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# FACTORS AFFECTING *TRANS* FATTY ACID CONTENT IN CHINESE FRIED DOUGH

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การศึกษานี้มีวัตถุประสงค์เพื่อประเมินปัจจัยที่มีผลต่อปริมาณกรดไขมันชนิดทรานส์ในอาหารทอด ด้วยวิธีแอตเทนนูเอเทดโททัลรีเฟลกชันฟูเรียทรานสฟอร์มอินฟราเรดสเปกโทรสโกปี โดยมีปาท่องโก๋เป็นตัวแทนของอาหารทอดเนื่องจากเป็นอาหารทอดที่รู้จักกันกว้างขวางและมักพบการใช้น้ำมันทอดซ้ำ ศึกษาปัจจัย 2 ด้านที่มีผลต่อปริมาณกรดไขมันชนิดทรานส์ ได้แก่ ผลของระยะเวลาที่ใช้ทอดโดยไม่มีการเติมน้ำมันใหม่ลงไป และผลของการเติมวิตามินอีในน้ำมันที่ใช้ทอด การเตรียมปาท่องโก๋ทำตามสูตรข้างฉลากของแป้งปาท่องโก๋สำเร็จรูป โดยการทอดแต่ละครั้งใช้ส่วนผสมทั้งหมดรวม 80 กรัม แบ่งออกเป็นปาท่องโก๋ 10 ชิ้น ทอดในน้ำมันปาล์ม 6,000 กรัม อุณหภูมิ 170 องศาเซลเซียส เป็นเวลา 3 นาที ระยะห่างของการทอดแต่ละครั้งคือ 10 นาที ทอดต่อเนื่องไปจนครบ 5 ชั่วโมงโดยไม่มีการเติมน้ำมันใหม่ลงไป มีการเก็บตัวอย่างเพื่อนำไปวิเคราะห์ทุก 1 ชั่วโมงรวมทั้งการทอดในครั้งแรกด้วย รวมทั้งสิ้น 6 ครั้ง และแบ่งกลุ่มเพื่อศึกษาผลของการเติมวิตามินอีเป็น 3 กลุ่มได้แก่ กลุ่มควบคุม กลุ่มที่เติมวิตามินอีร้อยละ 0.23 และกลุ่มที่เติมวิตามินอีร้อยละ 0.45 ผลการวิเคราะห์พบว่า การเติมวิตามินอีในน้ำมันไม่สามารถป้องกันการเกิดกรดไขมันชนิดทรานส์ในน้ำมันที่ใช้ทอดซ้ำๆ ได้ นอกจากนั้นยังพบว่าการใช้น้ำมันทอดซ้ำมีผลต่อปริมาณกรดไขมันชนิดทรานส์โดยพบว่าปริมาณกรดไขมันชนิดทรานส์ในกลุ่มควบคุมของการทอดครั้งแรกน้อยกว่าการทอดที่ชั่วโมงที่ 4 และ 5 อย่างมีนัยสำคัญทางสถิติ ดังนั้นผู้บริโภคควรหลีกเลี่ยงการรับประทานอาหารทอดที่มีการใช้น้ำมันทอดซ้ำ

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KEYWORDS: *TRANS* FATTY ACID / CHINESE FRIED DOUGH / FRIED FOOD/ VITAMIN E / REPEATED FRYING OIL

WATCHARAPORN JAIKRASANE: FACTORS AFFECTING TRANS FATTY ACID CONTENT IN CHINESE FRIED DOUGH.
ADVISOR: ASST.PROF.LINNA TONGYONK, D.Sc.,
CO-ADVISOR: ASST.PROF.CHAMNAN PATARAPANICH, Ph.D., 88 pp.

This study aimed to evaluate the factors affecting *trans* fatty acid in deep fried food by the attenuated total reflection fourier transform infrared spectroscopy (ATR-FTIR) method. Chinese fried bread (or Pa-tong-ko in Thai), the well-known fried food in Thailand, was chosen to be a representative of fried food in this study because the repeated used of frying oil was mostly found in this kind of food. The factors which were investigated in this study were frying time without refilling new oil added and vitamin E added in frying oil. Chinese fried bread was made following the instant wheat powder package label. 80 g dough was prepared and was separated into 10 equal pieces for once frying. Then they were fried with 6,000 g palm oil, at 170°C temperature controlled for 3 minutes. Therefore ten pieces of dough were fried every 10 minutes continually until reach 5 hours without refilling new oil added. Samples were divided into 3 groups; 1) control group 2) 0.23% (w/w) vitamin E added oil group and 3) 0.45% (w/w) vitamin E added oil group. The fried sample of each group was collected every 1 hour including the first of frying. So after 5 hours frying, there were total 6 times collected. The results showed that adding of vitamin E in frying oil (at dose of 0.23% and 0.45%) could not prevent the formation of *trans* fatty acid in oil that was repeated used. Moreover, this study showed that frying time affects *trans* fatty acid content in fried food. It was found that *trans* fatty acid content (% of total fat) of Chinese fried bread at first time frying (hr0) was significantly lower than those at hr4 and hr5. Therefore, consumers should avoid consuming food that used repeated frying oil.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ATR-FTIR | attenuated total reflection fourier transform infrared spectroscopy |
| BHA | butylated hydroxyanisole |
| CHD | coronary heart disease |
| CRP | c-reactive protein |
| FAME | fatty acid methyl ester |
| g | grams |
| GC | gas chromatography |
| HDL | high density lipoprotein |
| hr | hour |
| IL-6 | interleukin-6 |
| IR | infrared spectroscopy |
| LDL | low density lipoprotein |
| ml | milliliters |
| ND | not determine |
| NO. | number |
| TE | trielaidin |
| TFA | *trans* fatty acid |
| TO | triolein |
| TNFR | tumor necrosis factor receptor |
| TNF | tumor necrosis factor |
| ZnSe | zinc selenide |

