is extensive overlap of the geometric isomers (Kodali and List, 2005). Elimination of GC peak overlap usually

requires prior separation of the *cis* and *trans* 18:1 geometric isomers by silver ion-TLC (Wolff and Precht, 2002; Kramer et al, 2002).

The effect of this GC peak overlap on the accuracy of TFA determinations for ruminant fats and partially hydrogenated vegetable oil depends on identifying all the observed GC peaks in widely different and complex chromatographic profiles, after calibration with as many reference standards as available commercially or otherwise (Kodali and List, 2005).

2.9.2 Infrared (IR) spectroscopic method

The rapid determination of total TFA by IR spectroscopy has been a widely used standard procedure. This methodology is based on the measurement of the height of or area under the 966 cm^{-1} C–H out-of-plane deformation band, which is uniquely characteristic of isolated double bonds with trans configuration. By contrast, conjugated trans double bonds absorb near 985 and 945 cm^{-1} (conjugated *cis*/*trans*) and near 990 cm^{-1} (conjugated *trans*,*trans*). Thus measuring the intensity of the absorption of the *trans* band effectively sums up all the fatty acids containing isolated trans double bonds, but excluding those with conjugated *trans* double bonds (Mossaba et al, 1991). IR procedure based on attenuated total reflection (ATR) fourier transform infrared (FTIR) spectroscopy was the official method AOAC 2000.10. The ATR-FTIR procedure is extremely fast and simple. The ATR technique allows for the measurement of 50 microliters of neat oils (without solvent) or melted fats (at approximately $65^{o}C$) without the time-consuming requirement of having to quantitatively prepare solutions in a volatile and toxic carbon disulfide solvent (Mossoba et al, 2009).

Infrared spectroscopic method 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 the vibration of a part of the sample molecule. FTIR 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)

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 (Figure 2).

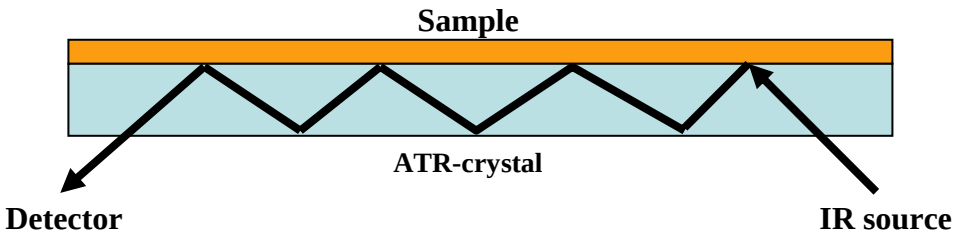


Figure 2. The path of infrared light that reflect inside of ATR crystal.

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 microliters of melted sample is spreaded 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 et al, 2004). In addition, this IR procedure requires the measurements to be carried out in the ATR mode rather than the conventional transmission mode. The ATR technique is advantageous in many respects. The effective pathlength in ATR is inherently precise because it depends solely on the number of internal reflections, the angle of incidence, the wavelength, and the refractive indices of the ATR crystal used and the test sample investigated at a given temperature.

However, the precision of this official ATR-FTIR method was limited to more than 5% *trans* fats of total fats. To improve sensitivity and accuracy and meet the labeling requirement, a new ATR–FTIR procedure called negative second derivative (-2D) was developed. A second derivative is traditionally used to enhance spectral features. Advantages of measuring the second derivative of the *trans* absorption band include: (a) problems associated with the baseline offset and slope no longer exist since the height of the second derivative is directly proportional to the amount of total trans fat in a test sample; (b) the need for a *trans*-free or any reference background oil is eliminated; and (c) a second derivative has a narrower bandwidth than an absorption band, and therefore allows the detection of interference bands that are adjacent to the 966 cm^{-1} band of interest. Finally, the ATR–FTIR measurement is rapid (5 min), and the negative second derivative procedure does not require any derivatization of the oil or fat test material to its corresponding FAMEs (Mossoba et al, 2009).

CHAPTER III

MATERIALS AND METHODS

3.1 Instruments

An ultrasonic bath (Transsonic Digital TP680DH, Elma, Germany) 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.

TFA content was determined by Fourier transform infrared spectrometer (Perkin Elmer Spectrum One FTIR, USA) and Zinc selenide crystal (ZnSe through 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, ethyl ether and ammonium hydroxide were purchased from J. T. Baker Chemicals Co. (Phillipsburg, NJ, USA) and anhydrous sodium sulfate was obtained from Merck (Darmstadt, Germany). 95% Ethanol was obtained from BDH (Poole, England).

3.3 Methods

3.3.1 Sample selection

Some kinds of deep fried foods including deep fried dough stick and deep fried banana were purchased from street vender in 3 difference places. French fries were bought from 3 well-known fast foods. Fried chicken was obtained from 3 well-known fast food and 3 others from 3 street venders in difference places. In milk and dairy products, they were selected from 3 difference brands. All of samples were purchased in Thailand during February 2009 and September 2009 and were recorded the place of purchasing.

3.3.2 Sample preparation

For deep fried foods, samples were crushed into small pieces and then homogenized again and stored in polyethylene bag at 4°C until use. After extraction, the fat was weighed and frozen at -10°C until analysis (within 1 day after extraction) For fried chicken breast, de-boned was done before chopping (meat and skin parts used).

Milk and dairy products were prepared according to AOAC official method 925.21 (AOAC, 2005). Briefly, test sample was thawed to 20°C in water bath and was mixed until homogeneous.

3.3.3 Experimental design

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

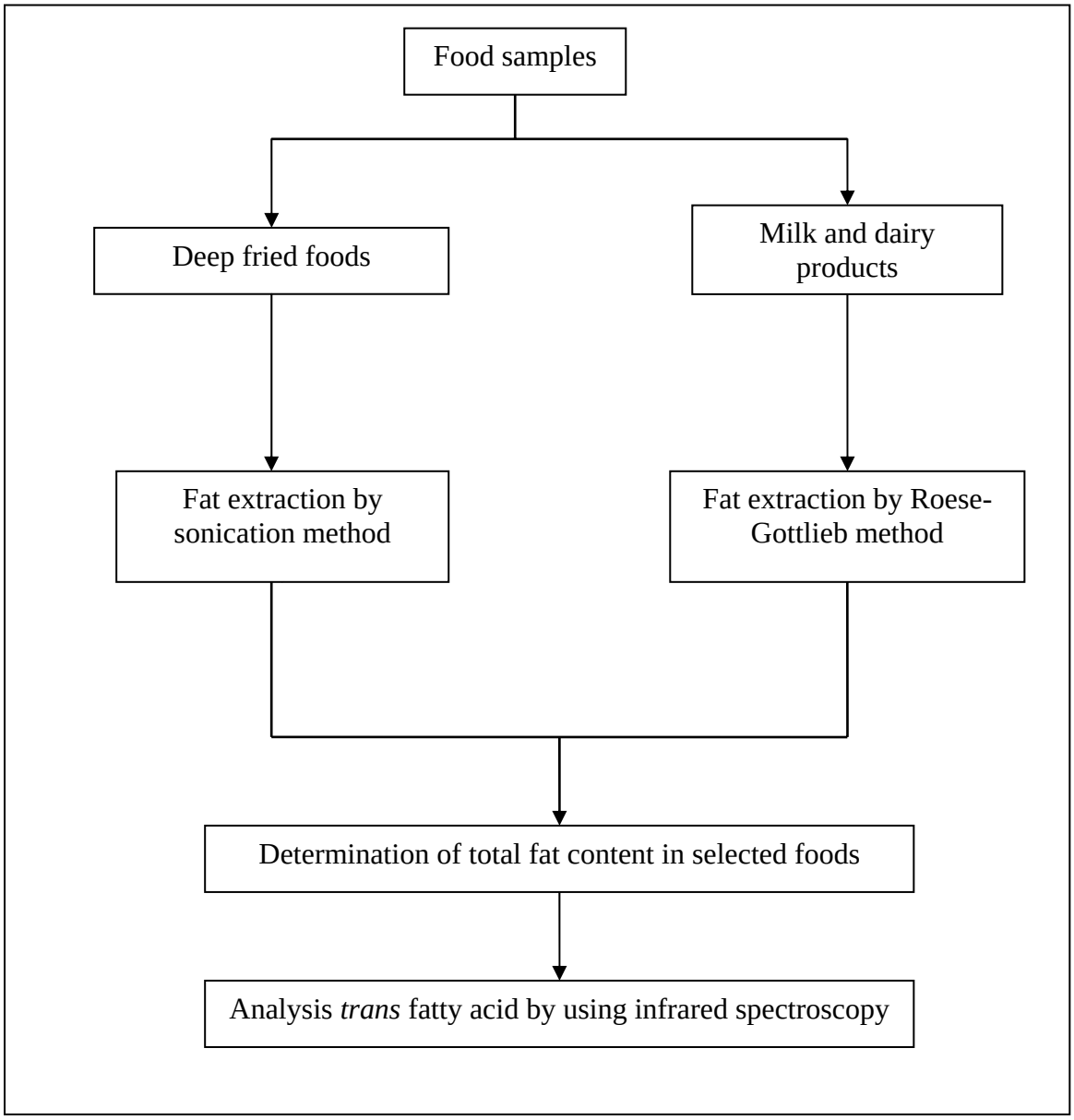


Figure 3. Overall experiment to determine total fat and *trans* fatty acids content in samples.

3.3.4 Fat Extraction

(I) Extraction of deep fried foods

To find out the optimum extraction conditions required for obtaining high lipid yield, two parameters including extraction time and ultrasonic intensity levels were investigated. Each condition was done in tripicate. The results obtained were compared with the values declared in the nutrition fact label of the products (90 – 110 % of label amount). The appropriate condition was further utilized to extract fat from other deep fried foods. Extractions were carried out according to the following procedure:

(a) Ultrasonic intensity levels

Four grams of each sample and beads were placed in a 250 ml round bottom flask containing 60 ml of *n*-hexane. The sample-solvent suspension was immersed into the ultrasonic bath using at 20%, 40%, 60%, 80% and 100% ultrasonic intensity levels for 120 minutes. After extraction, the mixture was filtered through filter paper, Whatman no.42. If the filtrate was cloudy it should be centrifuge at 2000 rpm for 10 minutes. The filtrate was evaporated by rotary evaporator and the extract was dried in vacuum desiccator for 90 minute. Finally, chose the intensity that obtained the highest lipid yield.

(b) Extraction time

The extraction was carried out in the same manner as described in section (a) using the selected ultrasonic intensity level that obtained high lipid yield for 30, 60, 90, 120 and 150 minute. The appropriate time which gave the highest lipid yield was selected.

(II) Extraction of milk and dairy products

All samples were extracted by Roese-Gottlieb method. In briefly, ten greams of sample was weighed, to nearest mg, into a tube and added 1.5 ml NH_4OH and then was mixed thoroughly. After that, ten of 95% ethanol was added and was mixed well. Then twenty five milliliters of ethyl ether was added, stopper with stopper and was shaken very vigorously 1 minute. Twenty five ml of petroleum ether was added and was repeated vigorous shaking and was stood until upper layer is practically clear. The upper solution was decanted into 125 ml pear-shaped flask. The lower layer remaining in tube was repeated extraction twice and 15 ml of each solvent

was used in each time and distilled water was added, if necessary. The 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 minutes.

3.4 Determination of *trans* fatty acids content of deep fried foods, milk and dairy products

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 for the calibration curve preparation. 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, 5, 10, 30 and 50% *trans* calibration standards.

3.4.3 Preparation of test samples

The fat sample was melted gently on water 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 operational 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. Each repeated fat extract was scanned 3 times. After each analysis, ATR cell was cleaned by rinsing with acetone.

3.4.5 Calculations

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

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

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

CHAPTER IV

RESULTS

4.1 Optimization of fat extraction conditions for deep fried foods

In this study, sonication method was used for the isolation of fat from deep fried foods. In order to obtain lipid yields, the appropriate conditions for lipid extraction including ultrasonic intensity levels and extraction time parameter were investigated. In this study, the optimum condition for fat extraction was investigated by using french fries as a representative of deep fried foods.

4.1.1 Effect of ultrasonic intensity levels on lipid yields and TFA contents

The effect of ultrasonic intensity levels on lipid yield by *n*-hexane as a solvent at 60^{o}C is shown in Table 2. It was found that lipid yield at 40% intensity for 120 minutes was the highest and TFA content at this intensity level was detected significantly higher than other levels.

Table 2. Effect of ultrasonic intensity levels on lipid yields and TFA contents.

Ultrasonic intensity level (%)	Average lipid yield [a] (g/100g sample) N=3	Average TFA content [a] (g/100g sample) N=3
20	8.83	2.44
40	19.00	5.63
60	15.58	4.44
80	14.47	4.15
100	13.86	3.98

[a] lipid yield and TFA content of French fries are extracted by *n*-hexane as solvent for 120 minutes

4.1.2 Effect of extraction times on lipid yields

The effect of extraction times on lipid yields by *n*-hexane as a solvent at 60^{o}C at 40% ultrasonic intensity is given in Table 3. The data showed 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 deep fried foods.

Table 3. Effect of extraction time on lipid yields.

Extraction time (min)	Average lipid yield [a] (g/100g sample) N=3
30	7.59
60	8.33
90	8.74
120	9.18
150	8.61

[a] lipid yield of french fries is extracted by *n*-hexane as solvent at 40% ultrasonic intensity

4.2 Determination of total fat contents in deep fried foods, milk and dairy products

Total fat contents of deep fried foods, milk and dairy products were determined by sonication method and Roese-Gottlieb method, respectively. 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 triplicate (Table 4). Different brands contained the different amount of fat due to difference fats and oil source to make their products. In group of deep fried foods, the highest amount of average total fat content was found in fried chicken from well-known fast food (20.73 ± 4.20 g/100g food) and the lowest was found in french fries (14.12 ± 24.73 g/100g food). And the milk and dairy products group, average total fat content of butter (80.12 ± 25.47 g/100g food) was the highest and pasteurized milk was the lowest (4.11 ± 12.27 g/100g food) in this group.

Table 4. Total fat contents of deep fried foods, milk and dairy products.

Products	Total fat content (g/100g food)	
	Range [a]	Mean ± SD
Fried chicken from well-known fast food (n=3)	20.25 – 21.04	20.73 ± 4.20
Fried chicken from street vender (n=3)	10.52 – 23.58	15.00 ± 74.33
French fries (n=3)	11.27 – 15.74	14.12 ± 24.73
Deep fried dough stick (n=3)	16.25 – 31.00	22.60 ± 75.84
Deep fried banana (n=3)	15.37 – 18.34	16.98 ± 15.02
Pasteurized milk (n=3)	2.97 – 5.41	4.11 ± 12.27
UHT milk (n=3)	4.77 – 5.34	5.14 ± 3.18
Ice-cream (n=3)	8.22 – 17.74	11.43 ± 54.65
Whipping cream (n=3)	35.52 – 44.83	39.16 ± 49.78
Cheese (n=3)	18.69 – 27.55	24.09 ± 47.36
Butter (n=3)	77.42 – 82.48	80.12 ± 25.47

[a] Data are expressed as mean of triplicate extraction

4.3 Determination of *trans* fatty acid content of deep fried foods, milk and dairy products

4.3.1 Total *trans* fatty acid contents in deep fried foods, milk and dairy products

The total *trans* isomers contents of selected foods were determined by 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 5-6). Graph of the negative second derivative of the *trans* absorption band relative to air of these samples are shown in Appendix B and C.

Table 5. The height of the negative second derivative of *trans* absorption band of deep fried foods.

Products			**Peak Height (-)** [a]	**Average peak height (-)**
	brand	**flask**		
Fried chicken from well-known fast food	1	a	0.0007	0.0006
		b	0.0006	
		c	0.0005	
Fried chicken from well-known fast food	2	a	0.0006	0.0006
		b	0.0005	
		c	0.0006	
Fried chicken from well-known fast food	3	a	0.0007	0.0007
		b	0.0007	
		c	0.0007	
Fried chicken from street vender	1	a	0.0005	0.0004
		b	0.0003	
		c	0.0004	
Fried chicken from street vender	2	a	0.0004	0.0004
		b	0.0004	
		c	0.0004	
Fried chicken from street vender	3	a	0.0004	0.0004
		b	0.0004	
		c	0.0004	

[a] Data are expressed as mean of triplicate scans

Table 5. The height of the negative second derivative of *trans* absorption band of deep fried foods (continued).

Products	brand	flask	Peak Height (-) [a]	Average peak height (-)
French fries	1	a	0.0007	0.0007
		b	0.0007	
		c	0.0007	
French fries	2	a	0.0006	0.0006
		b	0.0005	
		c	0.0006	
French fries	3	a	0.0005	0.0005
		b	0.0005	
		c	0.0006	
Deep fried dough stick	1	a	0.0006	0.0005
		b	0.0005	
		c	0.0005	
Deep fried dough stick	2	a	0.0006	0.0006
		b	0.0006	
		c	0.0006	
Deep fried dough stick	3	a	0.0004	0.0004
		b	0.0003	
		c	0.0004	
Deep fried banana	1	a	0.0004	0.0004
		b	0.0004	
		c	0.0004	
Deep fried banana	2	a	0.0005	0.0005
		b	0.0005	
		c	0.0005	
Deep fried banana	3	a	0.0003	0.0003
		b	0.0003	
		c	0.0003	

[a] Data are expressed as mean of triplicate scans

Table 6. The height of the negative second derivative of *trans* absorption band of milk and dairy products.

Products	brand	flask	Peak Height (-) [a]	Average peak height (-)
Pasteurized milk	1	a	0.0027	0.0027
		b	0.0027	
		c	0.0025	
Pasteurized milk	2	a	0.0026	0.0026
		b	0.0026	
		c	0.0026	
Pasteurized milk	3	a	0.0029	0.0027
		b	0.0027	
		c	0.0026	
UHT milk	1	a	0.0029	0.0029
		b	0.0029	
		c	0.0029	
UHT milk	2	a	0.0021	0.0021
		b	0.0020	
		c	0.0021	
UHT milk	3	a	0.0013	0.0014
		b	0.0014	
		c	0.0014	
Ice-cream	1	a	0.0010	0.0008
		b	0.0009	
		c	0.0007	
Ice-cream	2	a	0.0007	0.0006
		b	0.0006	
		c	0.0005	
Ice-cream	3	a	0.0035	0.0034
		b	0.0034	
		c	0.0034	

[a] Data are expressed as mean of triplicate scans

Table 6. The height of the negative second derivative of *trans* absorption band of milk and dairy products (continued).

Products	brand	flask	Peak Height (-) [a]	Average peak height (-)
Whipping cream	1	a	0.0031	0.0031
		b	0.0031	
		c	0.0031	
Whipping cream	2	a	0.0027	0.0027
		b	0.0027	
		c	0.0027	
Whipping cream	3	a	0.0026	0.0026
		b	0.0026	
		c	0.0026	
Cheese	1	a	0.0032	0.0032
		b	0.0032	
		c	0.0032	
Cheese	2	a	0.0047	0.0046
		b	0.0045	
		c	0.0045	
Cheese	3	a	0.0047	0.0047
		b	0.0047	
		c	0.0047	
Butter	1	a	0.0053	0.0053
		b	0.0053	
		c	0.0052	
Butter	2	a	0.0034	0.0034
		b	0.0034	
		c	0.0034	
Butter	3	a	0.0017	0.0017
		b	0.0017	
		c	0.0017	

[a] Data are expressed as mean of triplicate scans

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

Table 7. Composition of triolein and trielaidin 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

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 0.9998 (Figure 4).

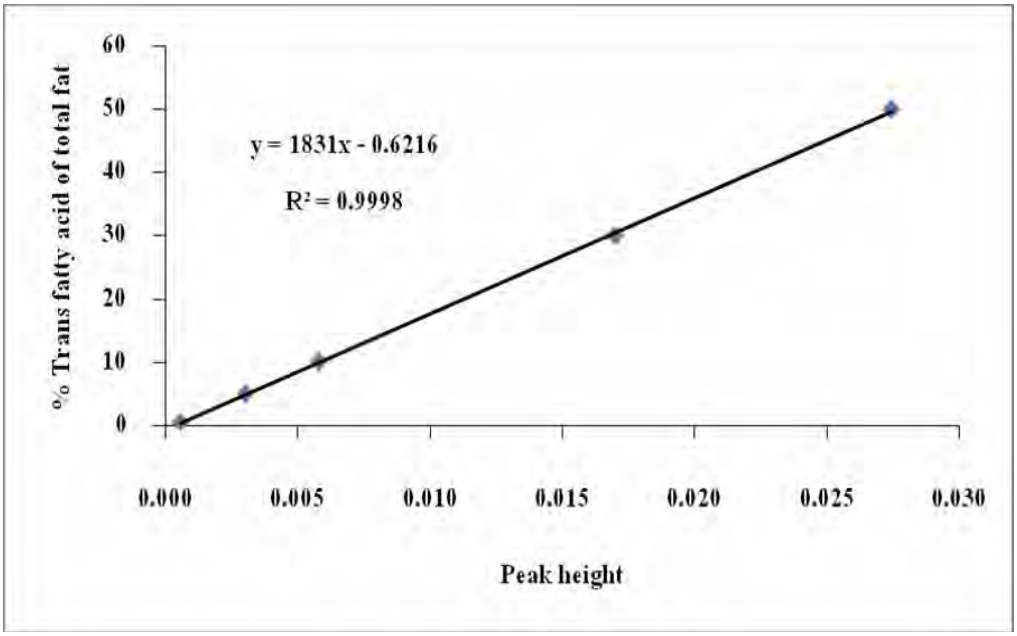


Figure 4. The graph of standard calibration of *trans* fatty acid.

The percentage of *trans* isomers was calculated by using the regression equation of *trans* standards mixtures calibration. Table 8 shows the results of *trans* fatty acid contents in selected foods. The total *trans* fatty acids contents of deep fried

foods ranged from 0.12 to 0.51% total fat or 0.01 to 0.11 g/100 g food and milk and dairy products ranged from 2.35 to 7.00% total fat or 0.17 to 4.52 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 8. *Trans* fatty acid contents in deep fried foods, milk and dairy products.