# CHAPTER I

# INTRODUCTION

*Trans* fatty acid (TFA) is unsaturated fatty acid in the *trans* configuration which the next two hydrogen atoms are bound to opposite sides of the double bond with at least one double bond. There are two major sources of *trans* fatty acid (Mozaffarian, 2006; Remig et al., 2010), firstly natural sources that are produced by bacteria in ruminants stomach (biohydrogenation) are found in milk or meat of ruminants such as sheep, goats as low levels of *trans* fatty acid (Gebauer et al., 2007). The other source is mostly found in partially hydrogenated vegetable oil during hydrogenation or refining process of unsaturated fats and oils. *Trans* fat is used in food industry because of its better functional properties such as long shelf life, oxidative stability, semisolidity (Kim et al., 2007; Saunders et al., 2008; Ratnayake et al., 2009). Moreover, *trans* fatty acid is also found in several foods such as bakery products, margarine, shortening, deep-fried fast food, packed snack foods, and crackers (Saunders et al., 2008; Adhikari et al., 2010).

In addition, deep fat frying causes physical and chemical changes in food such as oxidation, pyrolysis, polymerization, hydrolysis and isomerization. Therefore, *trans* fatty acid content in fried food might be increased because of trans isomerization that made configuration change from *cis* to *trans* form. Moreover, high levels of *trans* fatty acid might be dramatically increased while using repeated frying oil (Ariyapitipan, 2011).

The adverse health effects of *trans* fatty acid were reported and several studies of *trans* fatty acid increased due to their negative effects on human health. An increase in coronary heart disease (CHD) was the most important phenomenon since 2% energy increase from *trans* fatty acid containing food which equivalent to 4 g of 2,000 kcal standard diet may cause 23% CHD risk increase (Mozaffarian, 2006). Moreover, high *trans* fatty acid consumption was involved risk of cancer, an an increase in inflammatory marker, oxidative stress, body weight and insulin sensitivity (Lopez-Garcia et al., 2005; Gebauer et al., 2007; Martin et al., 2007; Saunders et al., 2008; Remig et al., 2010). Several countries have concerned about *trans* fatty acid consumption increasingly due to adverse health effects of them such as Japan (Kawabata et al, 2010), New Zealand (Saunders et al., 2008), and Italy (Sofi et al., 2009).

Now *trans* fatty acid level in food labels are regulated in several countries and the content of this fatty acid in many kinds of food was also determined. In the United State, products containing less than 0.5 g of *trans* fatty acid per serving can claim 0 g of *trans* fatty acid on food labels (Remig et al., 2010) while in Thailand, the amount of *trans* fatty acid on food labels still are not regulated. However, there are some studies to investigate *trans* fatty acid content in Thailand. Nackwichian (2009) analyzed *trans* fatty acid content expressed as grams of total *trans* fatty acid per 100 grams of food sample in shortening 1.84 – 3.37, margarine 1.54 – 1.89, butter cookies 0.25 – 5.27, brownie 0.18 – 0.67, sandwich chocolate cookie ND – 0.14, cake cream roll 0.16 – 0.73, croissant 0.14 – 0.83, rich butter bun 0.21 – 0.88, and crispy pie 0.41 – 0.58. Sunphan (2010) analyzed amount of *trans* fatty acid content expressed as grams of total *trans* fatty acid per 100 grams of food sample in 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 0.01 – 0.15, deep fried banana 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. Suwannakood (2011) analyzed amount of *trans* fatty acid content in snacks and non-dairy creamers.

The official methods for analysis of *trans* fatty acid content are gas chromatography (GC) and infrared spectroscopy (IR). GC method consists of several steps such as extracting samples, preparing fatty acid methyl esters (FAME) and internal standard that take a long time for separation (up to 1.5 hours) (Kim et al., 2007; Mossoba et al., 2009) while IR method is a rapid, easy and accuracy analytical method to determine the total *trans* fatty acid content. The IR method is based on the absorbance of isolated *trans* fatty acid double bond at wavelength number 966 $cm^{-1}$ (Kim et al., 2007).

Some studies showed that antioxidants decreased *trans* fatty acid occurring in frying oil. For example, phenolic extracts with butylated hydroxyanisole (BHA), synthetic antioxidant, added in amount 100 ppm or adding only the phenolic extracts in amount 200 ppm could reduce the amount of *trans* fatty acid in frying oil (Gamel, Kiritsakis, and Petrakis, 1999). Alpha-tocopherol, the most active form of vitamin E, also reduced *trans* isomerization during heating. The addition of alpha-tocopherol (1.0%) to triolein significantly prevented *trans*-isomerisation during heating (Tsuzuki et al., 2008).

Chinese fried dough (or Pa-tong-ko in Thai), one of the most well-known fried foods in Thailand, was chosen to be a representative of fried food in this study.

Because shops selling Chinese fried dough in Thailand tend to use repeated frying oil for frying the dough (Wananuwat, and Pattanapraison, 2011). Thus, the present study aims to determine the effect of vitamin E added into frying oil on the formation of *trans* fatty acid in Chinese fried dough using repeated frying oil. The content of *trans* fatty acid was analyzed by the ATR-FTIR (the attenuated total reflection fourier transform infrared spectrometry).