Products	***Trans* fatty acid content**			
	% of total fat		**g/ 100 g food**	
	Range	**Mean ± SD**	**Range**	**Mean ± SD**
Fried chicken from well-known fast food (n=3)	0.40 – 0.66	0.51 ± 0.13	0.08 – 0.14	0.11 ± 0.03
Fried chicken from street vender (n=3)	0.09 – 0.13	0.12 ± 0.02	0.01 – 0.02	0.01 ± 0.01
French fries (n=3)	0.36 – 0.64	0.47 ± 0.15	0.05 – 0.10	0.07 ± 0.03
Deep fried dough stick (n=3)	0.07 – 0.48	0.30 ± 0.21	0.01 - 0.15	0.07 ± 0.07
Deep fried banana (n=3)	ND – 0.27	0.19	ND – 0.02	0.04
Pasteurized milk (n=3)	4.08 – 4.38	4.28 ± 0.17	0.13 – 0.24	0.18 ± 0.06
UHT milk (n=3)	1.86 – 4.73	3.30 ± 1.44	0.09 – 0.25	0.17 ± 0.08
Ice-cream (n=3)	0.48 – 5.65	2.35 ± 2.86	0.04 – 1.00	0.37 ± 0.54
Whipping cream (n=3)	4.14 – 5.03	4.50 ± 0.47	1.54 – 2.26	1.78 ± 0.42
Cheese (n=3)	5.28 – 7.96	7.00 ± 1.49	1.38 – 2.14	1.67 ± 0.41
Butter (n=3)	2.49 – 9.02	5.70 ± 3.27	2.06 – 6.99	4.52 ± 2.47

ND = Non detectable at the level of traces

The highest average amount of total *trans* fat in foods 100 grams was found in butter and followed by whipping cream, cheese, ice-cream, pasteurized milk, UHT milk, fried chicken from well-known fast food fried, french fries, deep fried dough stick, deep fried banana and chicken from street vender, respectively.

4.3.2 Total *trans* fatty acid contents per serving in deep fried foods, milk and dairy products

Table 9. *Trans* fatty acid contents per serving in deep fried foods, milk and dairy products.

Products	Serving (g)	*Trans* fatty acid content (g/ serving)
Fried chicken from well-known fast food	60	0.07
Fried chicken from street vender	60	0.01
French fries	70	0.05
Deep fried dough stick	25	0.02
Deep fried banana	30	0.01
Pasteurized milk	200	0.36
UHT milk	200	0.34
Ice-cream	60	0.22
Whipping cream	20	0.36
Cheese	21	0.35
Butter	10	0.45

Table 9 shows that TFA content per serving of deep fried foods ranged from 0.01 to 0.07 grams and milk and dairy products ranged from 0.22 to 0.45 grams.

CHAPTER V

DISCUSSION

This study focused on two food categories, are widespread foods in city population, including deep fried foods which used frying oil, one of the major dietary source of industrial TFA, and milk and dairy products which contain TFA from natural. Two standard methods which are available in AOAC (2005) for determining total TFA content are gas chromatography (GC) and infrared (IR) absorption spectroscopic methods. In this study, the attenuated total reflection (ATR) fourier transform infrared (FTIR) spectroscopic method, which is more was advantageous than the GC method, was selected for determination total TFA content in samples. 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 determined by GC and ATR-FTIR were compared. In study of Ali et al (1996) showed that TFA content of the products contained greater than 5% of total fat, determined by ATR-FTIR were higher than those determined by GC but products with TFA content less than 5%, the results determined by the GC procedure were significantly higher than those obtained by the ATR-FTIR procedure. 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 *trans* fat higher than 5% (Milosevic et al, 2004). At low TFA levels, a potentially significant interference has been reported in products containing conjugated fatty acids. This interference is due to the presence of conjugated fatty acids which occur near 985, 950 and 990 cm^{-1}. However, to improve this problem and increase sensitivity, 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. This negative second derivative IR procedure was successfully used to eliminate both the baseline offset and slope of the *trans* IR

band. Moreover, this method also made it possible small shifts in IR band position and the presence of low interferences (Mossoba et al, 2007). Milosevic 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 the negative second derivative ATR-FTIR method is capable to determine low level of TFA content. In study of Bansal et al (2009), comparison of TFA content in frying oil samples showed that the negative second derivative ATR-FTIR method produced higher amounts than those obtained by GC. Therefore, negative second derivative ATR-FTIR method was used for determining total TFA content in this study.

Fat has a strong influence on the palatability of fried foods. The inclusion of cooking fat into crusty surface, which is developed by the frying process, helps in building up the crunchiness that is highly appreciated by consumers. On the other hand, the linkage between overconsumption of fat and several diseases has been well-documented (Bouchon, 2009). Several studies have provided evidence that TFA consumption has effects on both lipid and non-lipid risk factors of cardiometabolic health. Deep fat frying has been considered a source for TFA production. Formation of this fat during frying has been shown to be correlated with processing, temperature and time. In sunflower oil, the amount of TFA were found to be 1.10% when heated at 200^{o}C for 40 minutes as compared to 11.45% at 300^{o}C for the same duration of heating (Moreno et al, 1999) In this study, deep fried food category of Thailand had high total fat content but had TFA content less than 0.2 g/100 g food (ND – 0.15g/100g food). It was low when compared with a study of Pinkaew (2002) which found that deep fried dough stick had average 2.2% TFA of total fat while in this study found 0.3% TFA of total fat. It may be due to the difference source of oil used or/and frequency time of reused oil. Therefore, it is recommended that the frequent using of fresh oil in frying process help to reducing TFA content in deep fried foods.

From the results of deep fried foods in this study, the total fat content did not correlate with total TFA content. Food manufacture may use non-hydrogenated oils higher than partially hydrogenated oils in frying process in Thailand. Moreover, outlets, which was sampling, may not reuse oil several times in frying. However, current nutrition recommendations point to a reduction of total dietary fats, including

trans and saturated fatty acids. In addition, important nutritional compounds degrade during the process, and toxic molecules may generate either in the foodstuff or in the frying oil itself, whose intake should be at least limited. In April 2002, Swedish scientists sounded an alarm when they discovered that certain high levels of acrylamide, a chemical compound that is listed by the World Health Organization (WHO) as a probable human carcinogen (Mitka, 2002). This substance has been shown to be produced when food is heated above 120^{o}C due to a reaction between amino acids and reducing sugars (Mottram et al, 2002)

Ruminant-produced TFAs are made by bacterial metabolism of polyunsaturated fatty acids in the rumen of ruminants, and consequently present in all milk and dairy products from these animals. Concentration of TFA in ruminant fat varies with the feed of the animals and with the seasons (Jakobsen et al, 2006). The results in this study found that samples in this group had TFA 0.48 – 9.02 % of total fat (0.04 – 6.99g/100g food) which was quite variably. These results were similar to other countries. The amount of TFA in milk and butter from most European samples is generally below 5%. Australian and New Zealand butters have slightly higher proportions of TFA, at 6% and above (Parodi and Dunstan, 1971; Richardson et al., 1997; Saunders et al., 2008). TFA content of cheeses in most European and American cheeses contain 2 – 5% TFA. TFA contents of ice-cream samples made from dairy fats have 2.6 to 6% (Lai and Lo, 2006). However, in this study, TFA content in pasteurized milk, UHT milk and butter were 4-fold higher than the results of Pinkaew, which studied these samples in Thailand in 2002. It may be effected by altering the cows' feed.

Generally, results from epidemiological studies of intake of ruminant-produced TFA and risk of coronary heart disease (CHD) have indicated that intake ruminant TFAs is innocuous or even protective against CHD. Two prospective cohort studied have revealed an inverse association between energy-adjusted ruminant TFA intake and risk of CHD (Willett et al, 1993; Pietinen et al, 1997). However, Oomen et al (2001) found non-significant direct associations between the intake of ruminant produced TFA and risk of CHD. The effect of ruminant TFA on plasma lipid and lipoprotein risk-markers of CHD has been investigated in one human, randomized, cross-over study in 22 males and 24 females, fed either 11-12 g/day of ruminant TFA

or industrially produced TFA for 3 weeks, with a 1-week wash-out period in between. There was apparently no difference in the effect of ruminant TFA on LDL or HDL concentration compared with the effect of industrially produced TFA in males, whereas both LDL and HDL were significantly higher after intake of ruminant TFA in females (Chardigny et al, 2006). Unlike elaidic acid from industrially produced TFA, vaccenic acid from either industrially produced TFA or ruminant produced TFA can be converted to rumenic acid, most notably by ruminant animals, but also in nonruminant animals as well as in humans. Rumenic acid is a so-called conjugated linoleic acid (CLA) which may have positive metabolic effects, although the results in humans have been contradictory. It is noteworthy that a high intake of industrially produced TFA may provide more vaccenic acid than an average intake of ruminant produced TFA. If vaccenic acid has a beneficial effect on coronary risk, this must be more than counteracted by the harmful effect of the other industrially produced TFA to explain the negative association between the intake of industrially produced TFA and coronary risk (Stender et al, 2008). Weggemans et al. (2004) reviewed that, at higher intakes, both intake of total TFA and intake of industrially produced TFA were found to be associated with an increased risk of CHD, but there are insufficient data available on ruminant TFA.

The absence of a higher risk of CHD associated with the intake of ruminant TFA as compared with the intake of industrially produced TFA may be because of lower levels of intake (typically less than 0.5 percent of total energy intake), different biologic effects of different isomers, or the presence of other factors in ruminant products that balance any effects of the amount of TFA they contain. Although each of these potential explanations deserves further investigation, the sum of the current evidence suggests that the public health implications of consuming TFA from ruminant products are relatively limited (Mozaffarian et al, 2006).

The results of this study, when TFA contents per serving of foods was calculated, noticed that all of samples had TFA contents lower than 0.5 grams. Therefore, if the labeling regulation is ruled to indicate TFA content when products has its over 0.5 gram/serving, consumers will see “*Trans* fat 0 g” in label of these products.

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 aware and avoid risk of intake of TFA in deep fried foods by notice color and appearance of frying oil or color of fried products and limit the intake of high fat foods.

CHAPTER VI

CONCLUSION

This study was performed to determine the *trans* fatty acid contents in deep fried foods, milk and dairy products which distributed in Thailand. Attenuated total reflection fourier transform infrared spectroscopy and the negative second derivative IR procedure was used to achieve peak identification of *trans* fats. The results in the present study indicated that average total TFA content of deep fried foods was less than 0.2 g/100 g food. It was low when compared with the other countries. Average total TFA of fried chicken from well-known outlet was the highest. Furthermore, in milk and dairy products category, the results in this study found that they had TFA 0.04 – 6.99g/100g food. Average total TFA of butter was higher than whipping cream, cheese, ice-cream, UHT milk, and pasteurized milk respectively.

Current situation in Thailand, there are no TFA labeling regulations and the consumer has no possibility either to identify sources of TFA or to choose between low or high TFA content. In order to reduce the intake of TFA, we recommend that the government and health care providers should advice consumer about how to avoid the main foods containing TFA.

In this study, food samples were collected from supermarket, well-known fast food and street vender in Bangkok only. For further studies, food samples should be collected from a variety of location to provide more informative to represent the widely of Thailand marketplace and assess TFA contents in the various foods. In order to estimate intake and support decision making regarding risk management, there is a need to continue to assess the content of TFA in the various foods in Thailand.

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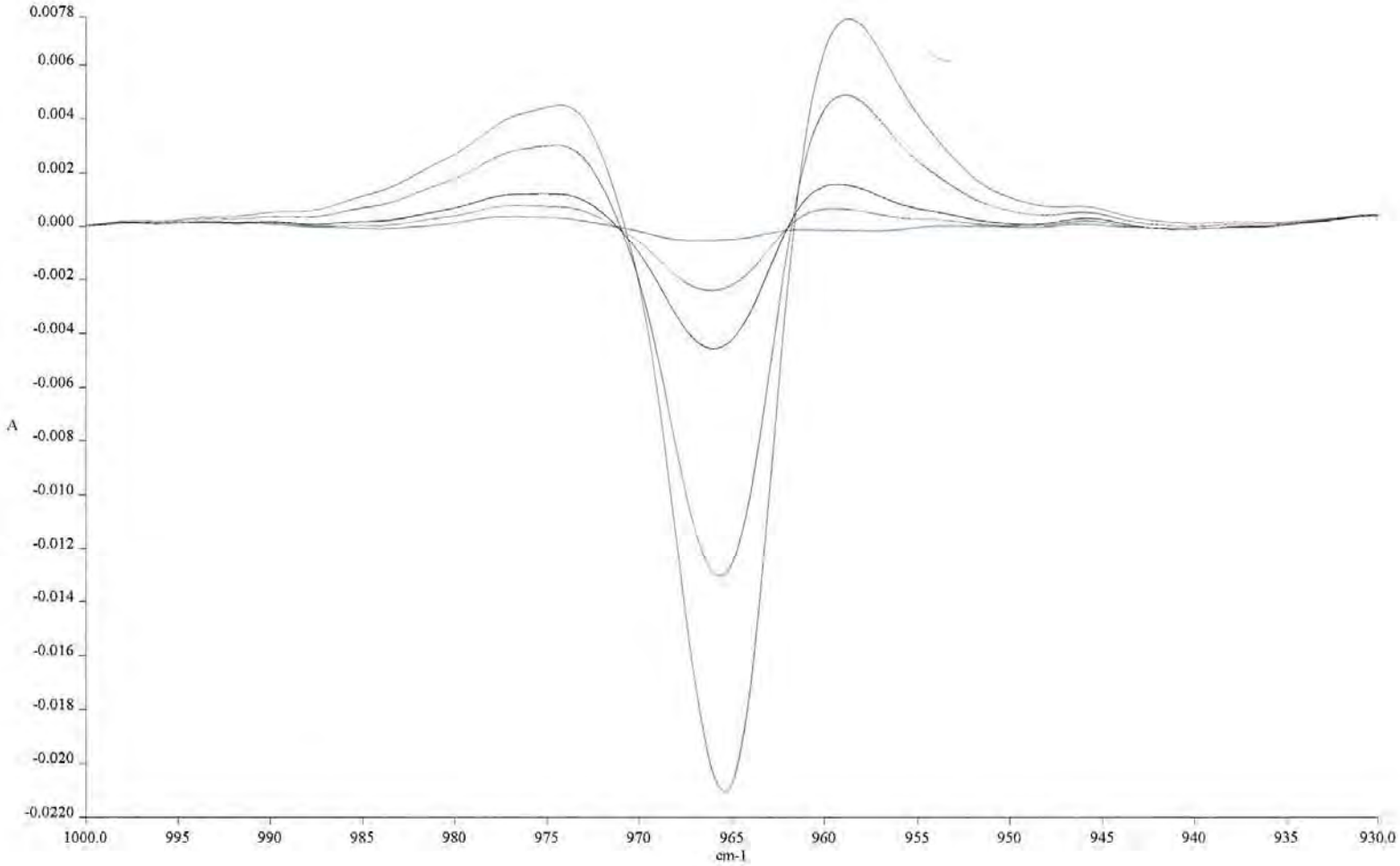
APPENDICES

APPENDIX A

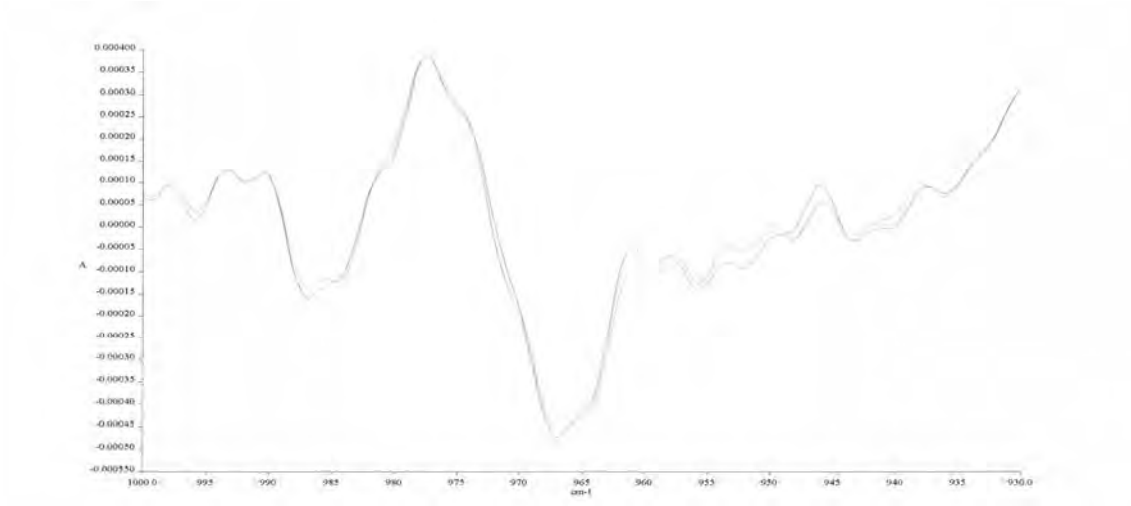
Graph of the negative second derivative of the *trans* absorption band relative to air of standard solution



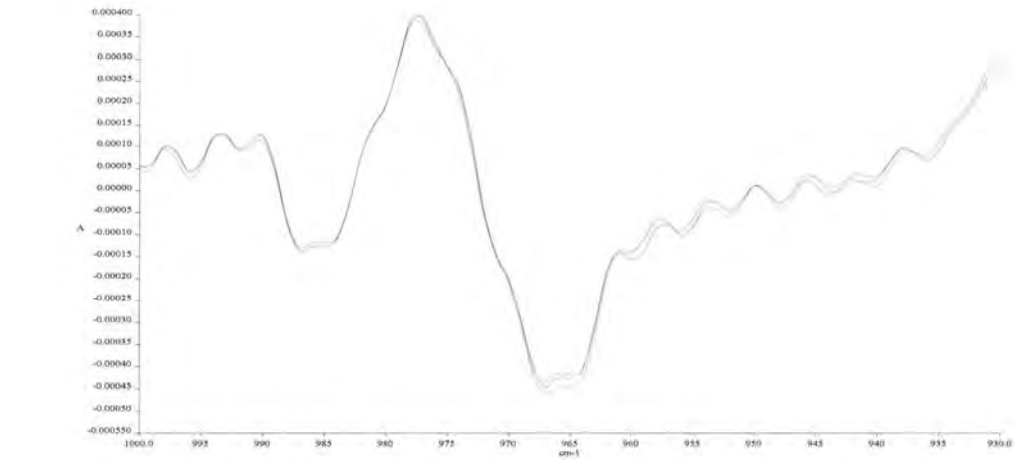
Graph of the negative second derivative of the *trans* absorption band relative to air of standard solution

APPENDIX B

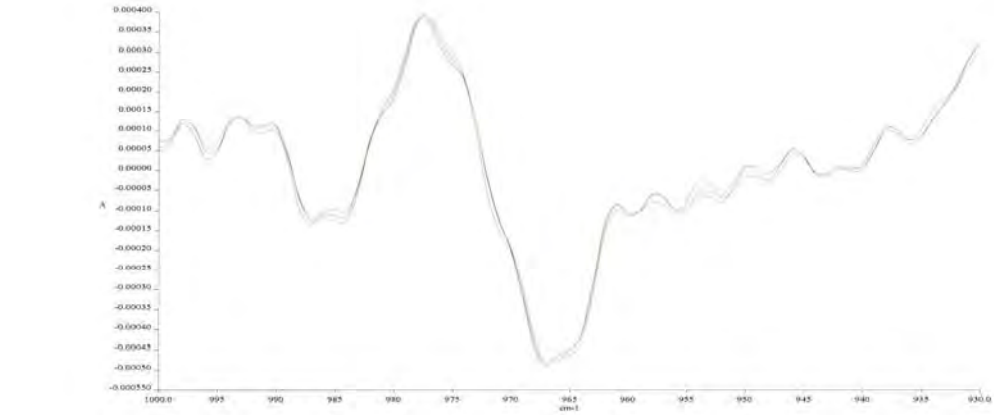
Graph of the negative second derivative of the *trans* absorption band relative to air of deep fried foods



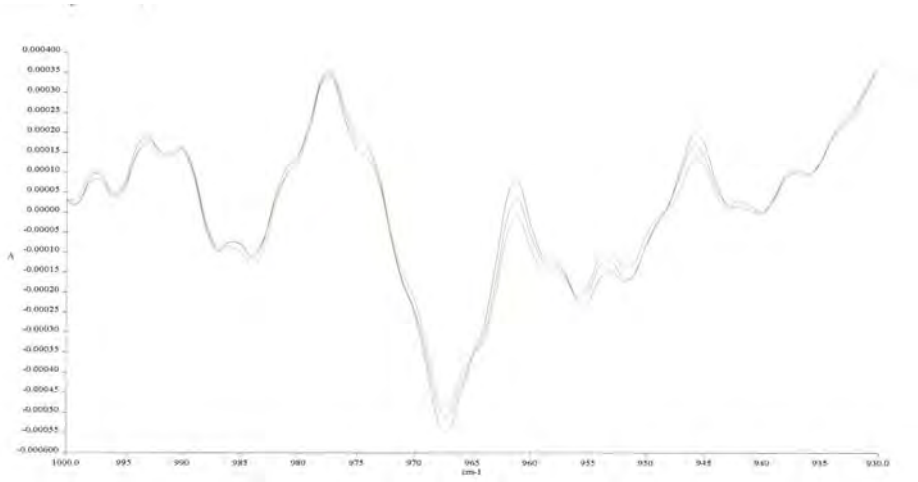
Fried chicken from well-known fast food brand 1 flask A