# CHAPTER II

# LITERATURE REVIEW

## 2.1 Physical and chemical properties of *trans* fatty acid

*Trans* fatty acid (TFA) is unsaturated fatty acid in the *trans* configuration which the next two hydrogen atoms are bound to opposite sides of the double bond with at least one double bond (Kim et al., 2007; Saunders et al., 2008; Ratnayake et al., 2009). Unlike *cis* fatty acid that the two hydrogen atoms bond to the carbon atom on the same sides of the double bond as *cis* configuration (Kim et al., 2007; Saunders et al., 2008; Ratnayake et al., 2009). The molecules of *trans* isomer are packed more linear structures and more closely so their structure were similar to the structure of saturated fatty acid (Figure 1). The melting point and the density of the *trans* configuration are higher than the *cis* configuration so their physical appearance are different. At room temperature, *trans* fatty acids are solid while *cis* fatty acids are liquid (Koletzko et al., 1997; Adhikari et al., 2010).

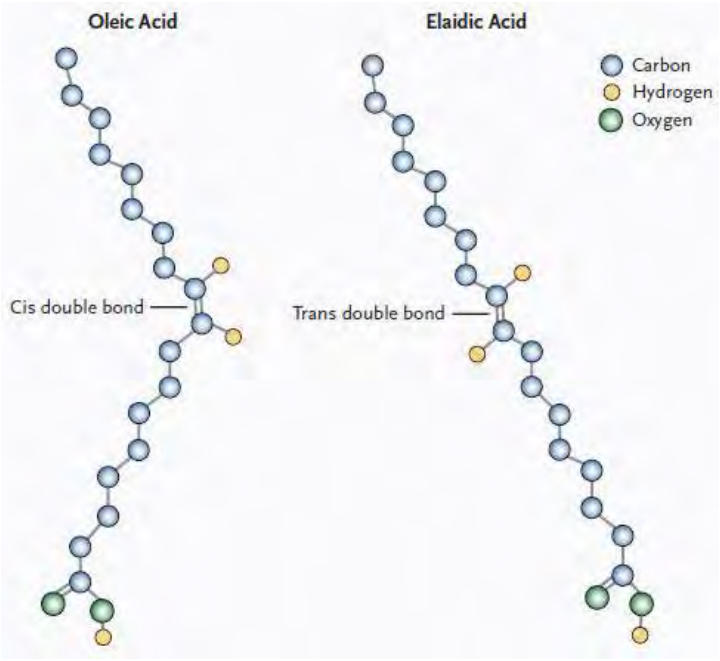


**Figure 1** *Cis* and *Trans* configuration of 9-octadecenoic acid

In industrial sources, *trans* fatty acids are mostly found in partially hydrogenated vegetable oil during hydrogenation or refining process of unsaturated fats and oils (Pfeuffer and Schrezenmeir, 2006). These processes are used for converting oil to semisolid fat for food industry in order to improve functional properties of food such as long shelf life, oxidative stability, and semisolidity (Kim et al., 2007; Saunders et al., 2008; Ratnayake et al., 2009). The partially hydrogenation process is heating oil under pressure with hydrogen and metal catalyst used (usually nickel) with stirring speed controlled condition (Martin et al., 2007). At the end of partially hydrogenation process, the mixture of *cis* and *trans* fatty acid were obtained (Remig et al., 2010). *Trans* fatty acid amount in products depend on the chemical composition of vegetable oil and controlled condition of hydrogenation. *Trans* fatty acid containing partially hydrogenation oils are mostly found in bakery

products, margarine, shortening, deep-fried fast food, packed snack foods, and crackers (McCarthy et al., 2008; Adhikari et al., 2010).

## 2.3 The transformation of *cis* to *trans* fatty acid in fried food

The formation of *trans* fatty acid could be induced during cooking or heat treatment such as frying. The amount of *trans* fatty acid forming in this process depend on frying condition, frying materials and the methods of *trans* fatty acid measurements. In addition, frying causes physical and chemical change in food such as oxidation, pyrolysis, polymerization, hydrolysis and isomerization. Therefore, *trans* fatty acid content in fried food might be increased because of *trans* isomerization that made configuration changed from *cis* to *trans* form. Moreover, high levels of *trans* fatty acid might be dramatically increased while using repeated frying oil (Ariyapitipan, 2011). In the previous study, triolein, trilinolein and trilinolein were used for studying the *trans* isomerization in the edible oil under 180 ºC for 4 and 8 hours as fried condition. The result implied that the *trans* isomerization of unsaturated fatty acid occurred during heating together with lipid oxidation (Tsuzuki et al., 2008).

## 2.4 The health effects of *trans* fatty acid

Several studies have been reported the negative effects of *trans* fatty acid consumption on human health. An increase in coronary heart disease (CHD) was the most important phenomenon since 2 % energy increased from *trans* fatty acid containing food which equivalent to 4 g of 2,000 kcal standard diet, may cause increased risk of CHD by 23% (Mozaffarian, 2006). Many studies have shown the positive relation between *trans* fatty acid consumption and coronary heart disease (CHD). The mechanism of *trans* fatty acid on CHD is well established (Gebauer et al., 2007; Fernande-San Jaun et al., 2009; Filip et al., 2010).

### 2.4.1 Coronary heart disease (CHD)

Coronary heart disease (CHD) resulted from impeded blood flow to the network of blood vessels surrounding the heart and serving the myocardium (Krummel, 2000). The risk factors of CHD are hypertension, dyslipidemia, smoking, and diabetes (Root and Anderson, 2004). Saturated fatty acid and *trans* fatty acid consumption were related to increase risk of CHD due to their effects to lipid profile and leading to dyslipidemia (Hayes, 2000).

*Trans* fatty acid intake was associated with the an increase in LDL cholesterol, triglycerides, LDL cholesterol to HDL cholesterol ratio and the decreasing of HCL cholesterol (Mensink et al., 2003; Mozaffarain, 2006; Gebauer et al., 2007; Sun et al., 2007; Brouwer et al., 2010; Remig et al., 2010; Wanders et al., 2010). Diet containing *trans* fatty acid can affect plasma lipoproteins in human than saturated fatty acid. Because the effects of *trans* fatty acid on human lipoprotein metabolism depress HDL cholesterol while raise LDL cholesterol. In contrast,

saturated fatty acids that increase LDL cholesterol also raise HDL cholesterol (Sundram et al., 1997; Hay, 2000).

### 2.4.2 The other health related effects of *trans* fatty acid

#### 2.4.2.1 Systemic inflammation

*Trans* fatty acid intake has been associated with the an increase in inflammatory marker such as c-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor (TNF) (Mozaffarain et al., 2004; Lopez-Garcia et al., 2005). Systemic inflammation is a risk factor for several diseases such as insulin resistance, diabetes, dyslipidemia, heart failure and coronary artery disease (Mozaffarain et al., 2004). *Trans* fatty acid intake and the concentrations of sTNF-R1 and sTNF-R2 were positively associated. High intake group (3.9 g/day) and low-intake group (1.8 g/day) were compared in the study of Mozaffarain et al. (2004). It was found that the sTNF-R1 and sTNF-R2 concentrations in the high intake group were 10% and 12% higher than the low intake group respectively.

#### 2.4.2.2 Diabetes

Some studies showed no significant effect of *trans* fatty acid intake on risk of type 2 diabetes (Louheranta et al., 1999; Riserus., 2006). In healthy young women, there was no difference in the insulin sensitivity between *trans* fatty acid intake and monounsaturated fatty acid intake groups (Louheranta et al., 1999). Moreover, Riserus (2006) found that after high *trans* fatty acid intake, the insulin resistance increased in type 2 diabetic patient increased.

#### 2.4.2.3 Cancer

Few studies reported the association between *trans* fatty acid intake and cancer. *Trans* fatty acid intake may increase risk of colorectal cancer (Slattery et al., 2001, Vinikoor et al., 2010) and prostate cancer (Chavarro et al., 2008). *Trans* fatty acid intake during the premenopausal year increased risk of postmenopausal breast cancer (Kim et al., 2006; Chajes et al., 2008).

## 2.5 The Regulation and the Nutrition Label of *Trans* Fatty acid

Now *trans* fatty acid content in food labels is regulated in several countries. *Trans* fatty acid content in many kinds of food were determined and the way to reduce *trans* fatty acid in food. In the United State, food products containing less than 0.5 g of *trans* fatty acid per serving can claim 0 g of *trans* fatty acid on food labels (Remig et al., 2010). In Thailand, the amount of *trans* fatty acid on food labels still are not be regulated. However, there are some studies that reported the *trans* fatty acid content in some kinds of food in Thailand. Nackwichian (2009) analyzed trans fatty acid content in shortening, margarine, butter cookies, brownie, sandwich chocolate cookie, cake cream roll, croissant, rich butter bun and crispy pie, Sunphan (2010) analyzed amount of *trans* fatty acid content in fried chicken from well-known fast food, fried chicken from street vender, French fries, Deep fried dough stick, deep fried banana pasteurized and UHT milk, ice-cream, whipping cream, cheese, and butter. Suwannakood (2011) analyzed the content of *trans* fatty acid content in snacks and non-dairy creamers.

## 2.6 Fat extraction methods

There are several extraction methods for the fat in food depending on the sample matrix such as Roese-Gottlieb, Mojonnier, Folch, Werner-Schmid and Bligh-Dyer methods are based on hydrolysis before solvent extraction step. Some other methods are used only the solvent extraction step. The solvent for fat extraction depends on the chemical property of sample and type of fat solvent such as ethers (petroleum ether, ethyl ether, isopropyl ether), hydrocarbon (hexane, benzene), alcohol (methanol, ethanol), acetone or the mixtures. Moreover, the solvent that used for fat extraction from food should have low boiling point and be rapidly evaporated without any residues remained and it also has less toxicity. (Shahidi and Wannasundara, 1998). Soxhlet method is the solvent method that requires a longer period, 4 - 24 hours for extraction (Garcia-Olmo et al, 2004). The ultrasonic extraction method allows extraction of the total fat amount in a time shorter than soxhlet method. The time for fat extraction of cookies by soxhlet method and ultrasonic method are 16 hours and 3 hours, respectively (Ruiz-Jimenez et al, 2004).

Ultrasonic extraction methods can lead the ultrasonic vibration to contact between sample and solvent and the ultrasonic irradiation of aqueous solution induces acoustic cavitation into liquid media, the wave of vibrating pressure can break the sample molecules so there is the penetration of solvent into sample (Capelo et al., 2005).