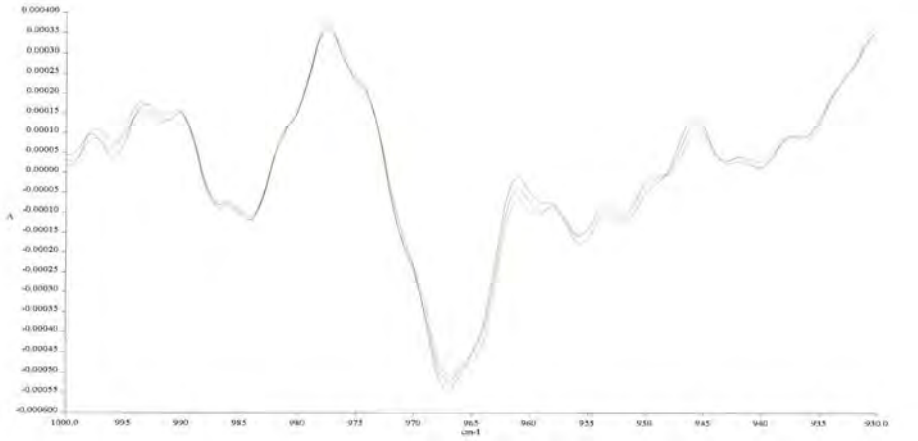
Fried chicken from well-known fast food brand 1 flask B



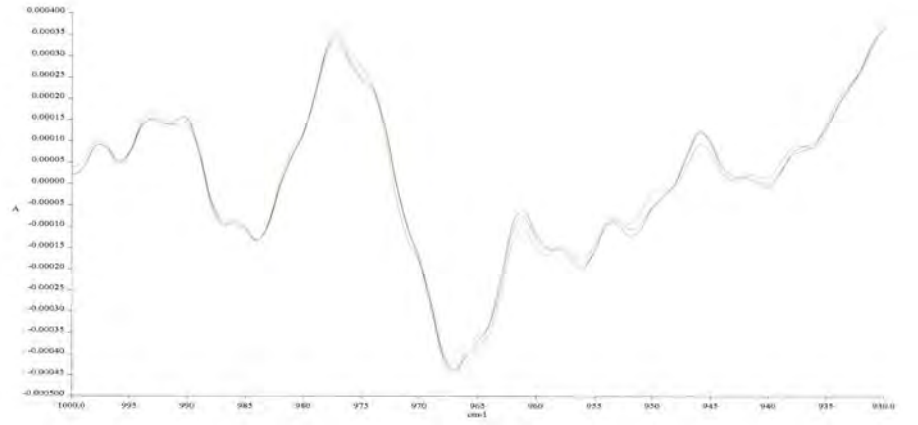
Fried chicken from well-known fast food brand 1 flask C



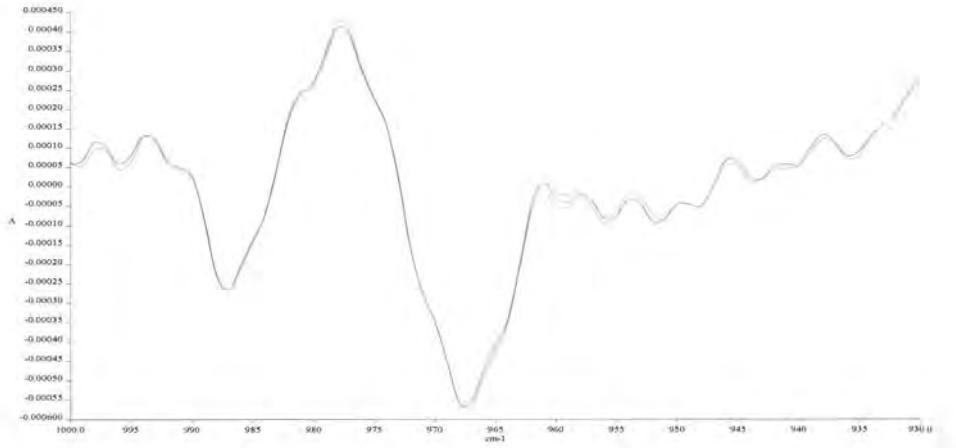
Fried chicken from well-known fast food brand 2 flask A



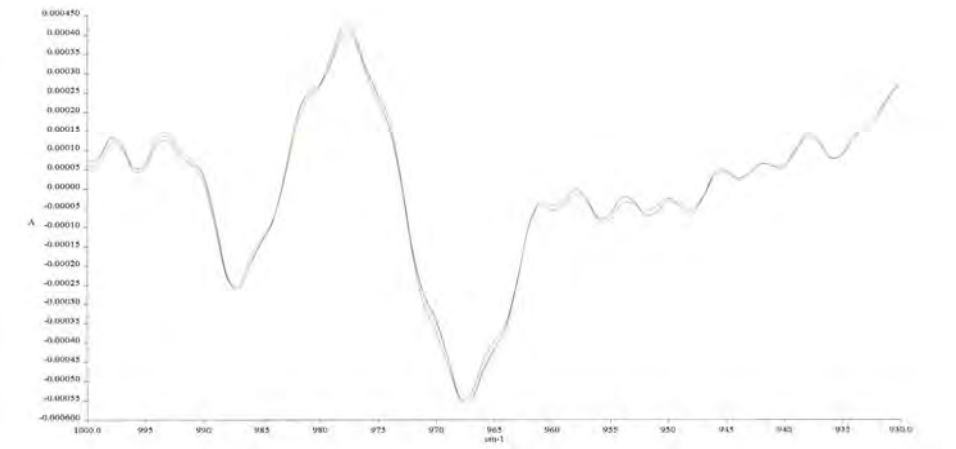
Fried chicken from well-known fast food brand 2 flask B



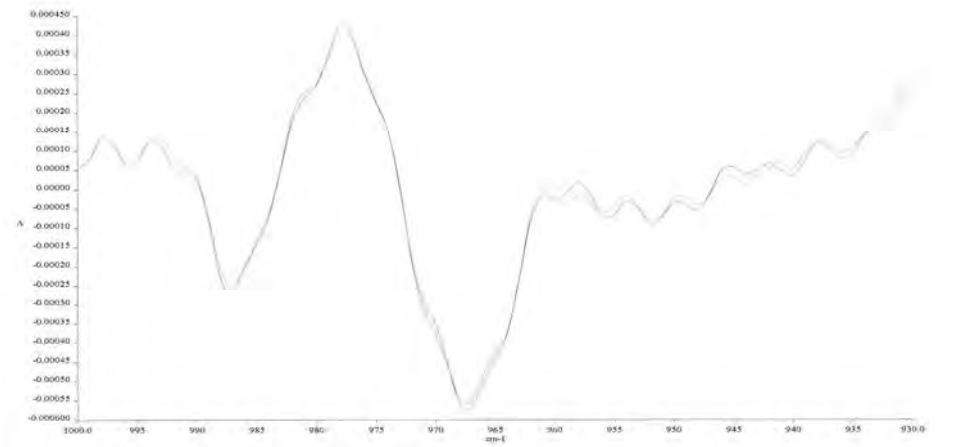
Fried chicken from well-known fast food brand 2 flask C



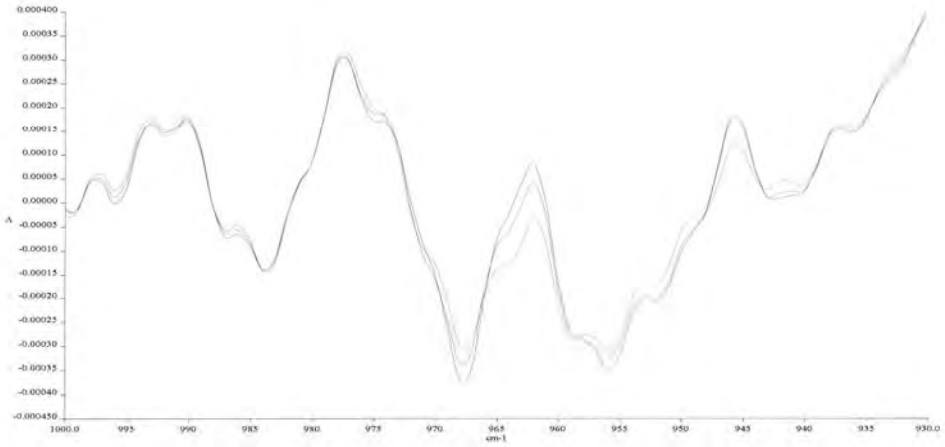
Fried chicken from well-known fast food brand 3 flask A



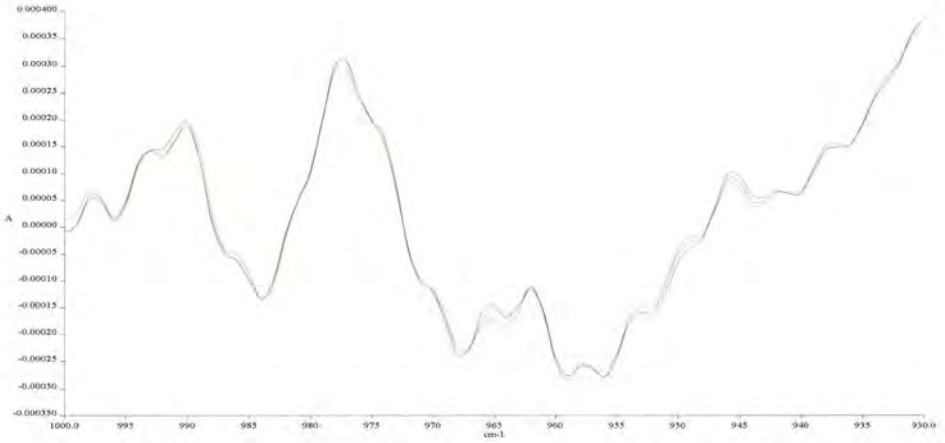
Fried chicken from well-known fast food brand 3 flask B



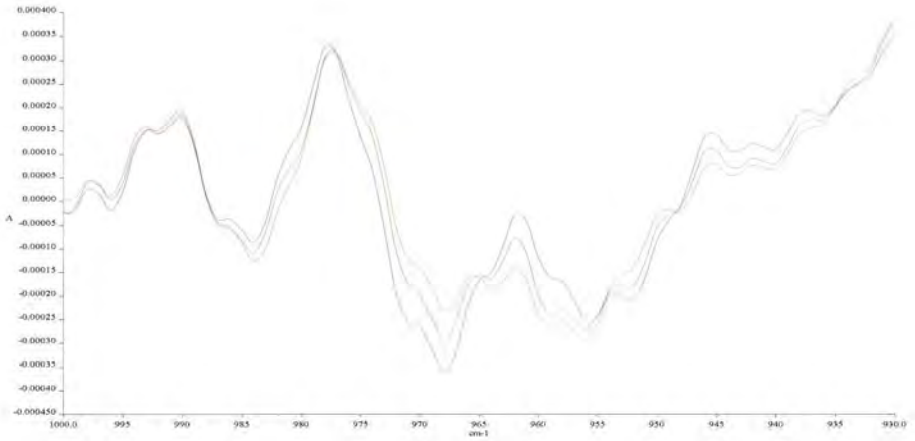
Fried chicken from well-known fast food brand 3 flask C



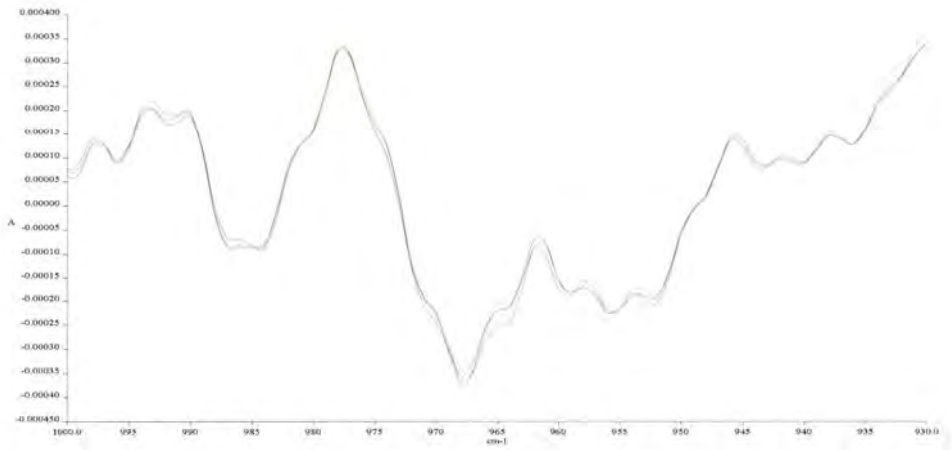
Fried chicken from street vender brand 1 flask A



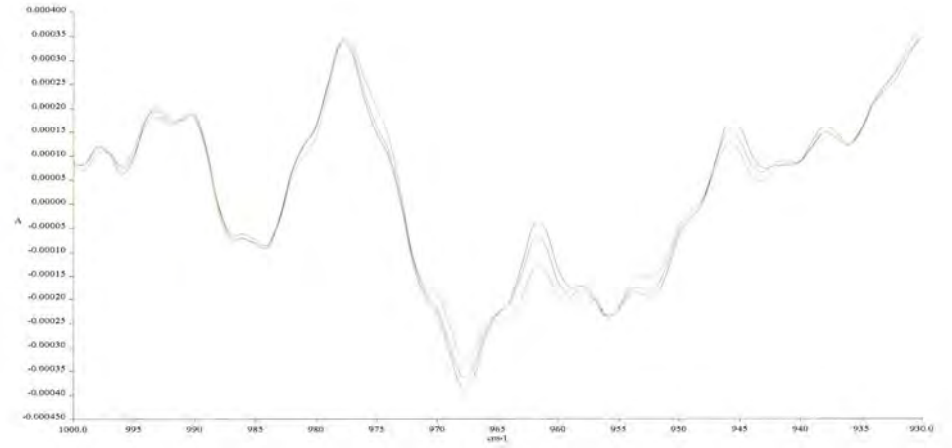
Fried chicken from street vender brand 1 flask B



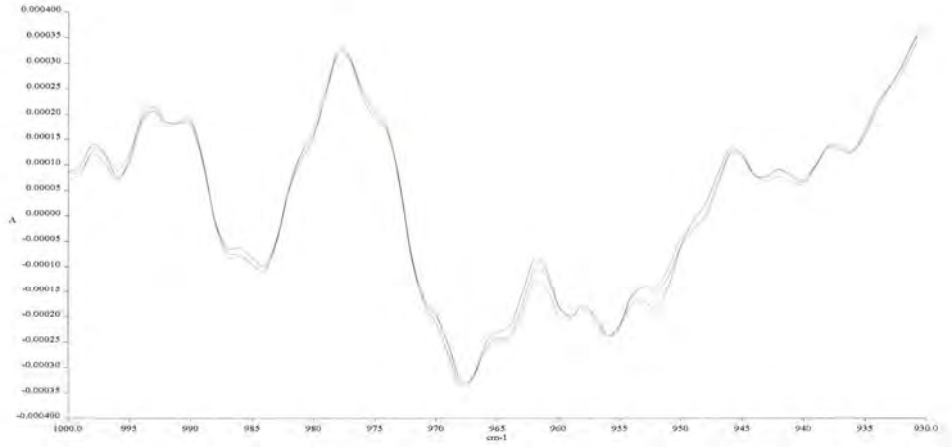
Fried chicken from street vender brand 1 flask C



Fried chicken from street vender brand 2 flask A

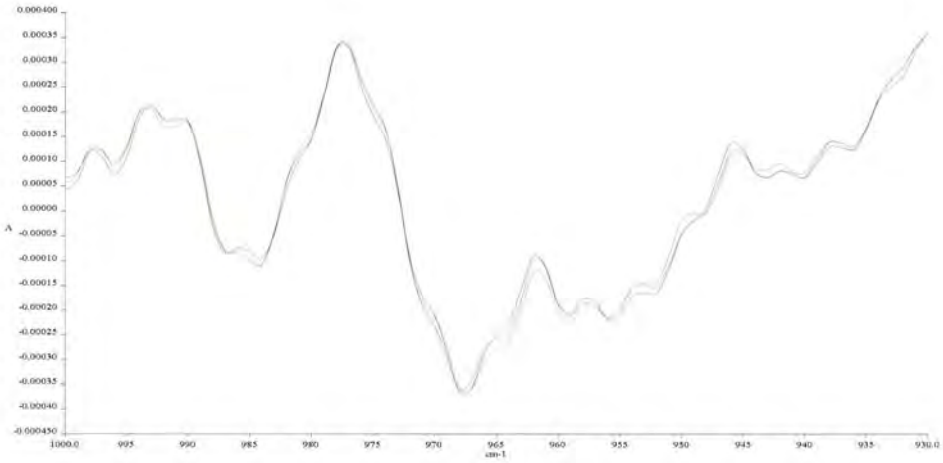


Fried chicken from street vender brand 2 flask B



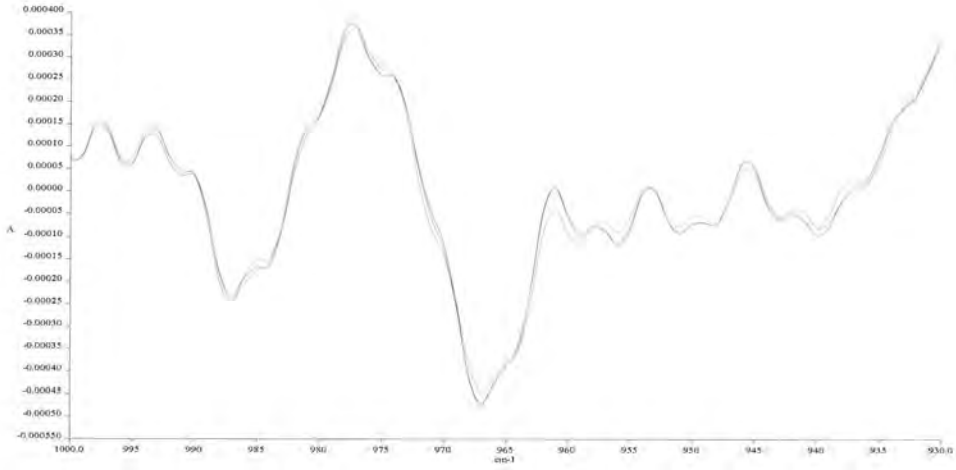
Fried chicken from street vender brand 2 flask C

Fried chicken from street vender brand 3 flask A

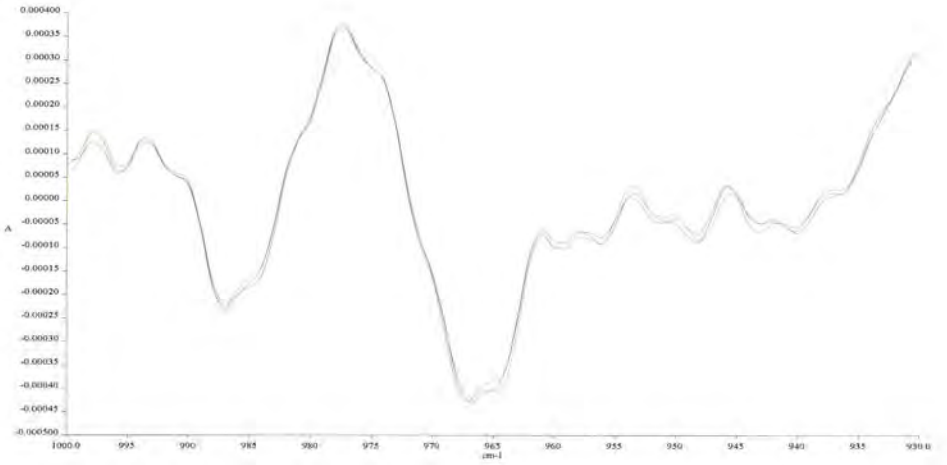


Fried chicken from street vender brand 3 flask B

Fried chicken from street vender brand 3 flask C



French fries brand 1 flask A



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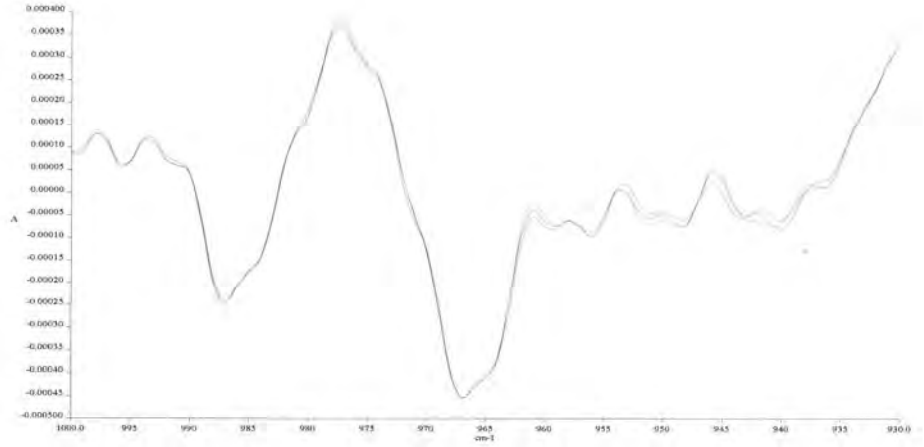

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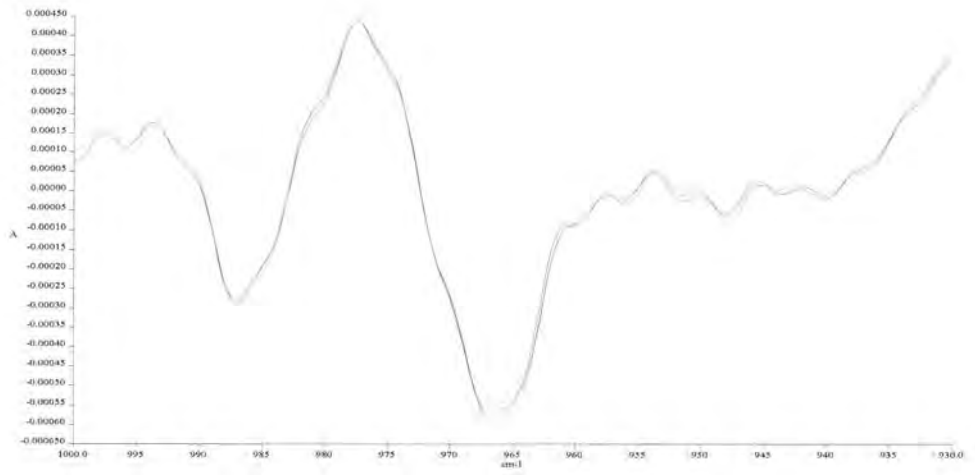