## 2.7 The official methods for analysis of *trans* fatty acid content

### 2.7.1 Gas chromotographic (GC) method

The GC method procedures are sample digestion, solvent extraction, fatty acid methyl esters (FAME) preparation, an internal standard addition (IS) and requires the specific column for separate FAME components (Mossoba., 2009). GC method can analyze individual identification and quantitation of each isomer (Ledoux, Laloux and Wolff, 2000; Mossoba., 2009) and the lower level of *trans* fatty acid can be detected (Favier and Bicanic, 1996). There is 100 m columns for determination of *trans* fatty acid content to give optimum separation of *trans* isomers (Kim et al., 2007).

### 2.7.2 Infrared spectroscopic (IR) method

The Infrared spectroscopy method has been worldwide used for *trans* fatty acid content determination. This method based on the vibrations of specific bonding molecule by passing infrared radiation through samples and the fraction of the radiation absorbed. The wave length that unique for detection of *trans* double bond is 966 $cm^{-1}$ (Mossoba et al., 2009; Mahesar et al., 2010). The AOAC (2000) chose the IR method based on an attenuated total reflection fourier transform infrared (ATR-FTIR) spectroscopy as the official method.

#### 2.7.2.1 Attenuated total reflection fourier transform infrared spectroscopy (ATR-FTIR) method

ATR-FTIR method is rapidly method for determining the amount of isolated *trans* double bonds in fats and oils at the wavelength number 966 $cm^{-1}$. ATR uses the phenomenon of total internal reflection, the IR electromagnetic radiation

beam penetrates into the melted fat which placed on the surface of an internal reflection crystal such as zinc selenide (ZnSe) or diamond (Mossoba et al., 2009) (Figure 2). This method is rapid, use a small amount of sample (about 50 µl), and not necessary to prepare the sample but the results of this method is the total *trans* fatty acid content of sample.

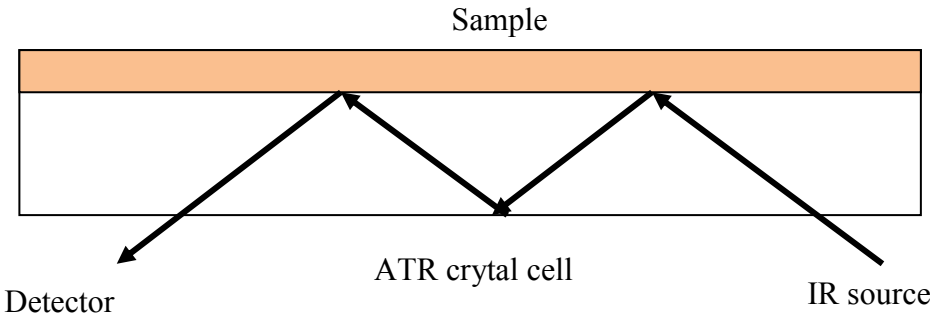


**Figure 2** The penetration of IR radiation beam through cell diagram

However, the ATR-FTIR method was limited to determine *trans* fatty acid content more than 5% of total fats (Mossoba et al., 2009). Therefore, a new procedure of ATR-FTIR which is called negative second derivative (-2D) was used. This method measures the height of the negative second derivative (-2D) of *trans* absorption band relative to air due to enhance the height of spectrum (Figure 3) (Mossoba et al., 2009). The negative second derivative IR procedure has been successfully to eliminate interferences from both conjugated and saturated fats (Mossoba et al., 2009). The precision of this method is 0.1% *trans* fatty acid as a percentage of total fat (Milosevic et al., 2004). Therefore, the negative second

derivative (-2D) methodology suitable for the rapid determination of total *trans* fatty acid at low levels (Mossoba et al., 2009).

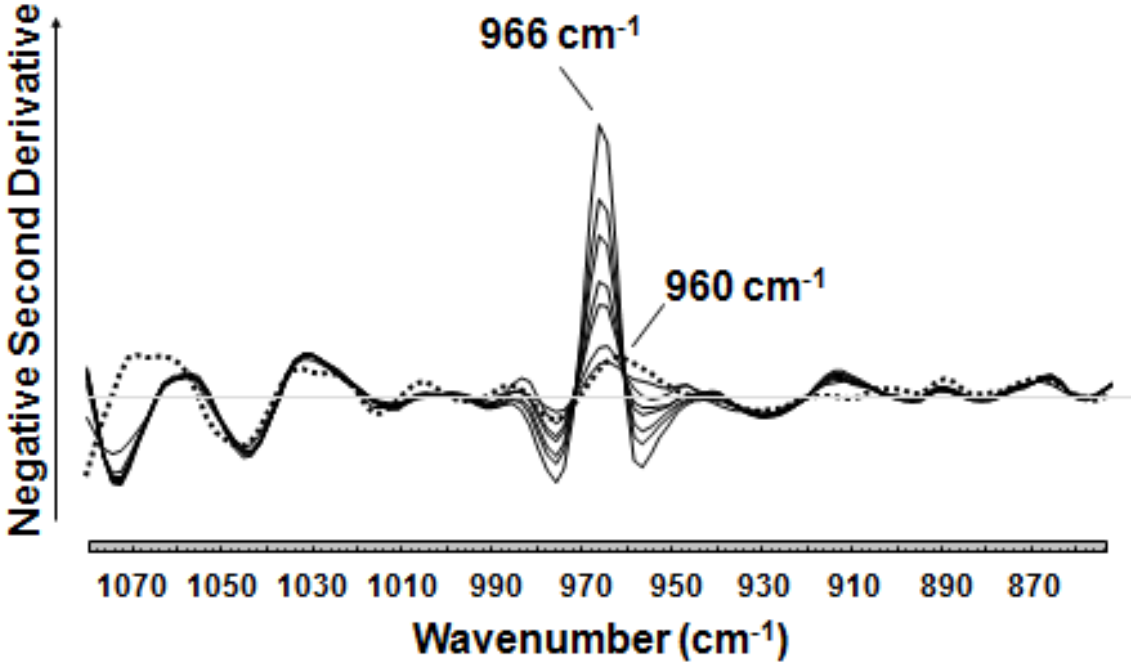


**Figure 3** Negative second derivative of ATR-FTIR spectra for *trans* fat sample measured relative to open beam (air)

## 2.8 Vitamin E

Vitamin E is a fat soluble vitamin which its antioxidant property slows the production of ROS formed when oxidation occur (Tucker and Townsend, 2005). There are two classes of biologically active form of vitamin E, tocopherols and tocotrienols (Figure 4). The most active form and strongly biological antioxidant is α-tocopherol and the synthetic form is only half as active as the natural form

(Gallagher, 2008). Synthetic forms of α-tocopherol are present as esters of either the natural *RRR*- or the synthetic mixture (*all rac*-) forms of α-tocopherol.

United States Pharmacopeia (USP) conversions which one IU is defined as 1 mg of *all rac*-α-tocopheryl acetate (USP, 1979, 1999). All of the conversions are based on rat fetal resorption assays that were conducted in the 1940s. The amounts of the free and succinate forms have been adjusted for their different molecular weights relative to the *all rac*-α-tocopheryl acetate.

To convert mg to $\mu$mol, divide the mg by the molecular weight of the vitamin E compound (α-tocopheryl acetate = 472; α-tocopheryl succinate = 530; α-tocopherol = 430) and multiply by 1,000. Because the amounts of free and succinate compounds are adjusted for their different molecular weights relative to α-tocopheryl acetate, these forms have the same conversion factors as the corresponding tocopherol compounds. To convert the $\mu$mol of the vitamin E compound to mg of α-tocopherol, multiply the $\mu$mol by the molecular weight of α-tocopherol (430) and divide by 1,000. The activities of the three synthetic α-tocopherol compounds have been divided by 2 because the 2*S*-stereoisomers contained in synthetic-α-tocopherol are not maintained in the blood. Factors for converting units of vitamin E are shown in Table 1 (Institute of Medicine, 2000).