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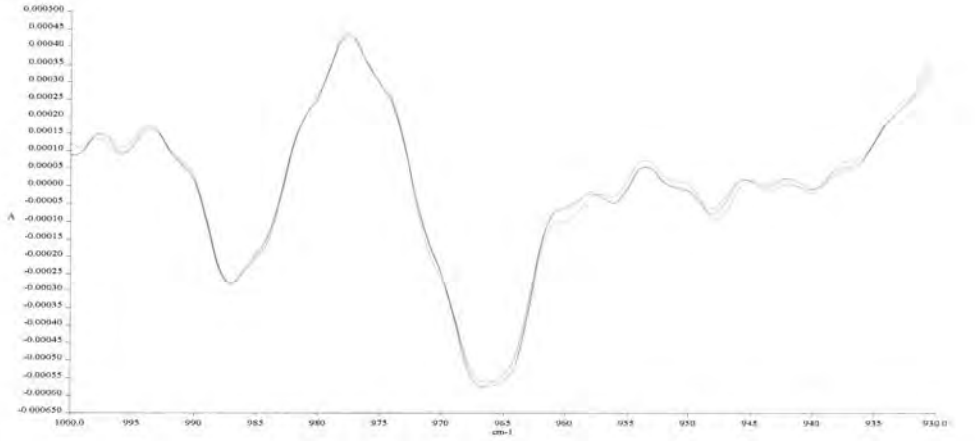
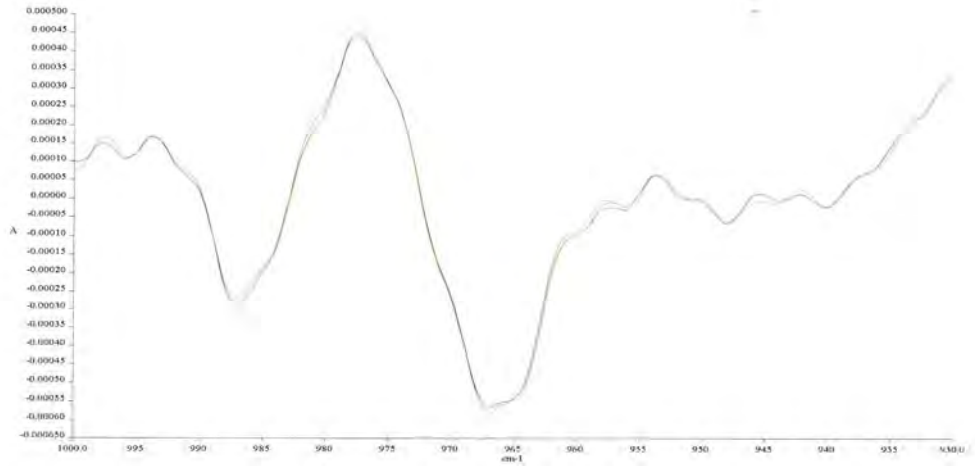
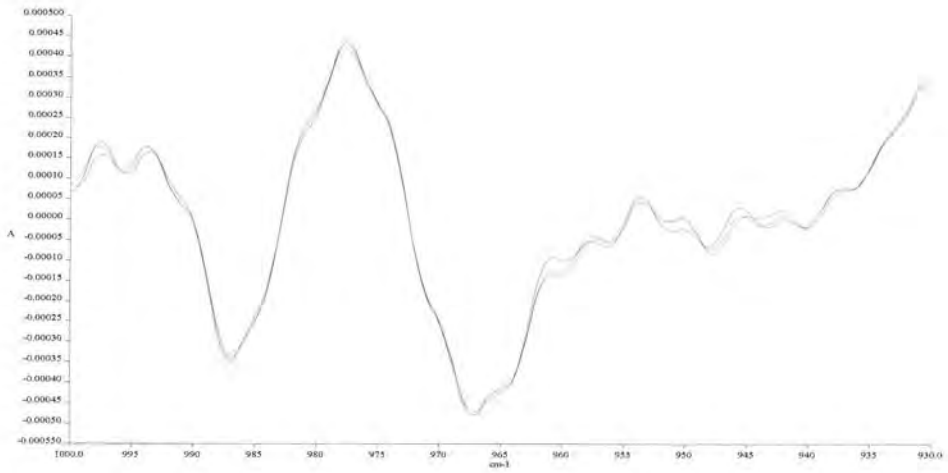


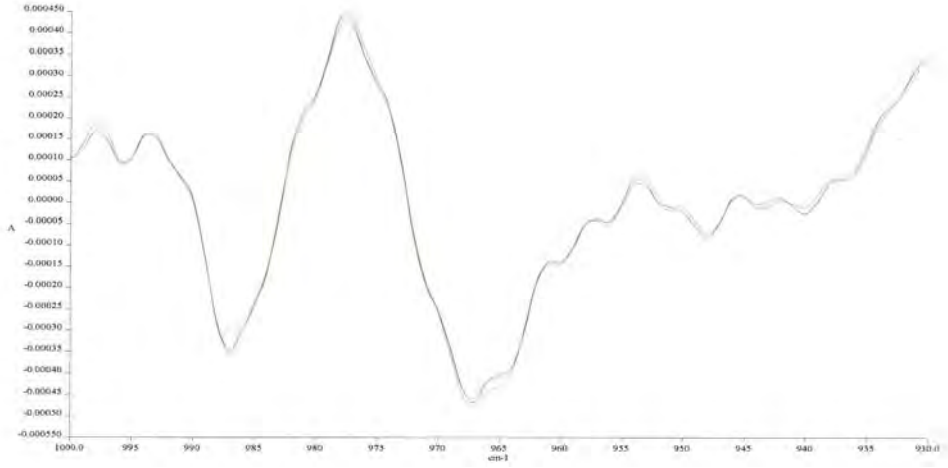
French fries brand 2 flask B



French fries brand 2 flask C



French fries brand 3 flask A



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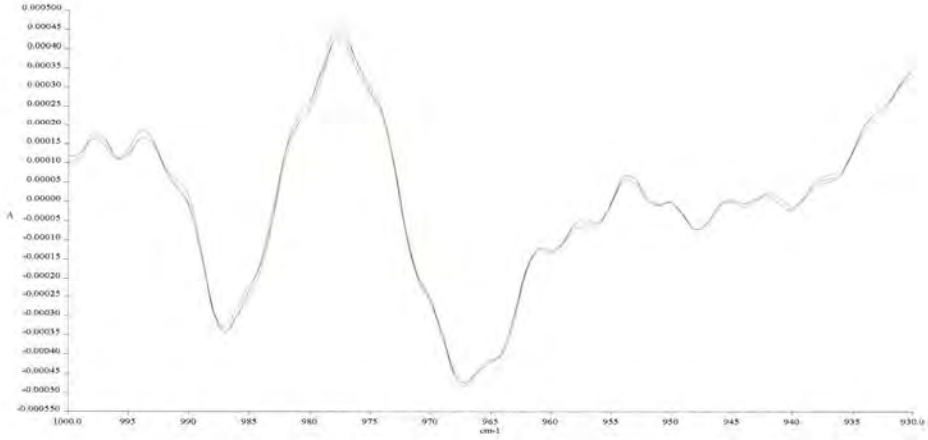
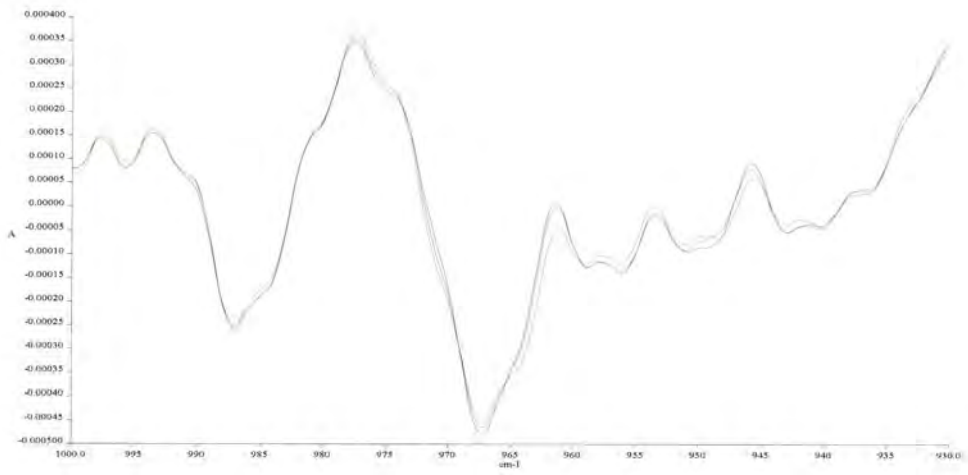
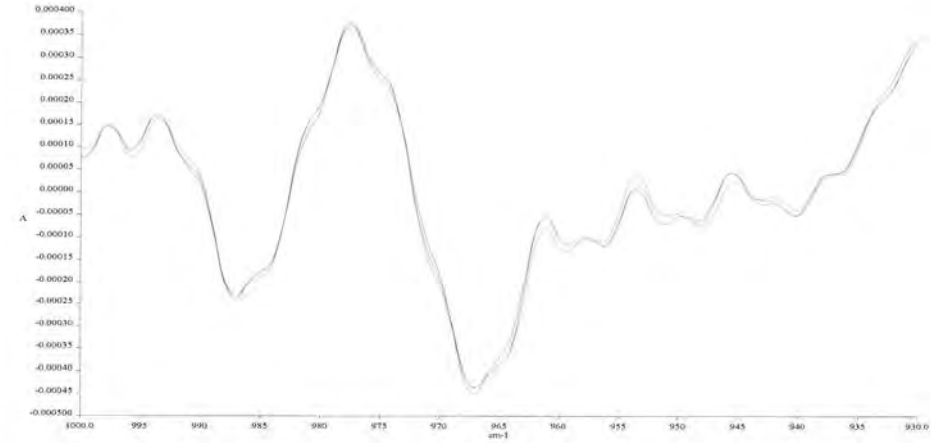


French fried brand 3 flask C



Deep fried dough stick brand 1 flask A



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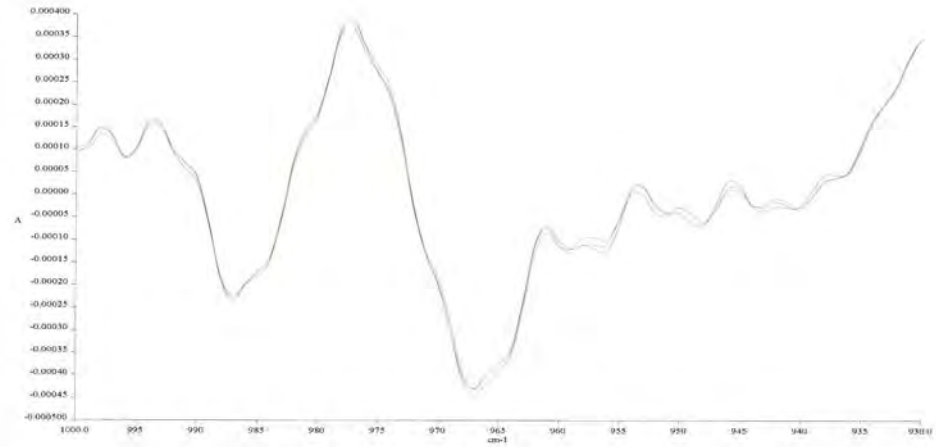

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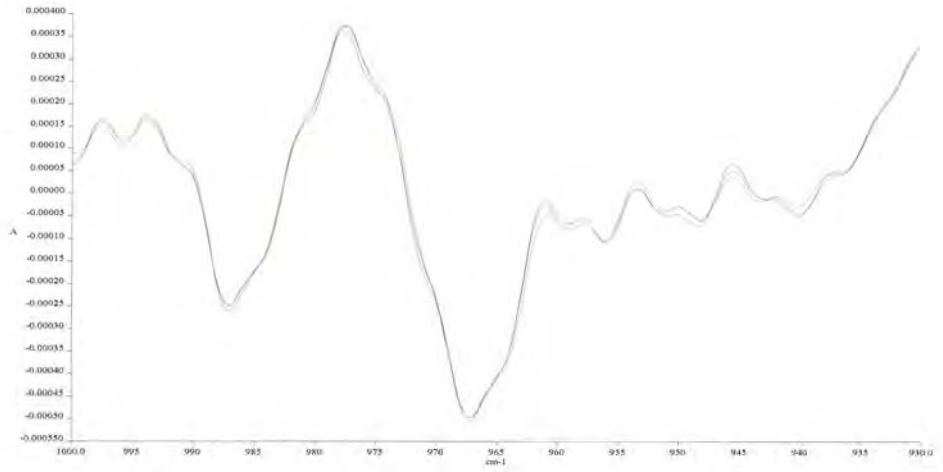

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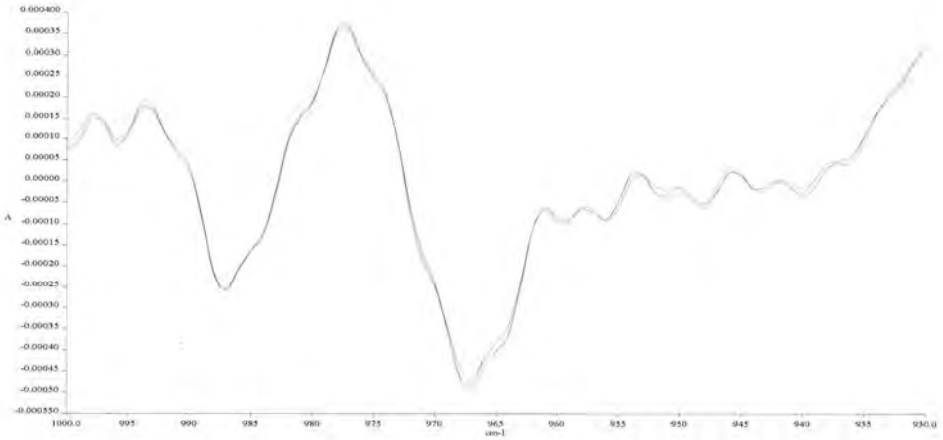

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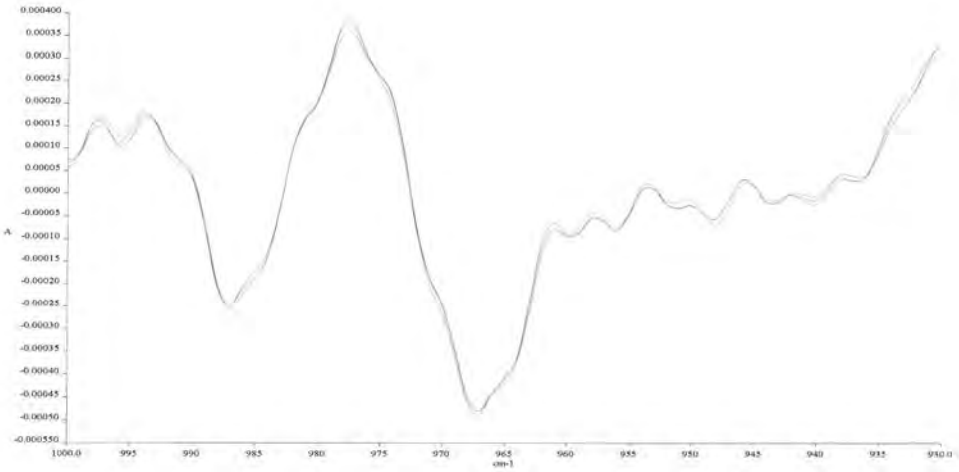
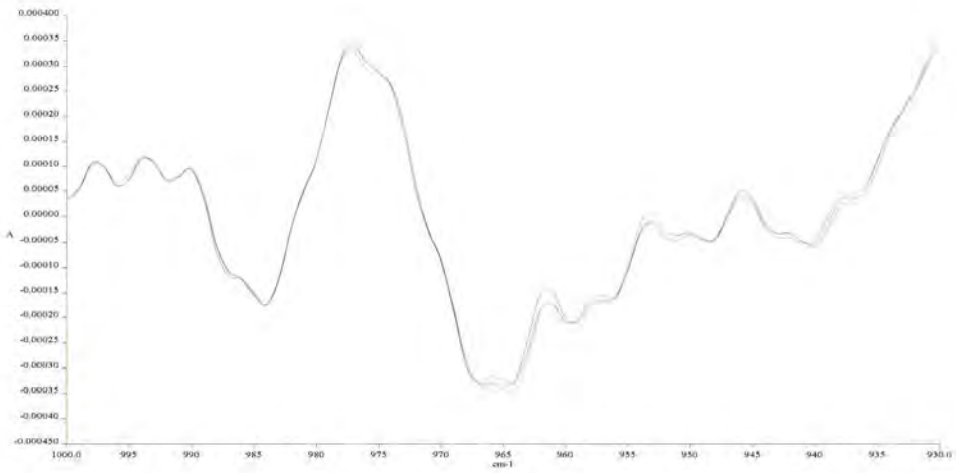
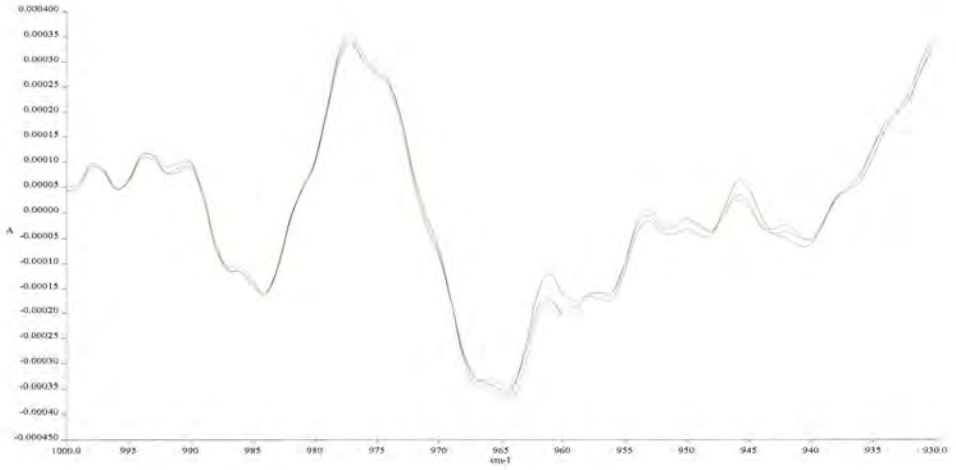


Deep fried dough stick brand 2 flask C



Deep fried dough stick brand 3 flask A



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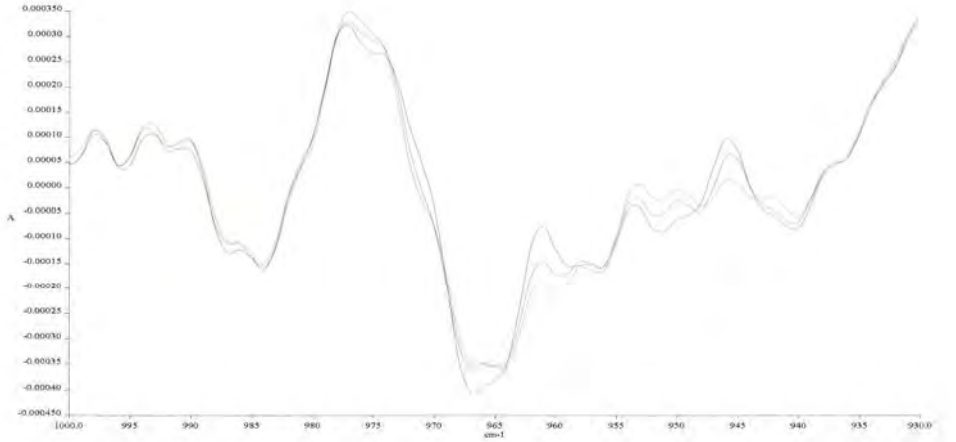
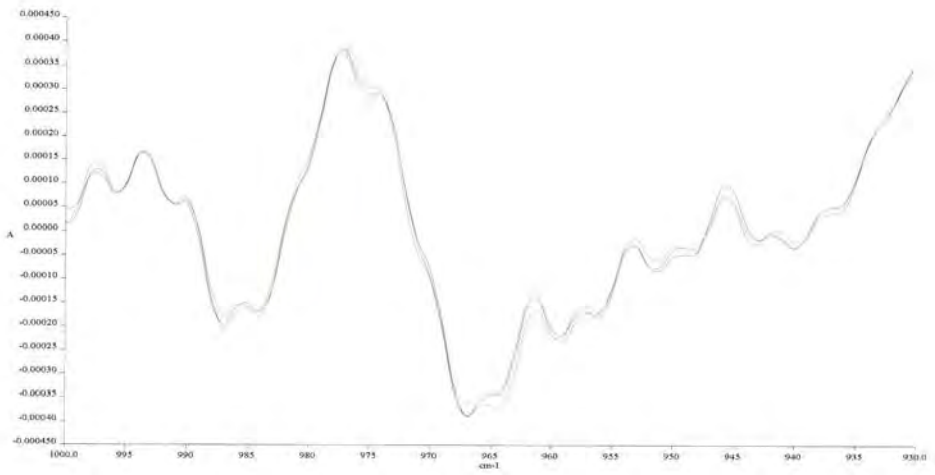
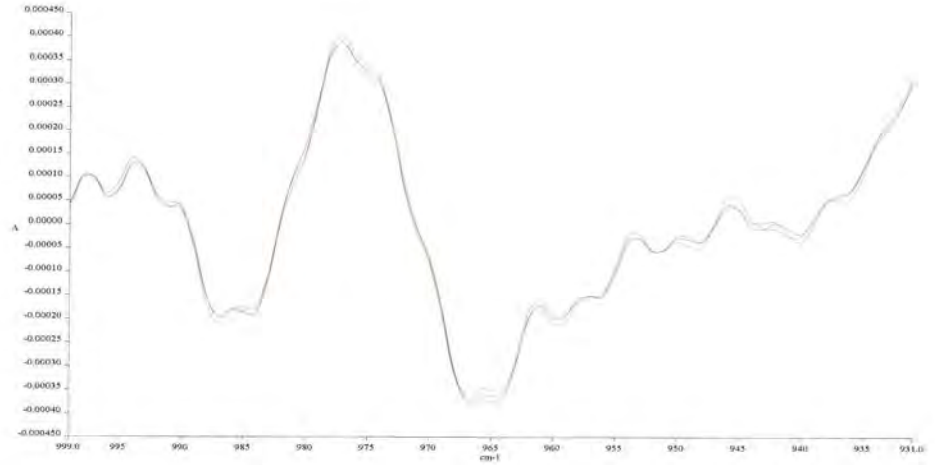