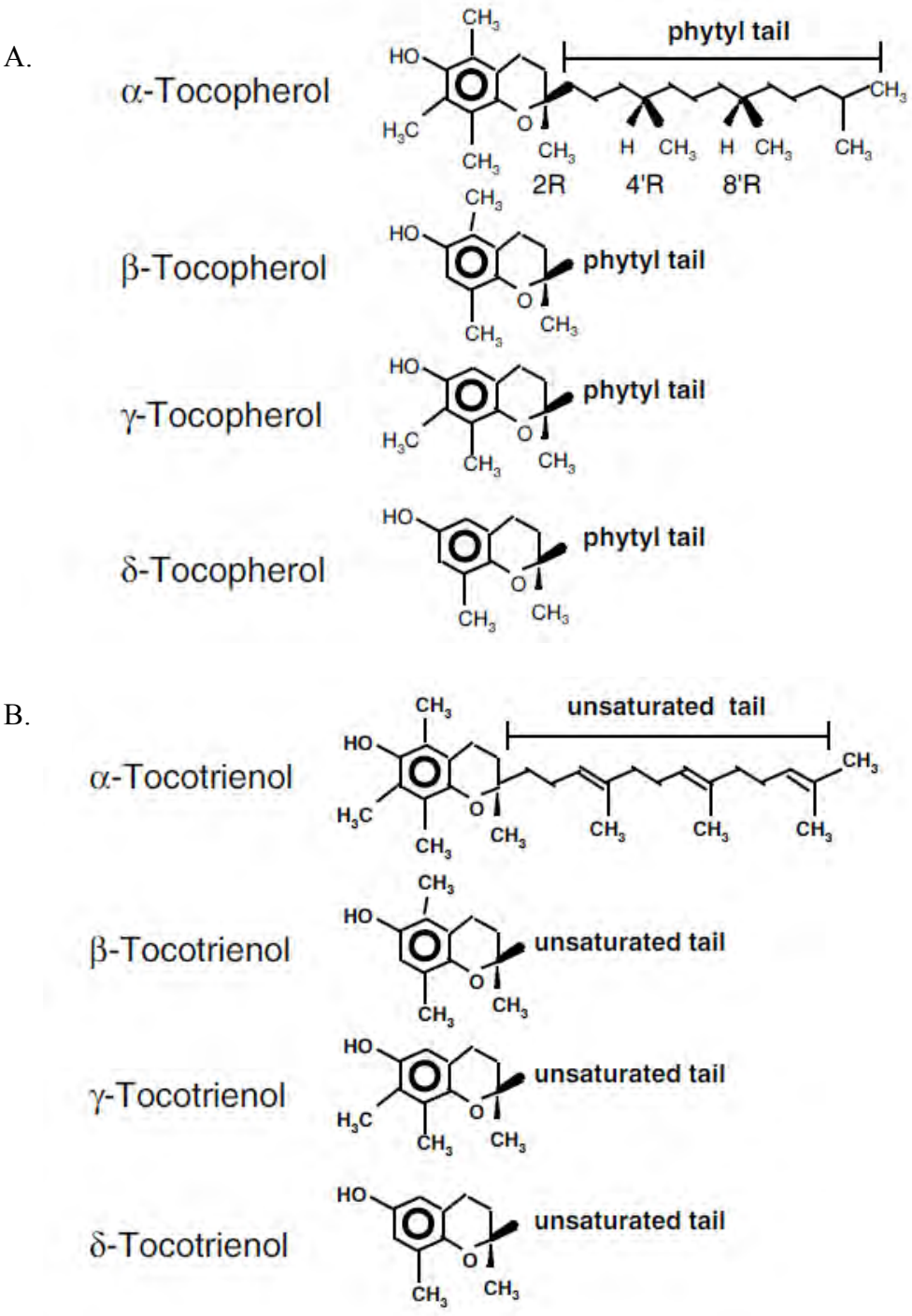


**Figure 4** Structures of tocopherols and tocotrienols. The four tocopherols are shown in A and the four tocotrienols in B. All tocopherols are in the *RRR*-form.

**Table 1** Factors for converting units of vitamin E

| | USP Conversion Factors | | Molar Conversion Factors | α-tocopherol Conversion Factors |
|---|---|---|---|---|
| | IU/mg | mg/IU | $\mu$mol/IU | mg/IU |
| Synthetic Vitamin E and Esters | | | | |
| *dl*-α-Tocopheryl acetate | 1.00 | 1.00 | 2.12 | 0.45 |
| *dl*-α-Tocopheryl succinate | 0.89 | 1.12 | 2.12 | 0.45 |
| *dl*-α-Tocopherol | 1.10 | 0.91 | 2.12 | 0.45 |
| Natural Vitamin E and Esters | | | | |
| *d*-α-Tocopheryl acetate | 1.36 | 0.74 | 1.56 | 0.67 |
| *d*-α-Tocopheryl succinate | 1.21 | 0.83 | 1.56 | 0.67 |
| *d*-α-Tocopherol | 1.49 | 0.67 | 1.56 | 0.67 |

# CHAPTER III

# MATERIALS AND METHODS

## 3.1 Instruments

A deep fryer (FR1265, Fritel, Belgium) and an ultrasonic bath (Transsonic Digital S, Elma, Germany) were used for sample preparation and fat extraction, respectively. A rotary evaporator (CH-9230, Buchi labortechnik AG, Switzerland) and vacuum desiccator (Heraeus, Germany) were used to evaporate the solvent from sample extracts. The Fourier transform infrared spectrometer (Perkin Elma Spectrum One FTIR, USA) and a zinc selenide crystal (ZnSe through plate 45º, Perkin Elma, USA) attenuated total reflection infrared cell was used for determination of *Trans* fatty acid.

## 3.2 Chemicals

Trielaidin [1,2,3, tris(*trans*-9-octadecanoate)] and triolein [1,2,3, tris(*cis*-9-octadecanoate)] with more than 99% purity were purchased from Sigma-Aldrich (St. Louis, MO, USA) as fatty acid standards. *n*-Hexane was obtained from Mallinckrodt Chemicals (Phillipsburg, NJ, USA) as fat extracted solvent. Fifty percent USP tocopheryl acetate obtained from DSM Nutritional Products (Swisszerland) was used in this study and 1 g of 50% USP tocopheryl acetate was calculated to be equivalent to 0.23 g of vitamin E (tocopherol form).

## 3.3 Methods

### 3.3.1 Experimental design

Chinese fried dough was chosen to be a representative of fried food in this study. Each batch of dough sample was prepared by deep fat frying and then fat were extracted from the fried dough by sonication method. Finally, *trans* fatty acid content was determined by using the attenuated total reflection fourier transform infrared spectrometer (ATR-FTIR). The overall experiment to determine *trans* fatty acid content is shown in Figure 5.

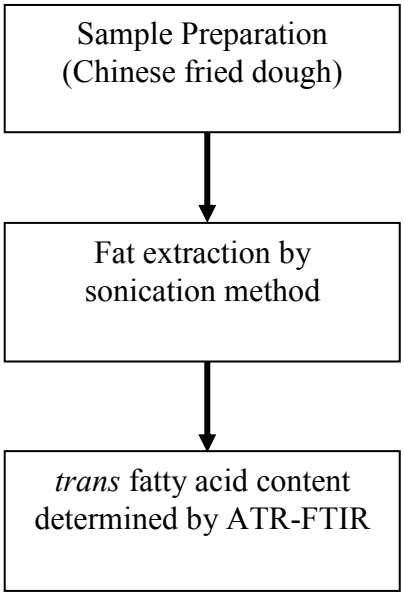


**Figure 5** Overall experiment to determine *trans* fatty acid content of Chinese fried dough.

### 3.3.2 Sample Preparation

Ingredients of Chinese fried dough including instant wheat powder, soybean oil and yeast were purchased from local supermarket in Bangkok. Palm oil (Yok®) was used as oil for frying and the recipe of Chinese fried dough was used following the instant wheat powder package label.