Deep fried dough stick brand 3 flask C



Deep fried banana brand 1 flask A



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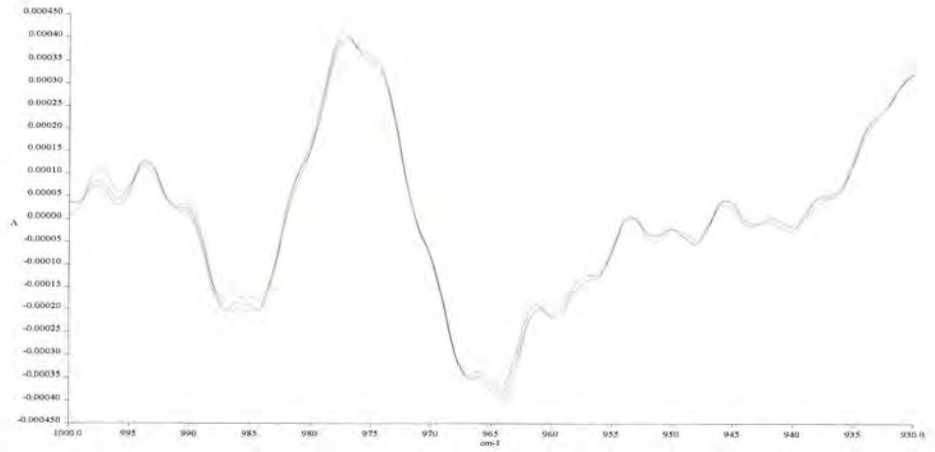
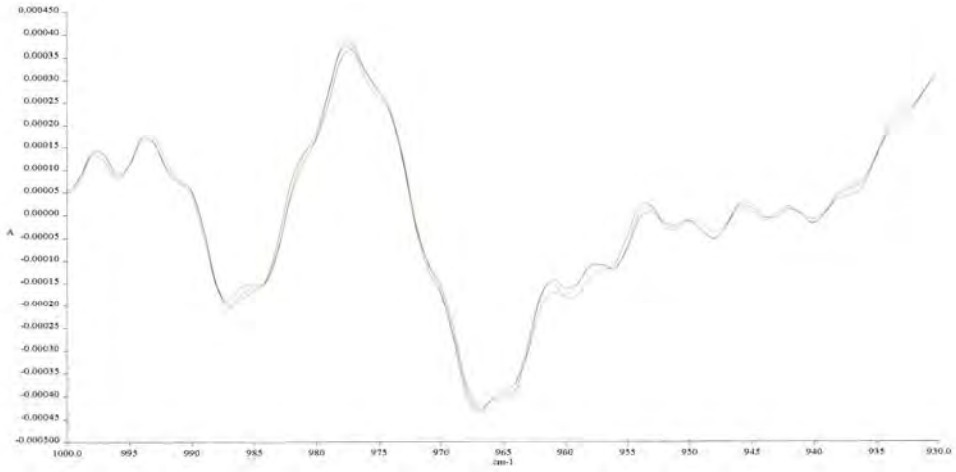
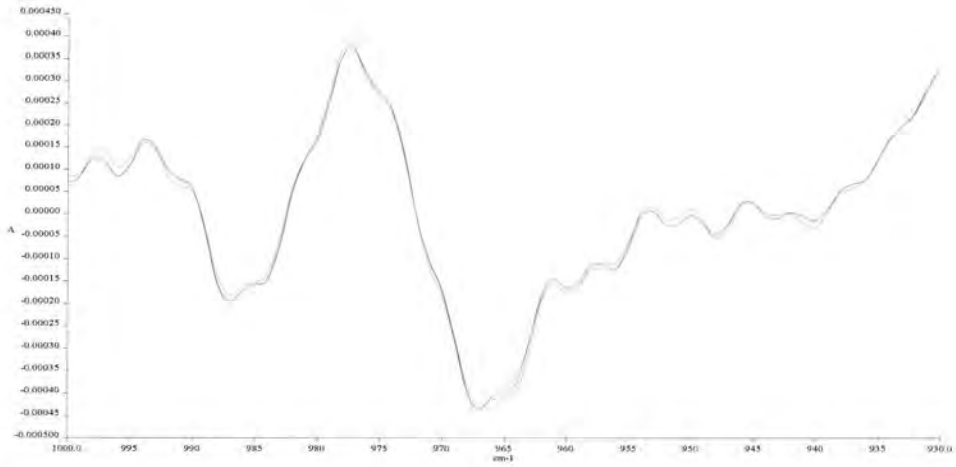


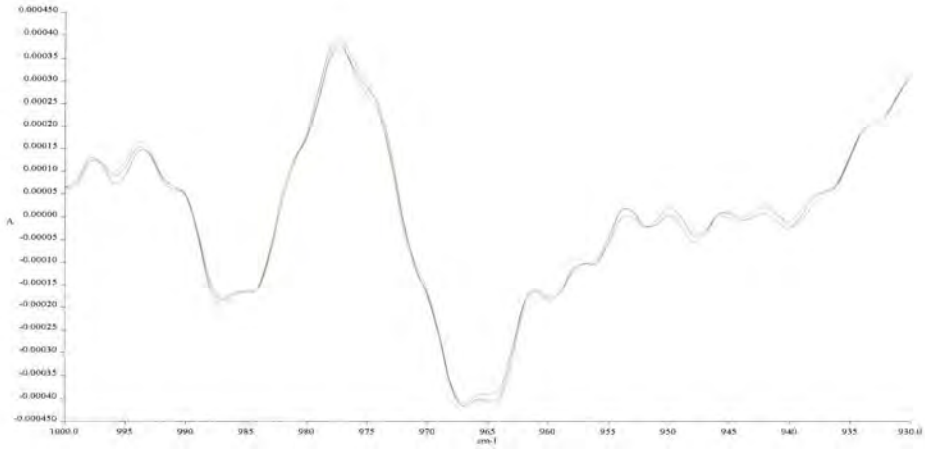
Deep fried banana brand 1 flask C



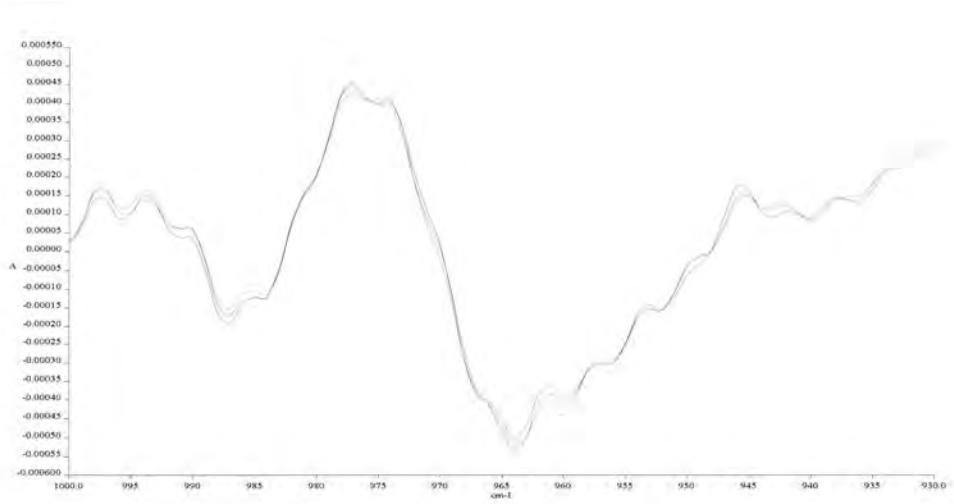
Deep fried banana brand 2 flask A



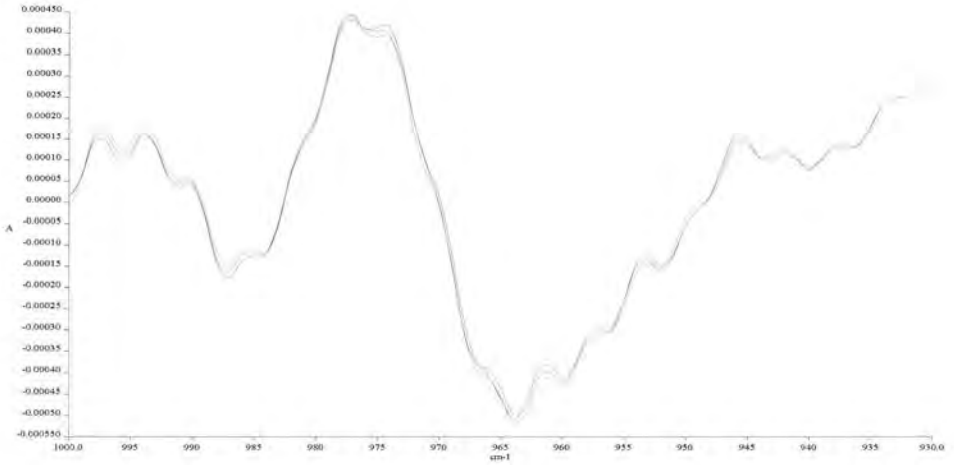
Deep fried banana brand 2 flask B



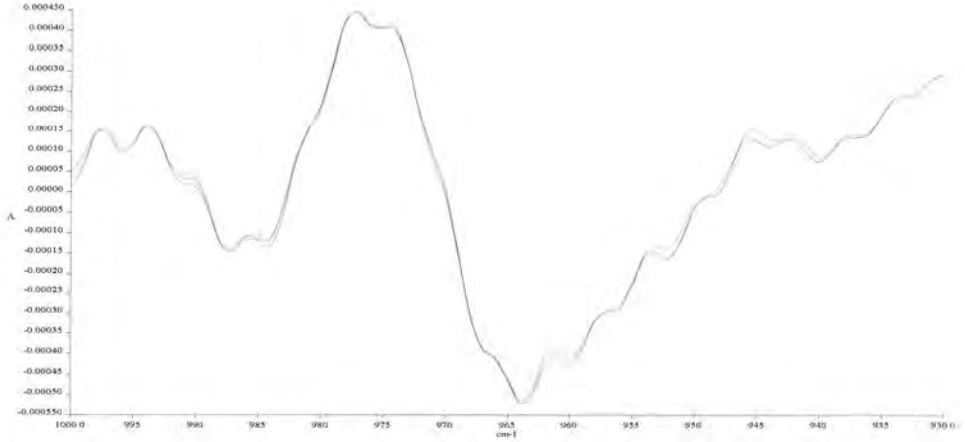
Deep fried banana brand 2 flask C



Deep fried banana brand 3 flask A



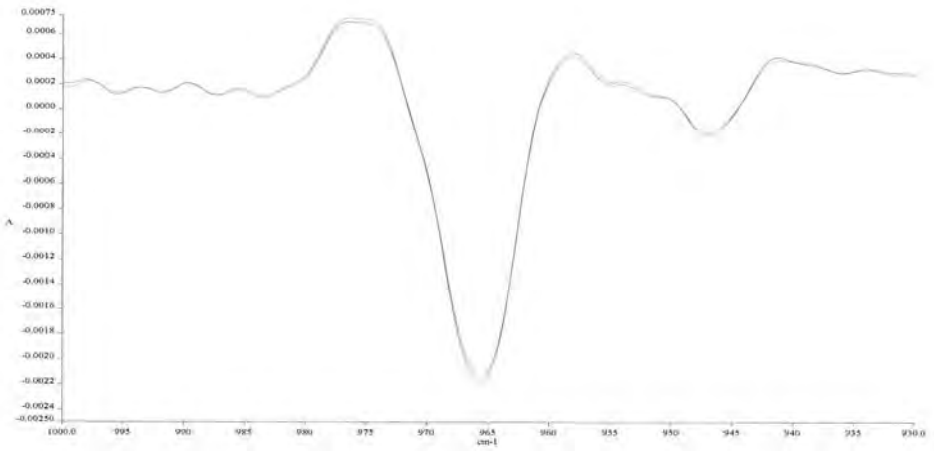
Deep fried banana brand 3 flask B



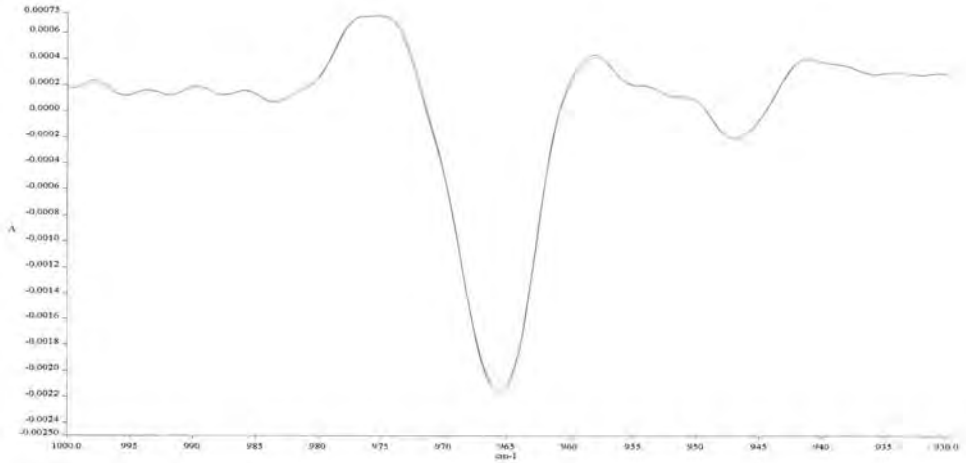
Deep fried banana brand 3 flask C

APPENDIX C

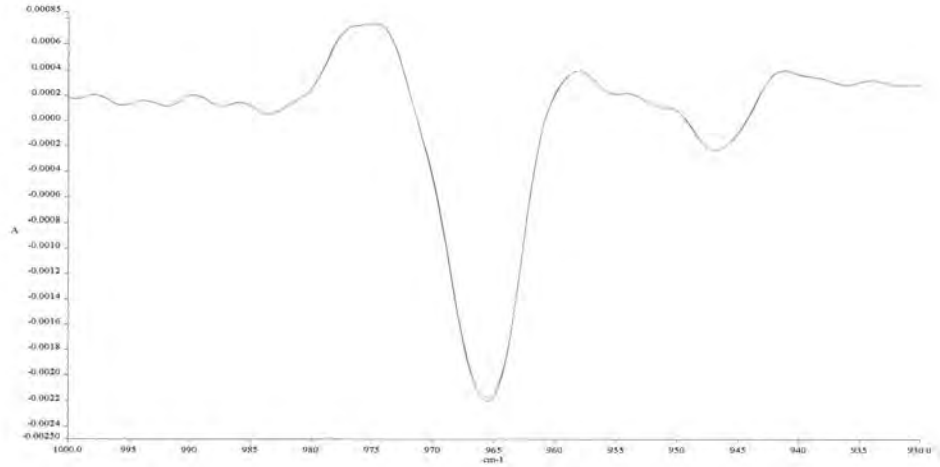
Graph of the negative second derivative of the *trans* absorption band relative to air of milk and dairy products



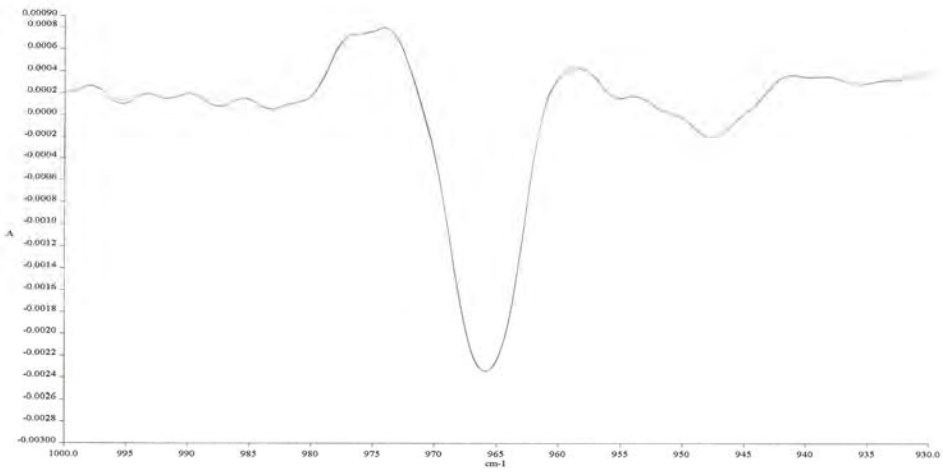
Pasteurized milk brand 1 flask A



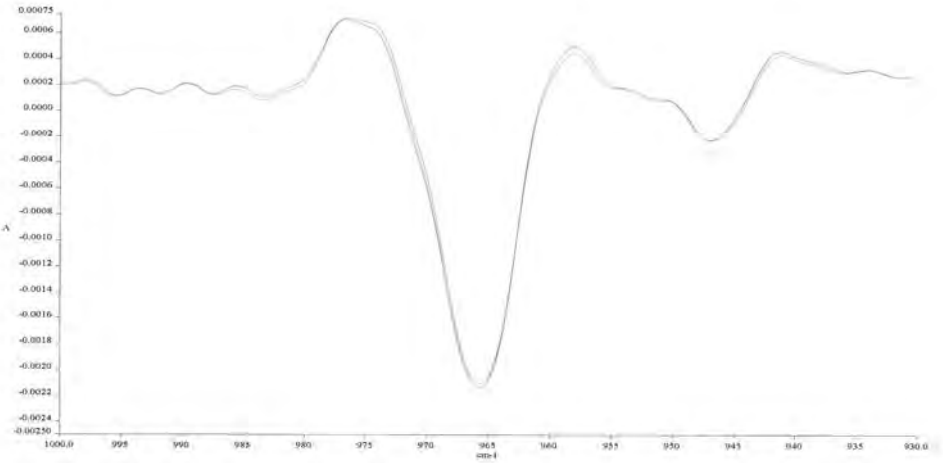
Pasteurized milk brand 1 flask B



Pasteurized milk brand 1 flask C



Pasteurized milk brand 2 flask A



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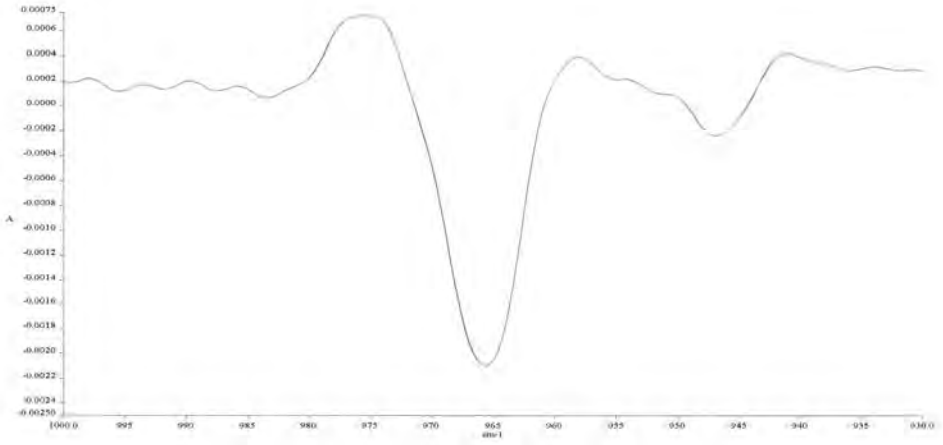
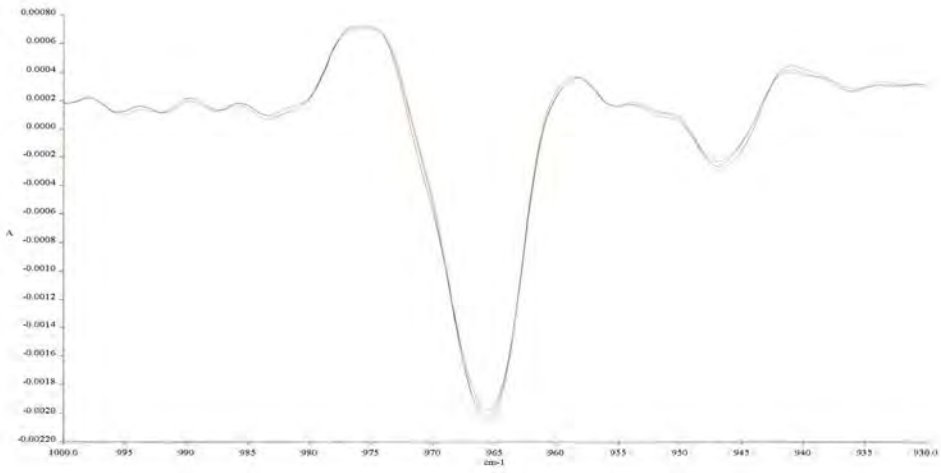
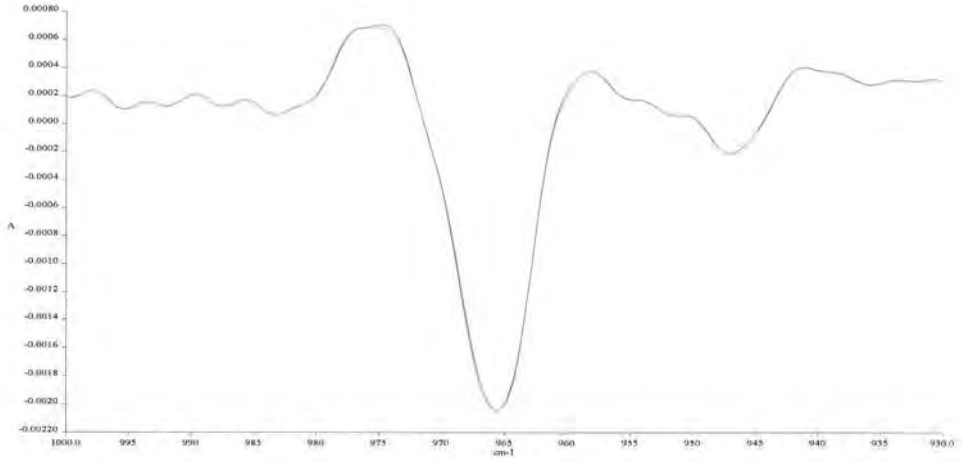


Pasteurized milk brand 2 flask C



Pasteurized milk brand 3 flask A



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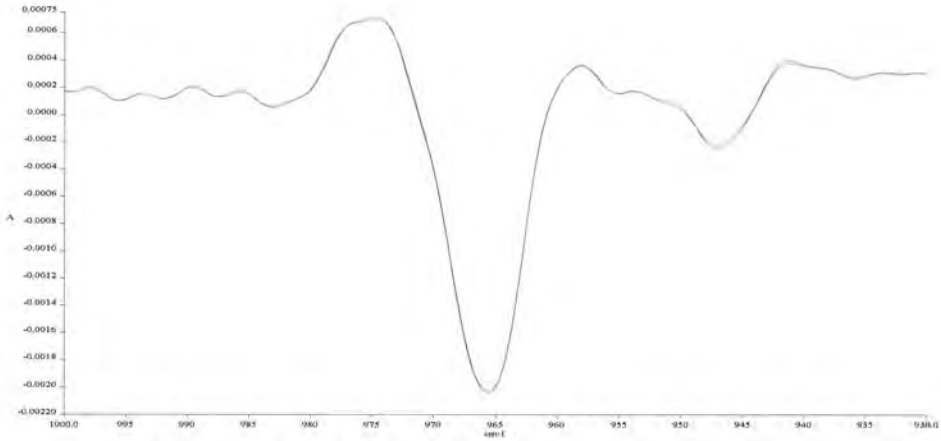
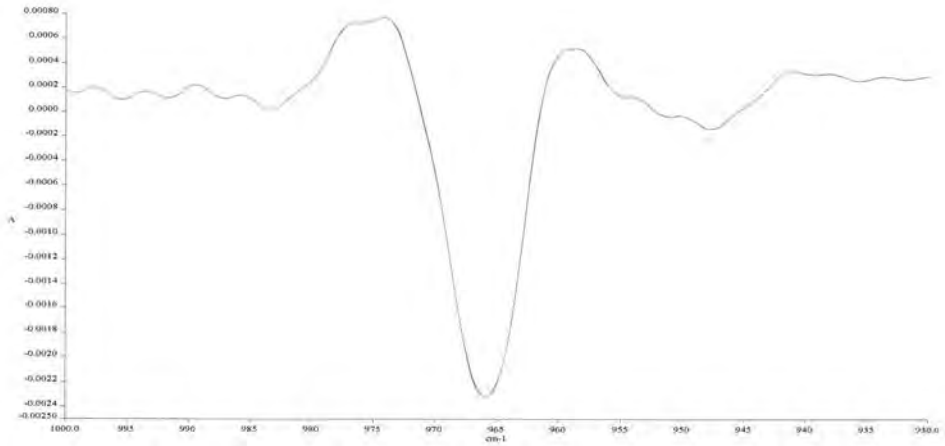
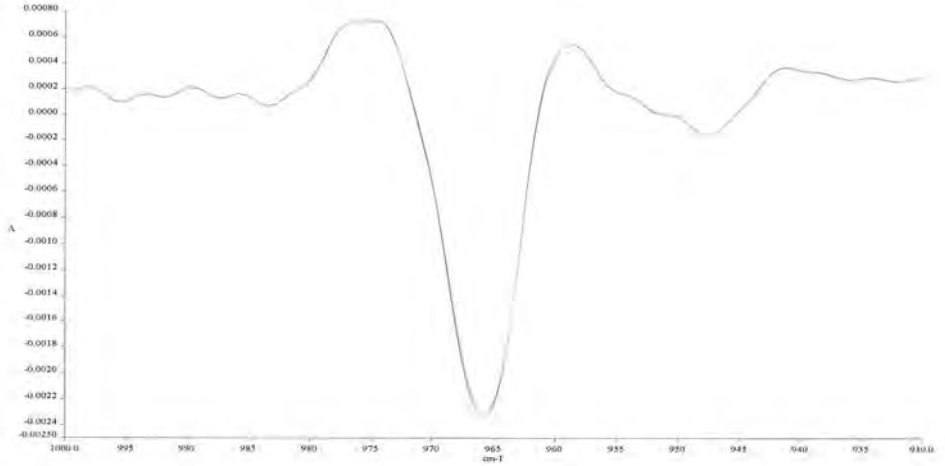


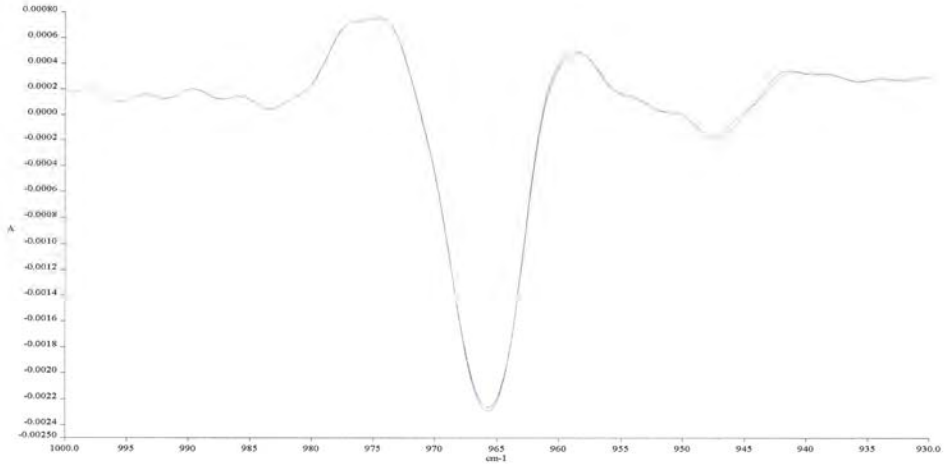
Pasteurized milk brand 3 flask C



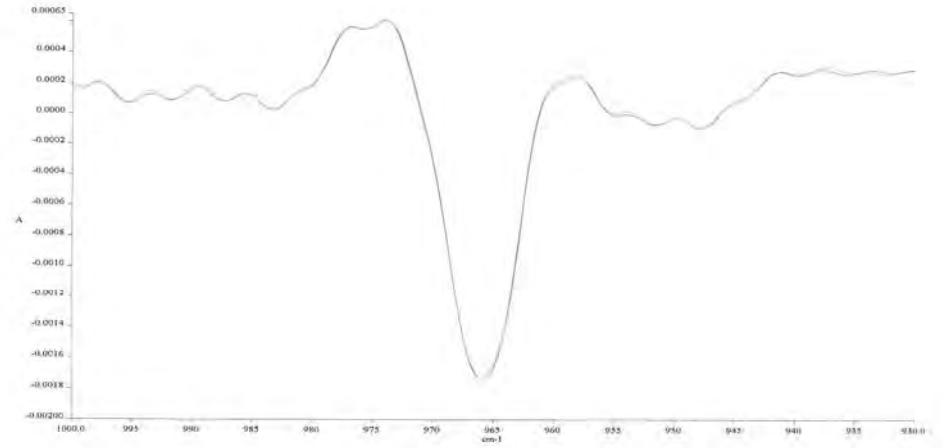
UHT milk brand 1 flask A



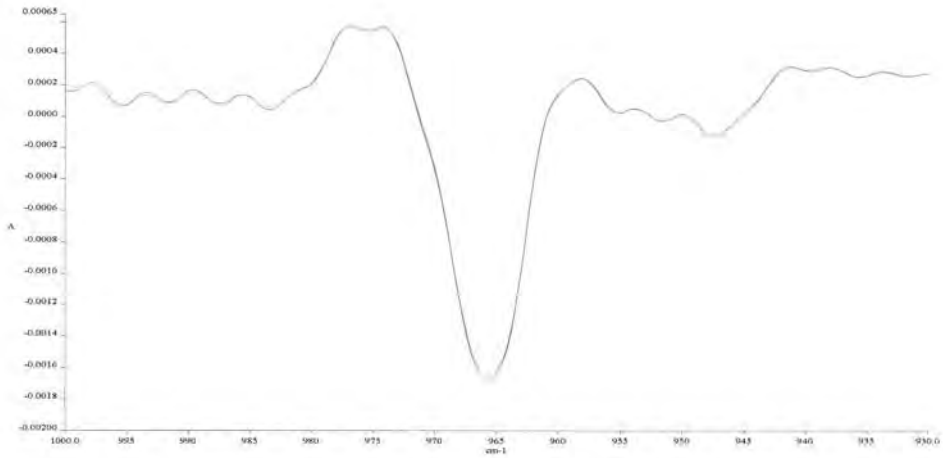
UHT milk brand 1 flask B



UHT milk brand 1 flask C



UHT milk brand 2 flask A



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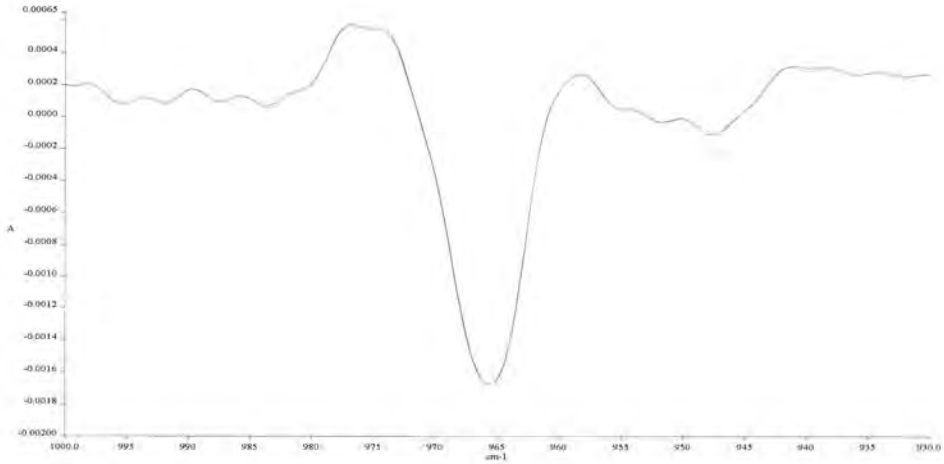
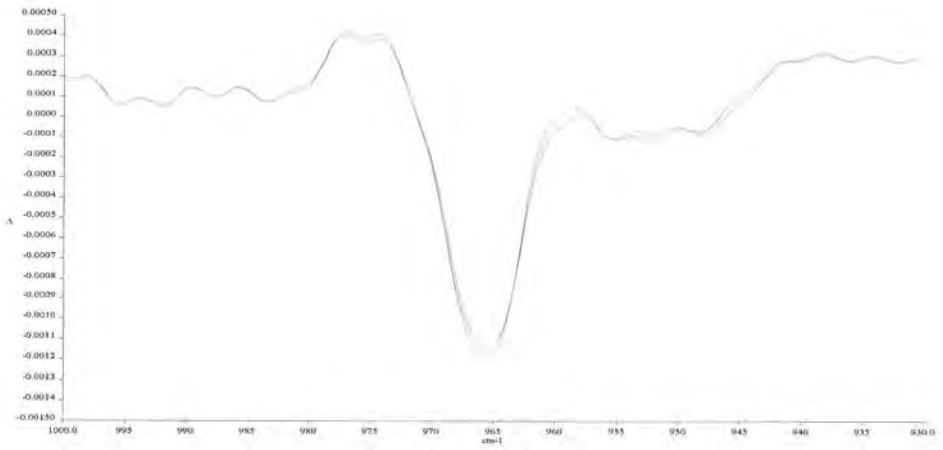
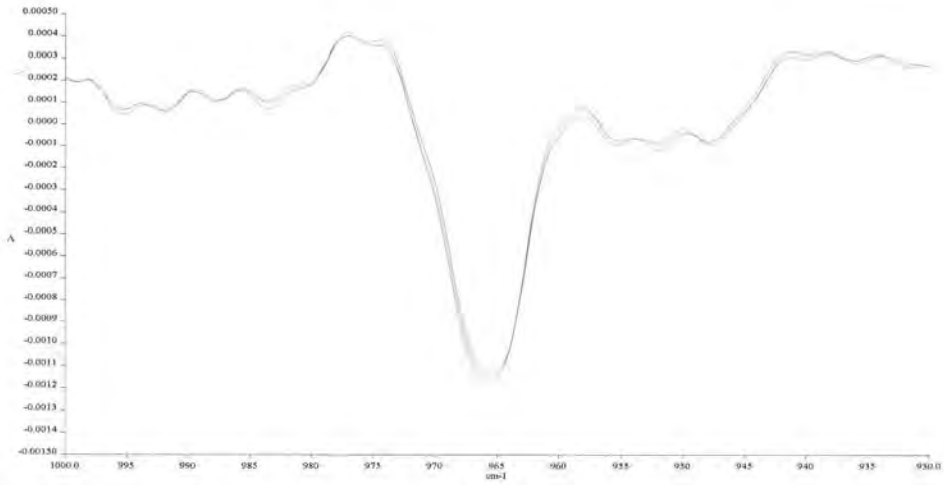


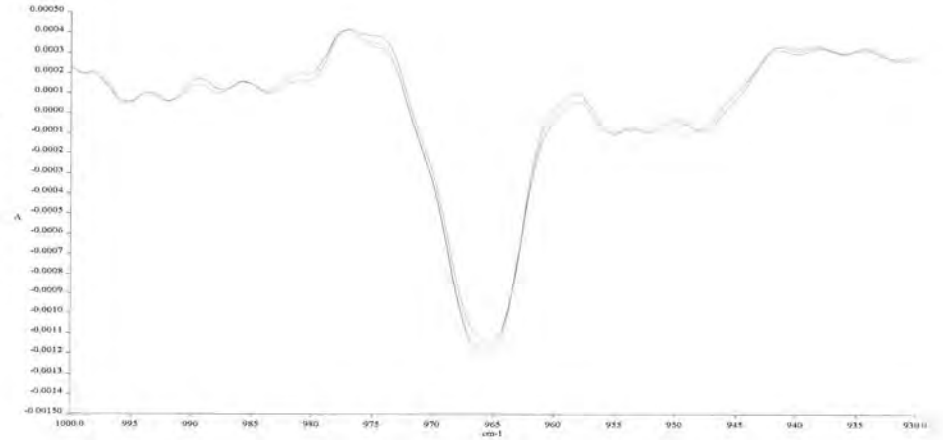
UHT milk brand 2 flask C



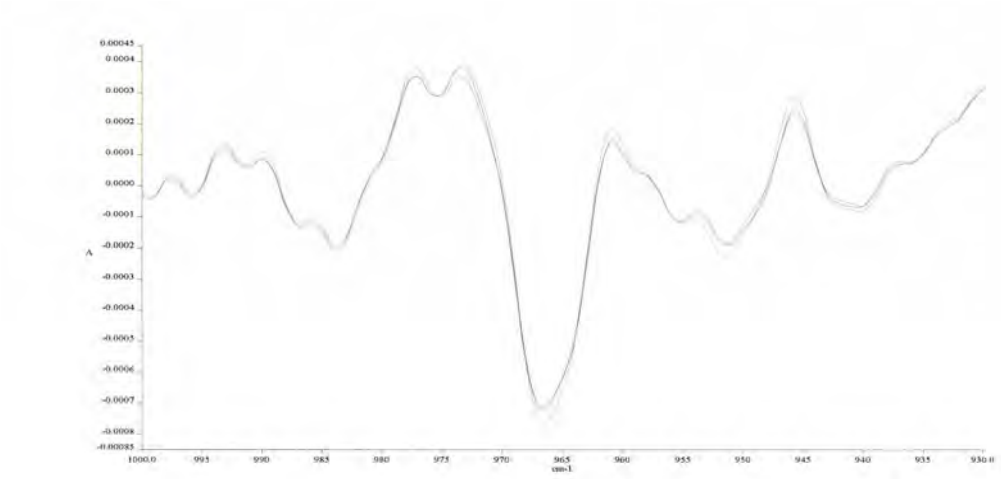
UHT milk brand 3 flask A



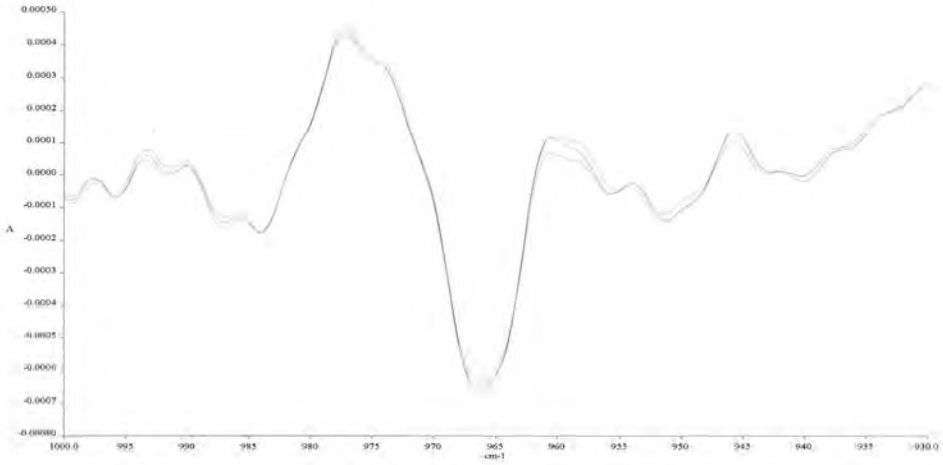
UHT milk brand 3 flask B



UHT milk brand 3 flask C



Ice-cream brand 1 flask A



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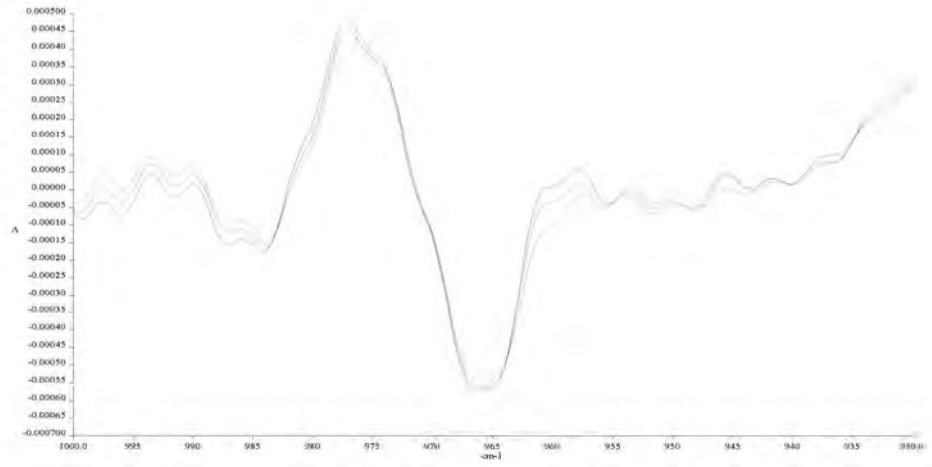
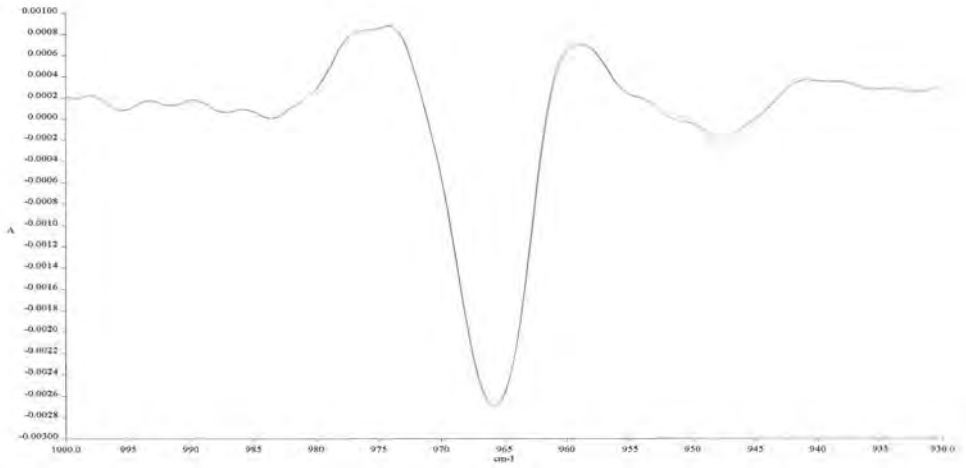


Ice-cream brand 1 flask C



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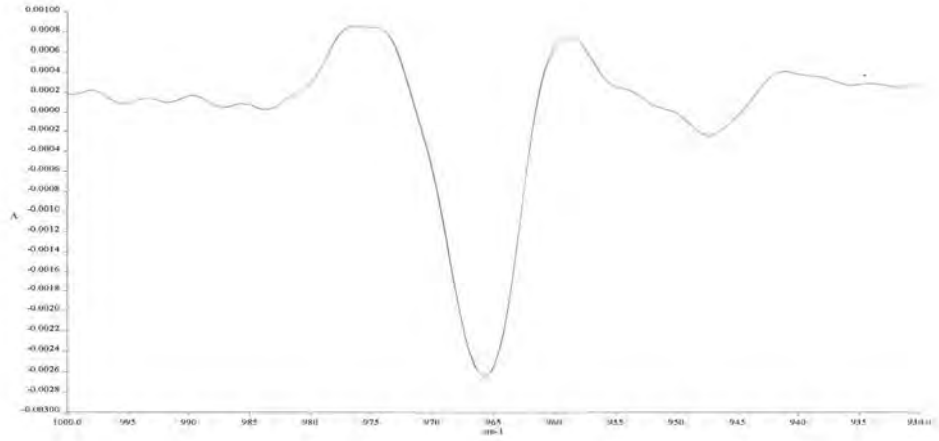

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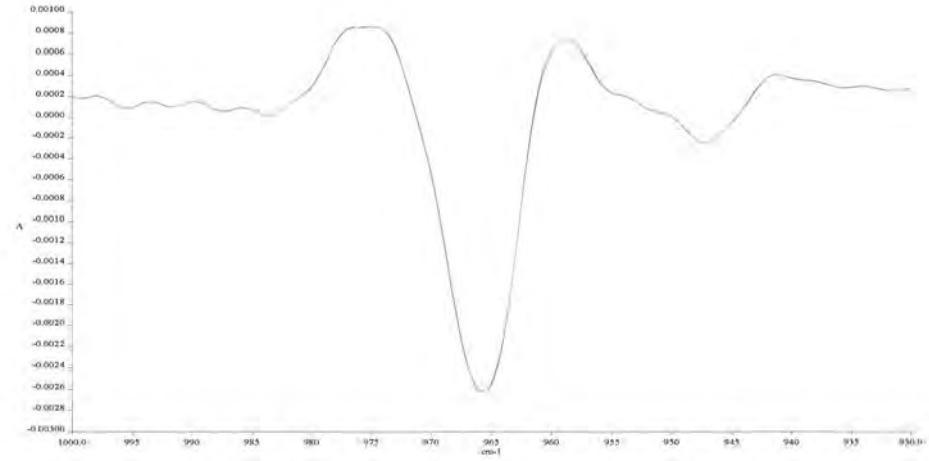

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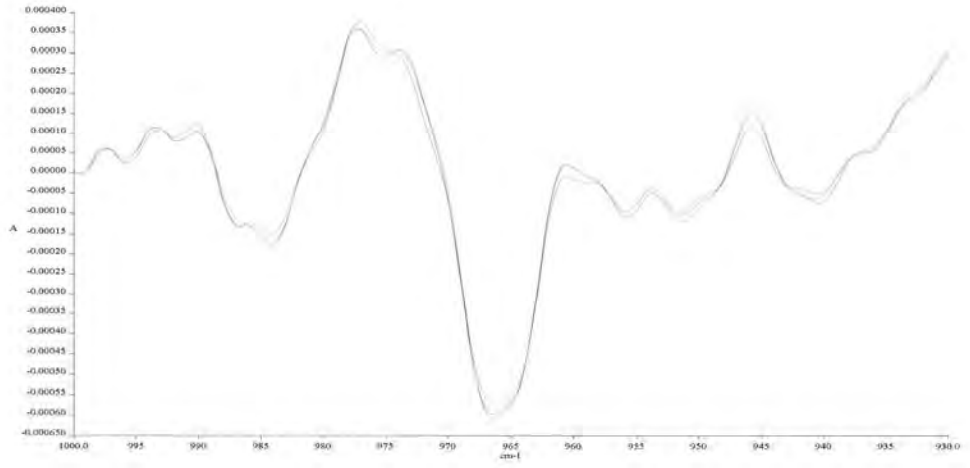

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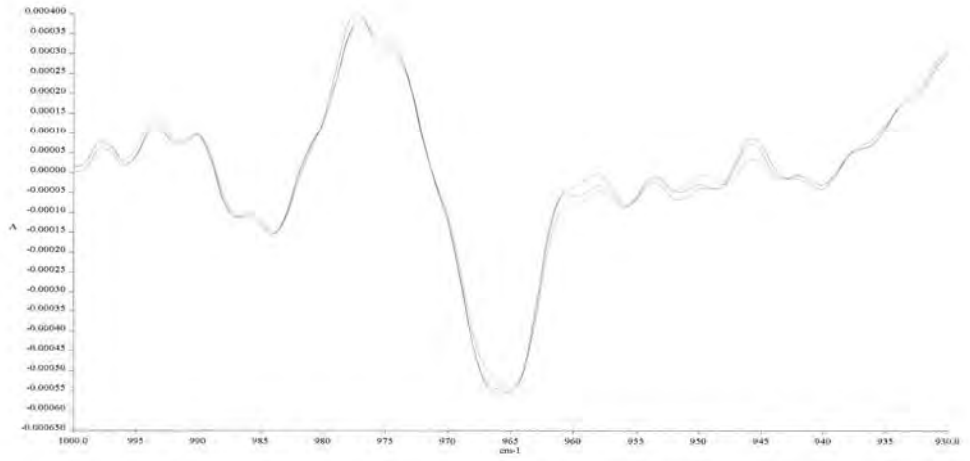

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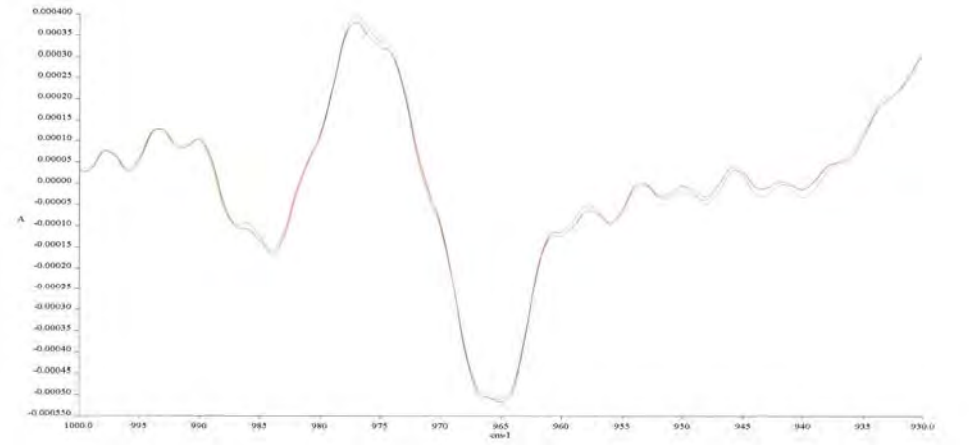

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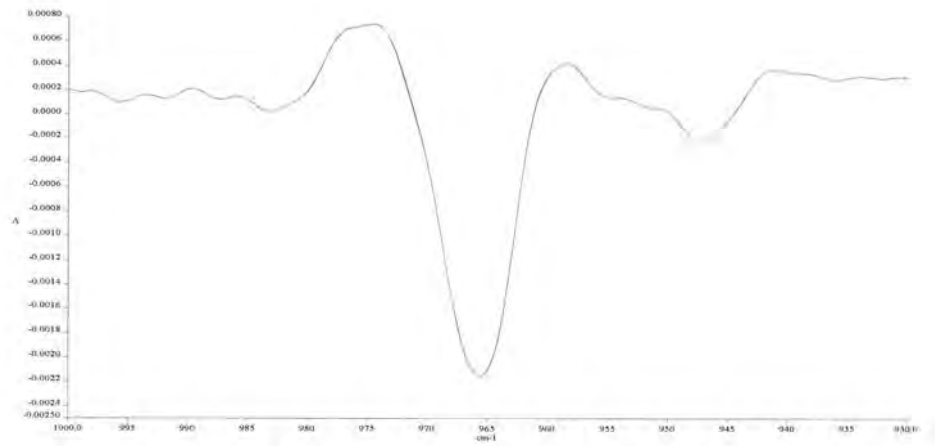
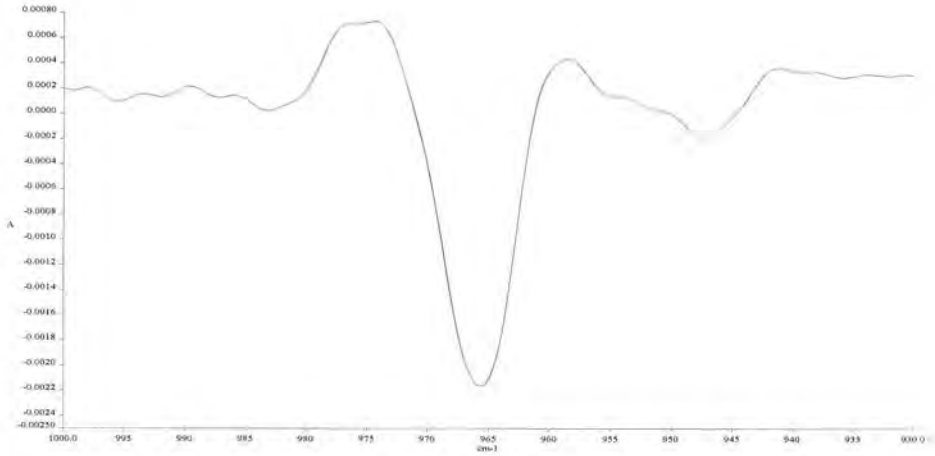
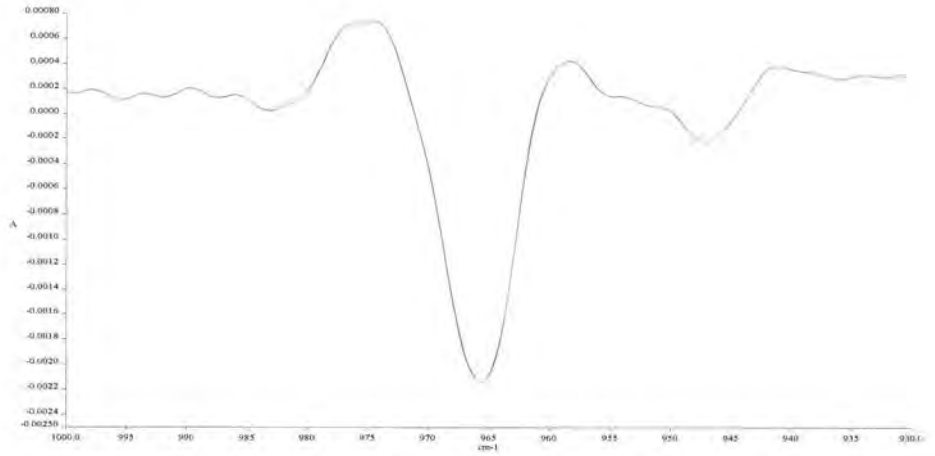


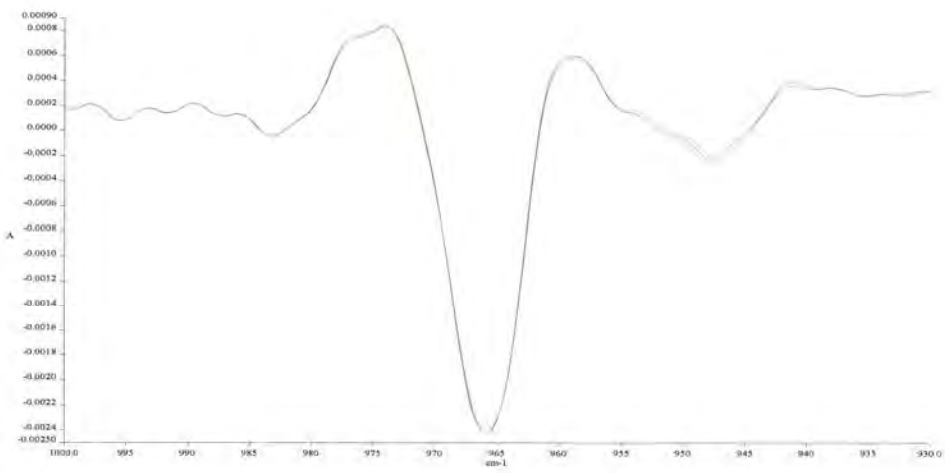
Whipping cream brand 1 flask A



Whipping cream brand 1 flask B



Whipping cream brand 1 flask C



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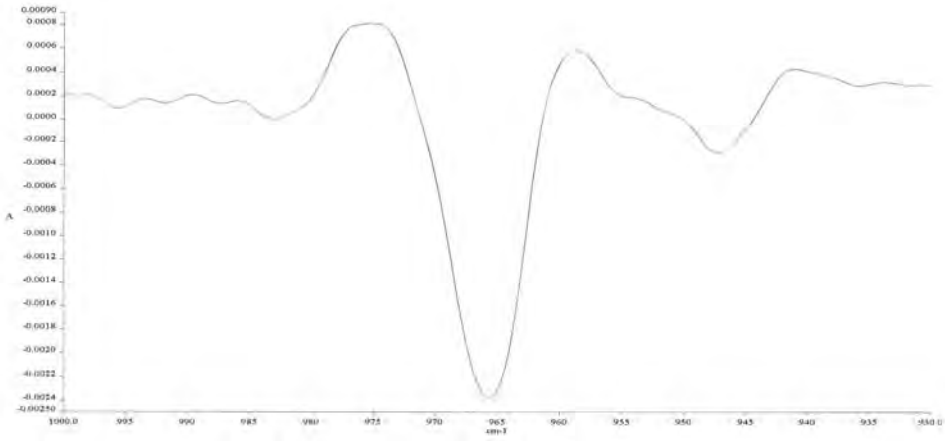

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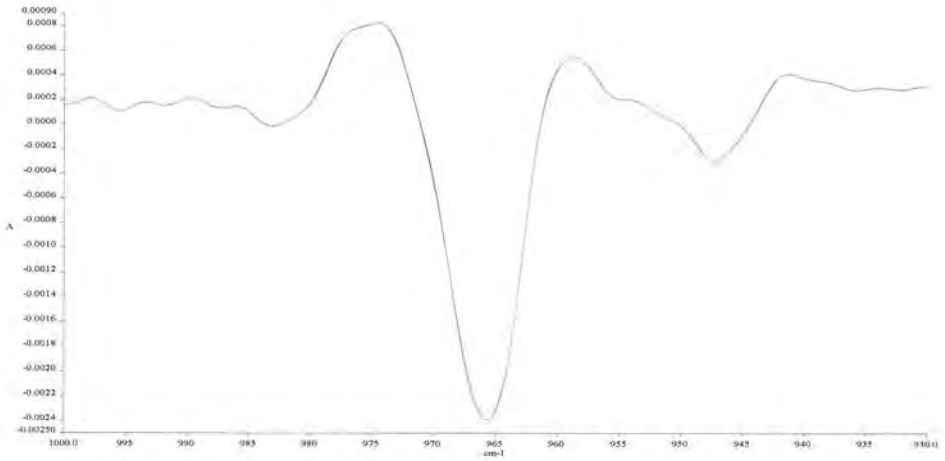

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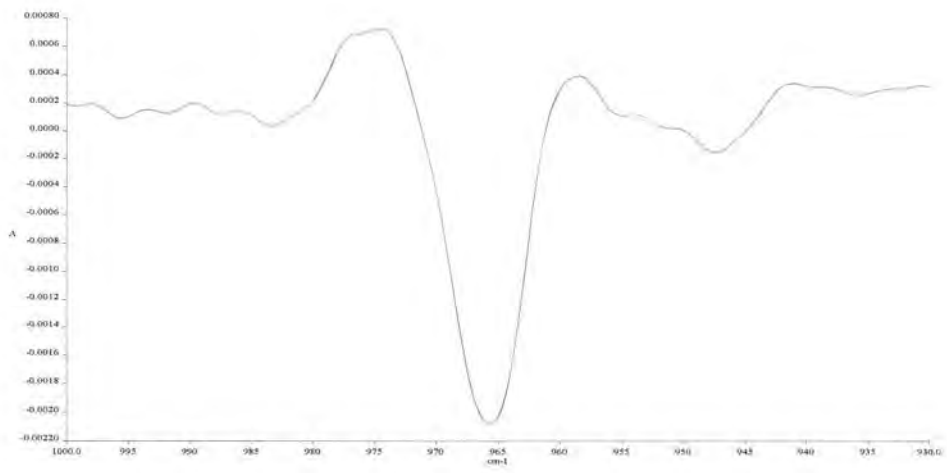

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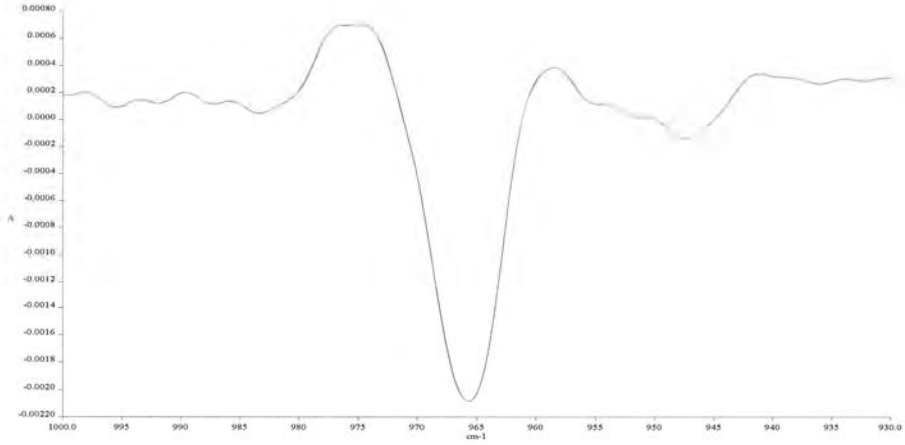

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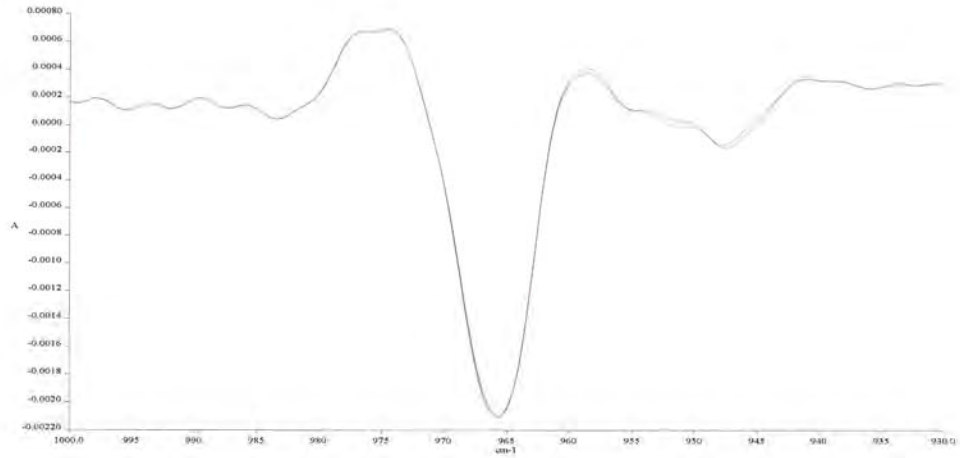

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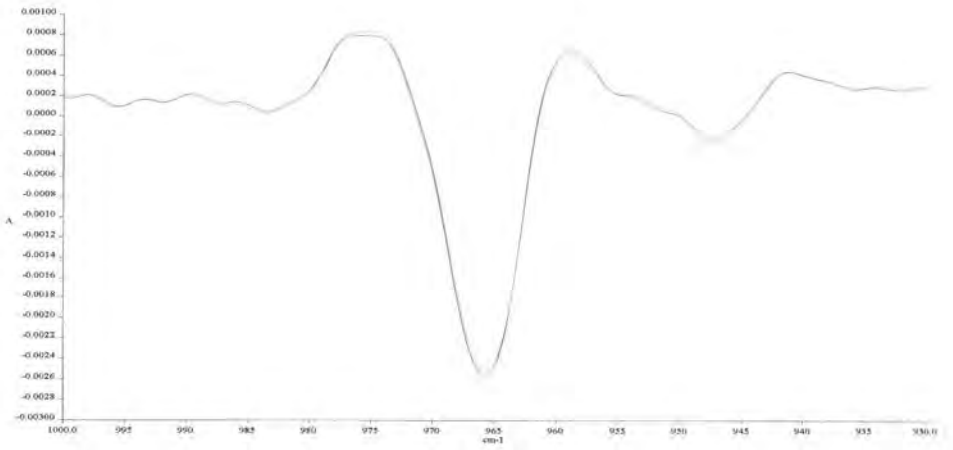
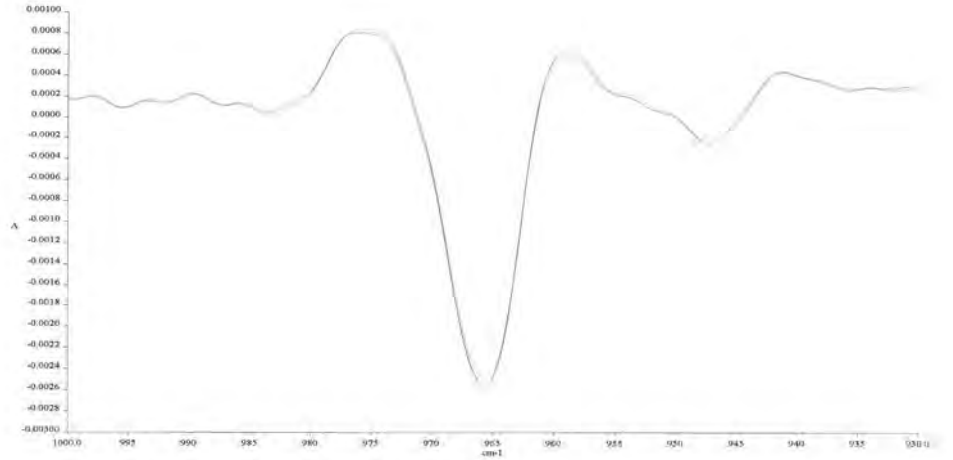
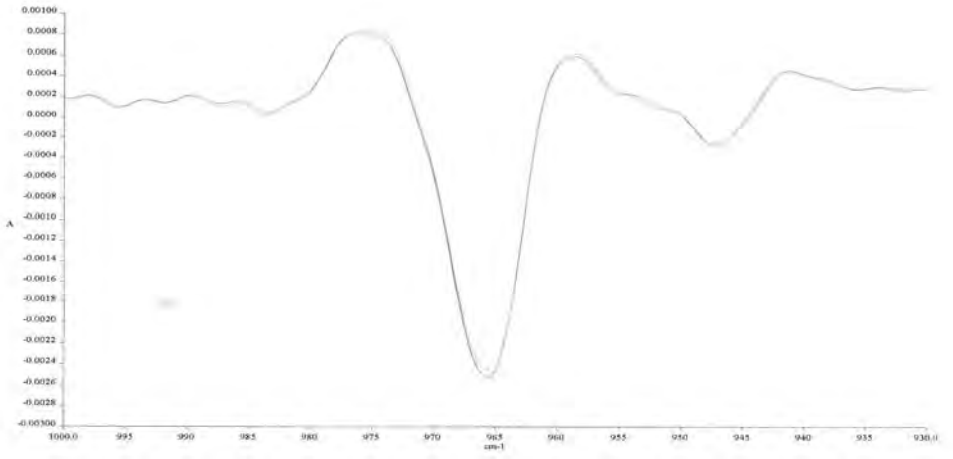


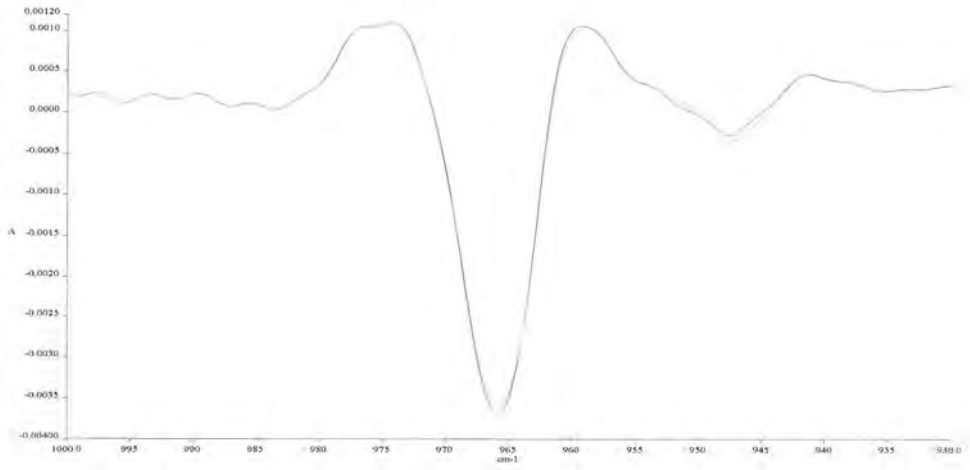
Cheese brand 1 flask A



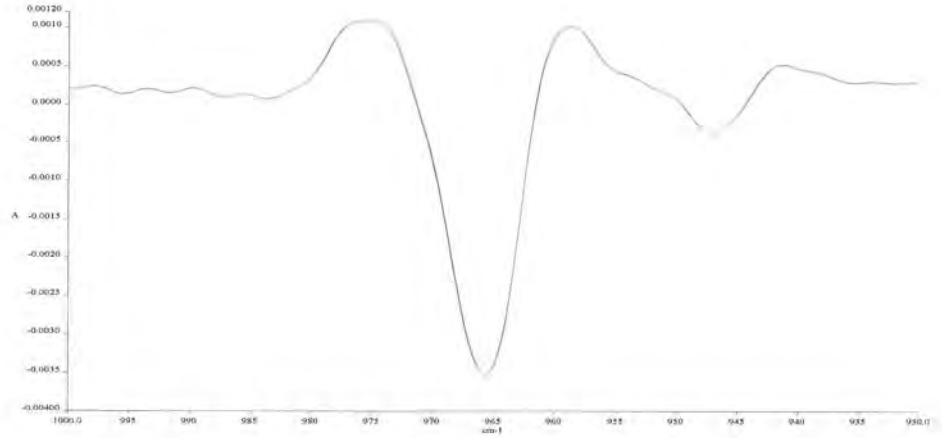
Cheese brand 1 flask B



Cheese brand 1 flask C



Cheese brand 2 flask A



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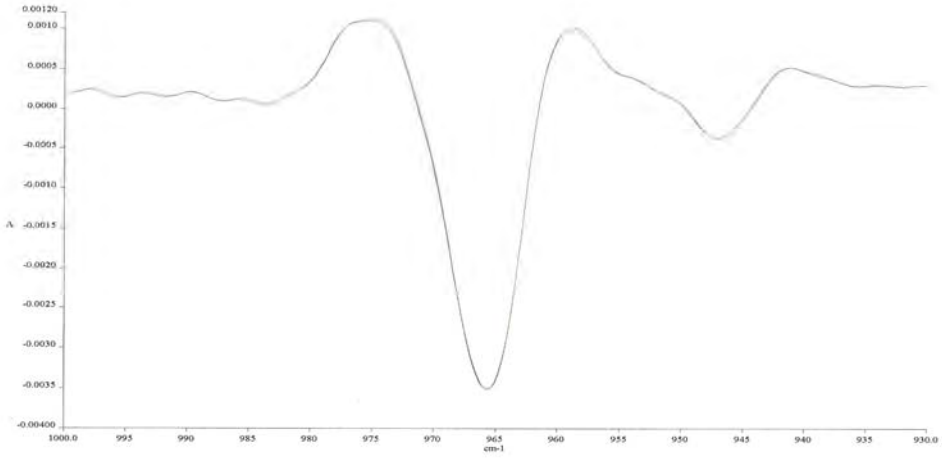
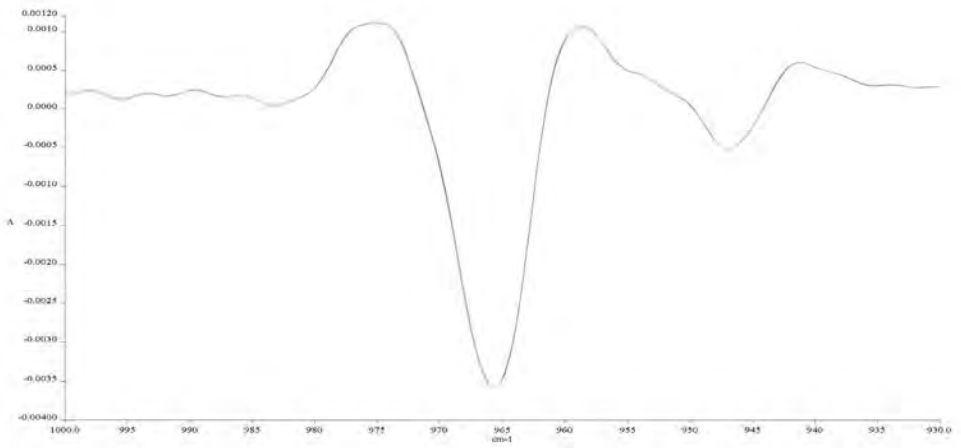
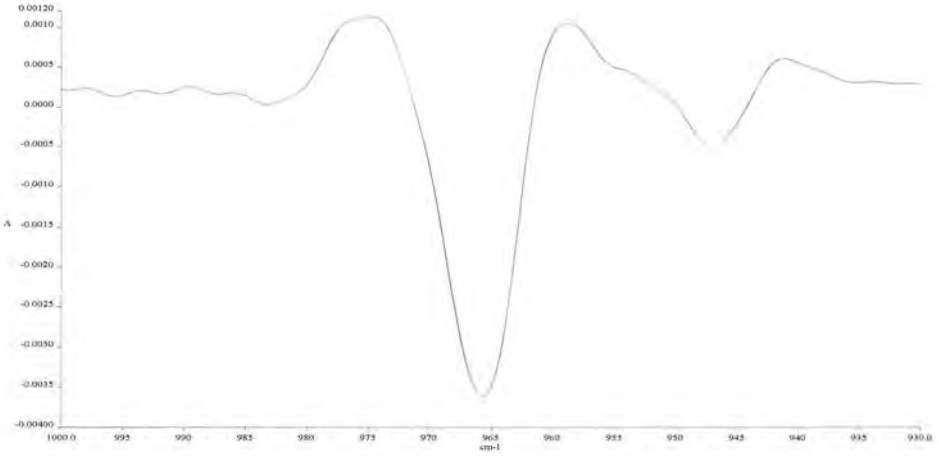


Cheese brand 2 flask C



Cheese brand 3 flask A



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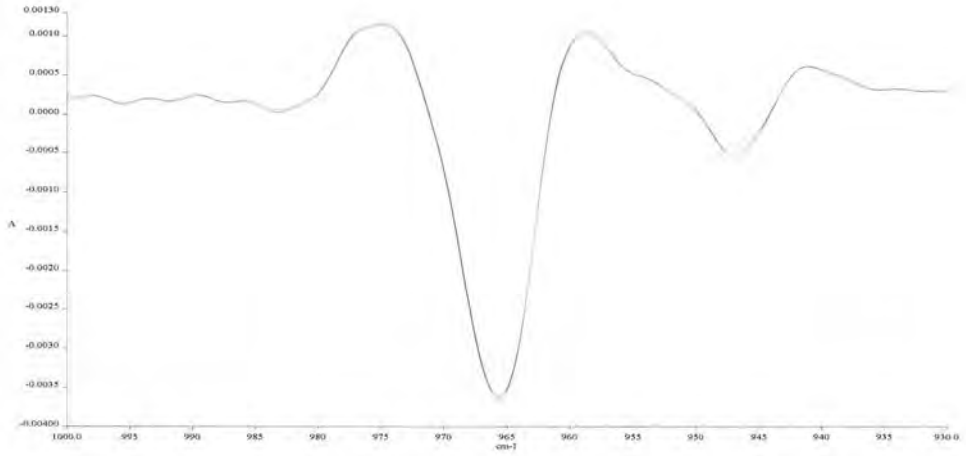
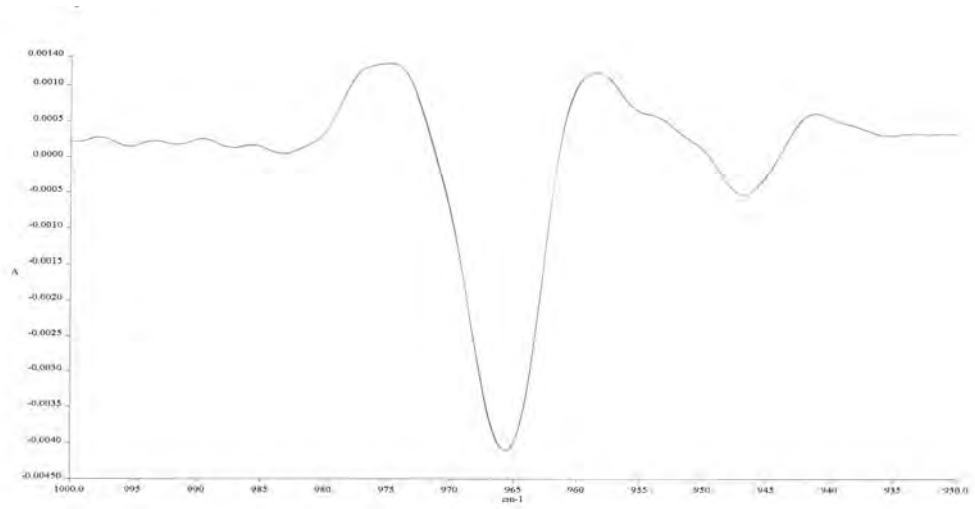
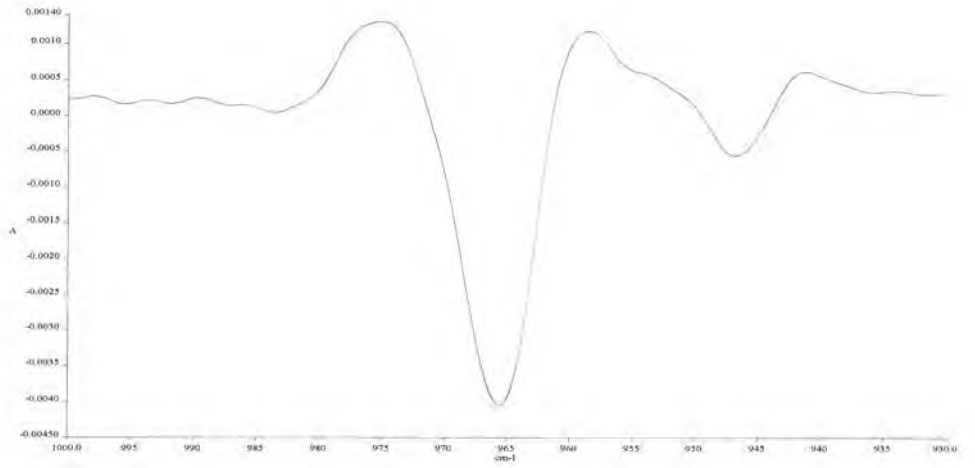


Cheese brand 3 flask C



Butter brand 1 flask A



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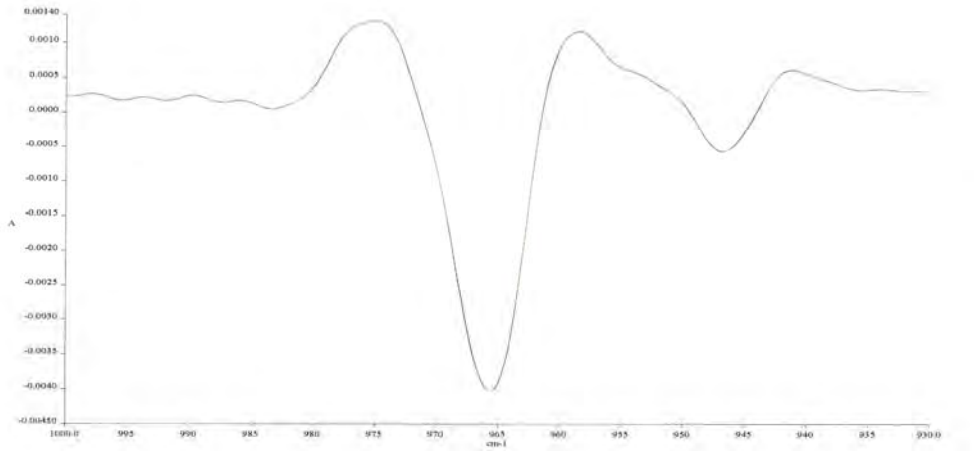

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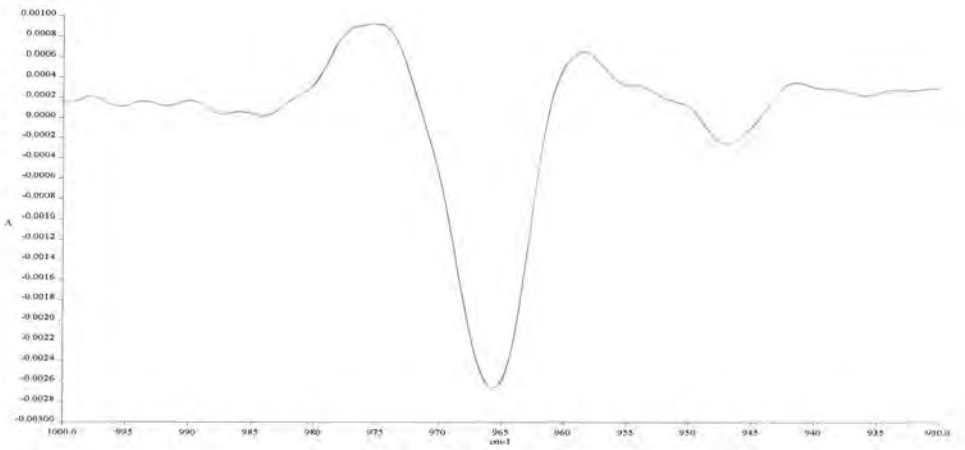

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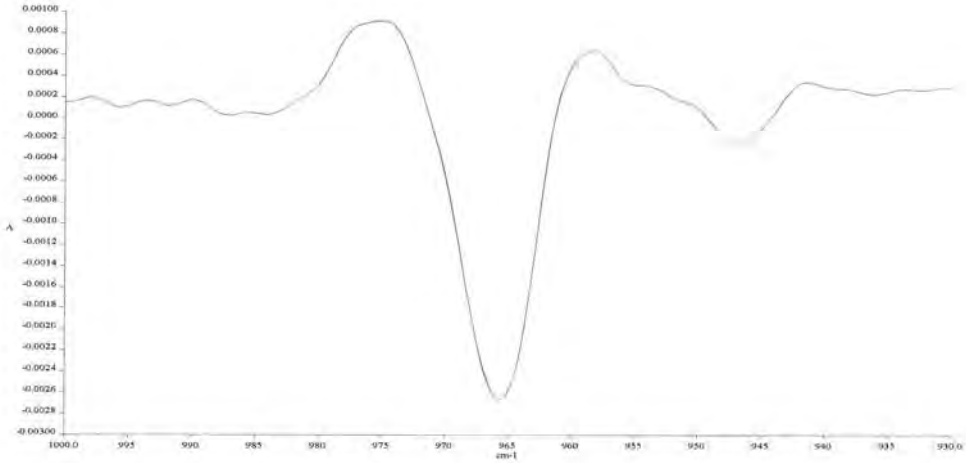

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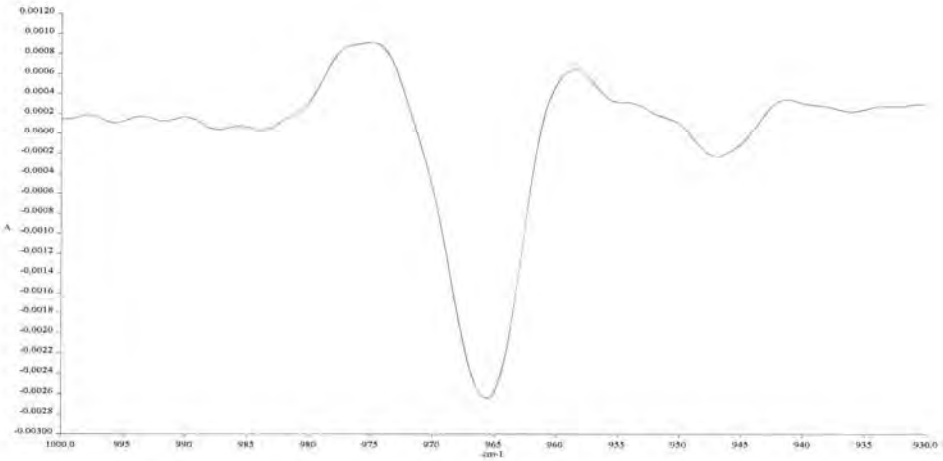
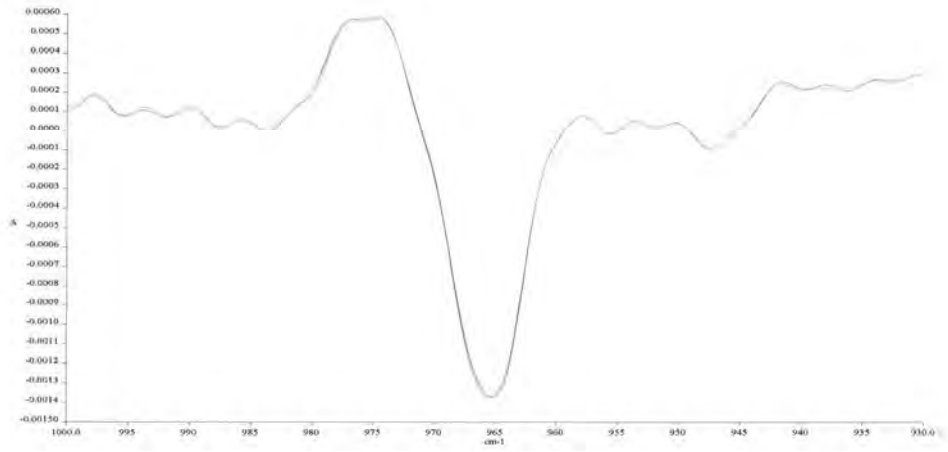


Butter brand 2 flask C



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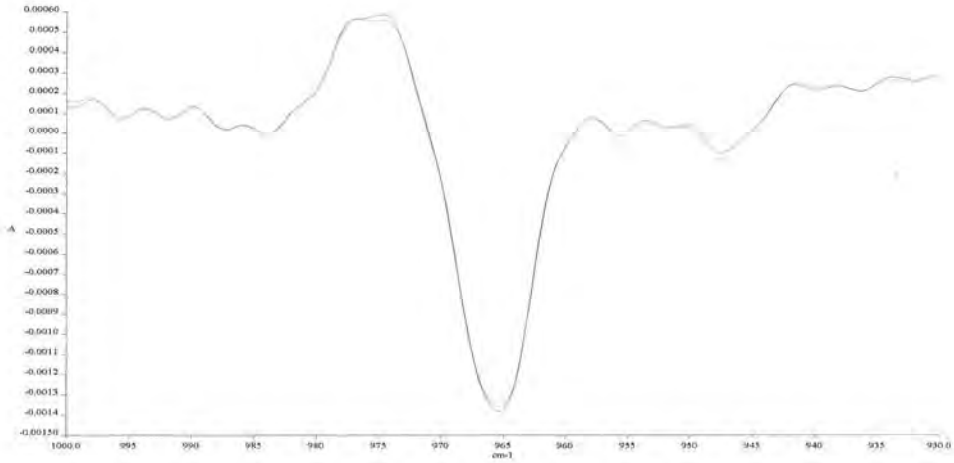
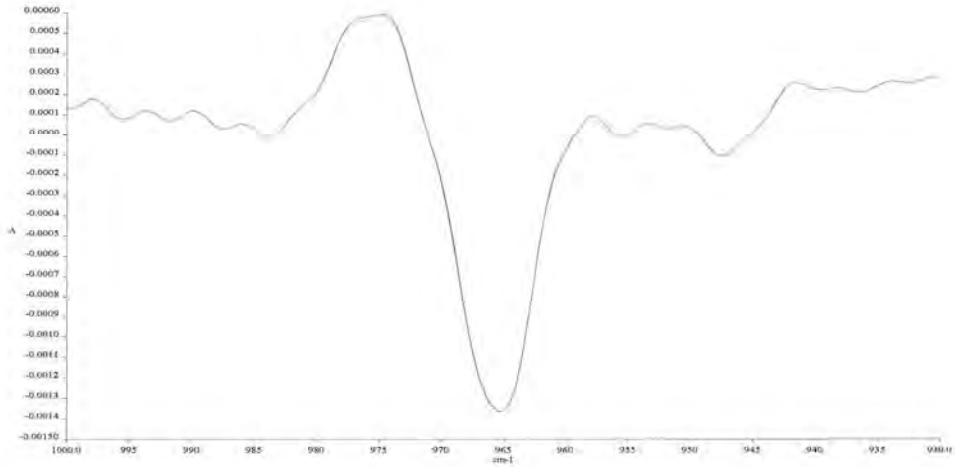


Butter brand 3 flask B



Butter brand 3 flask C

BIOGRAPHY

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DATE OF BIRTH	September 3, 1981
PLACE OF BIRTH	Prachinburi, Thailand
INSTITUTIONS ATTENDED	Silpakorn University, 1999-2004; Bachelor of Science in Pharmacy Chulalongkorn University, 2007-2010; Master of Science in Pharmacy (Food Chemistry and Medical Nutrition)
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