#### 3.3.2.1 Recipe of Chinese fried dough

| | | | |
|---|---|---|---|
| **(I)** | Instant wheat powder | 2,000 | grams |
| **(II)** | Water | 7 | cups |
| **(III)** | Vegetable oil | 4 | tablespoons |
| **(IV)** | Yeast | 4 | teaspoons |

#### 3.3.2.2 Procedure of Chinese fried dough preparation and storage

After all of the compositions were prepared, instant wheat powder was sifted and rested for a moment. Yeast and vegetable oil were mixed with water and stirred. In powder's bowl, the mixture was kneaded to homogeneous dough and rested in an oven at 45°C for 2 hours. Afterward, whole dough was separated into amount of 80 g dough for once frying. Then 80 g dough was separated into 10 equal pieces and one piece was pressed into 2-tail shape with 0.5x2x2.5 cm size per tail. The 2-tail shape of Chinese fried dough is shown in Figure 6. Then two tails of dough were joined together before frying in the deep fryer with 6,000 g palm oil. Ten pieces of dough were fried in onetime with 170°C temperature controlled, 3 minutes fried and then rested in a tray for a moment before kept in polyethylene bag at -20°C until fat extraction and *trans* fatty acid determination. The interval time of frying was 10 minutes therefore ten pieces of dough were fried every 10 minutes continually until

reach 5 hours without renew oil added. The experiment was adapted from the research of Wananuwat et al. (2011).

**Figure 6** The 2-tail shape of Chinese fried dough

#### 3.3.2.3 Sample groups and time of sample collection

Samples were divided into 3 groups; 1) control group (without vitamin E added), 2) 0.23% (w/w) vitamin E added oil group and 3) 0.45% (w/w) vitamin E added oil group. In each group, the dough was fried every 10 minutes and the samples were collected every 1 hour including the first of frying. So after 5-hour frying, there were total 6 times collected as hr0 (first time frying), hr1, hr2, hr3, hr4 and hr5. Each experiment was repeated for 3 times with the same manner. The overall of Chinese fried dough preparation and storage is shown in Figure 7. Sample groups and time of sample collection are shown in Figure 8.

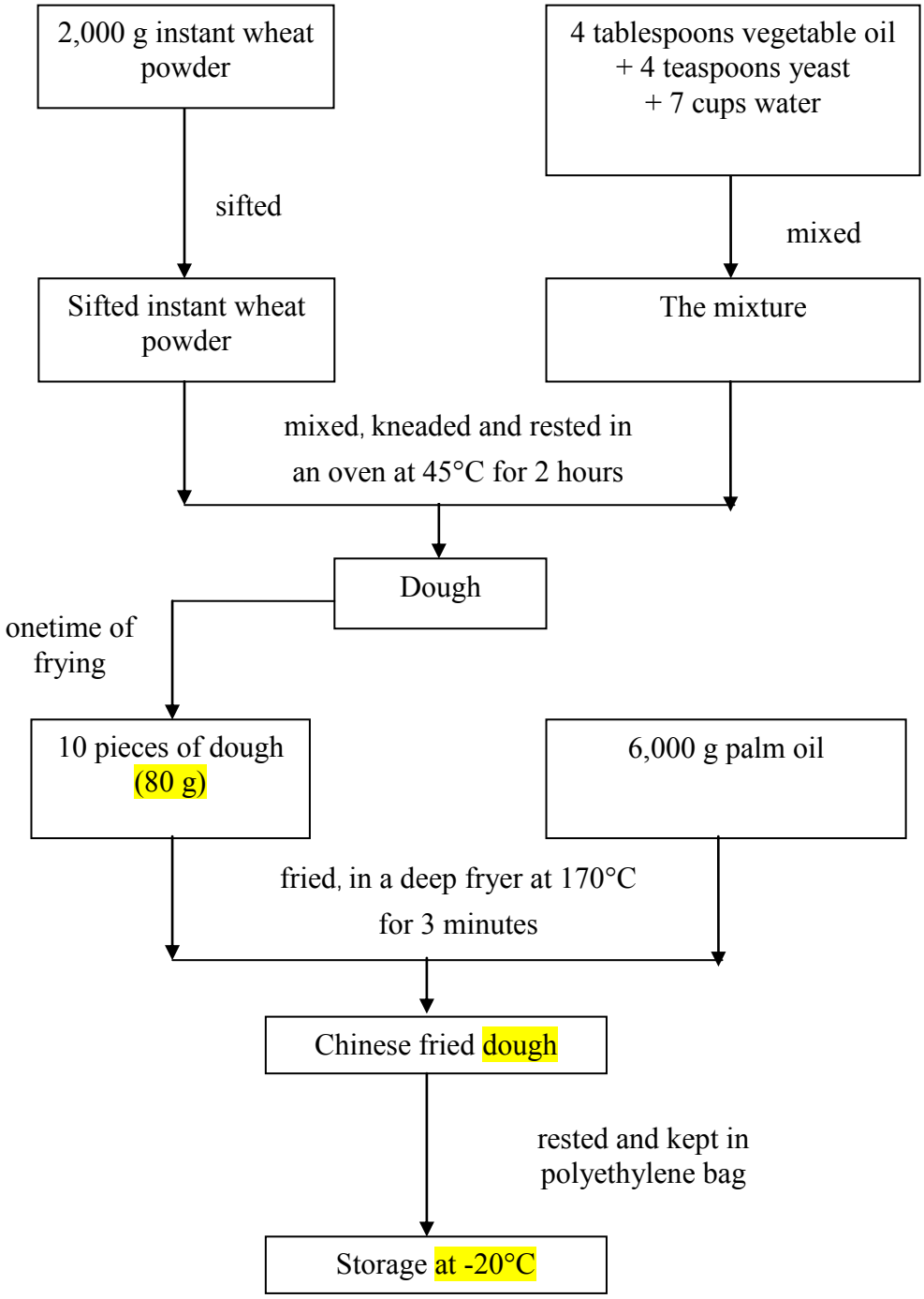


**Figure 7** The overall of Chinese fried dough preparation and storage

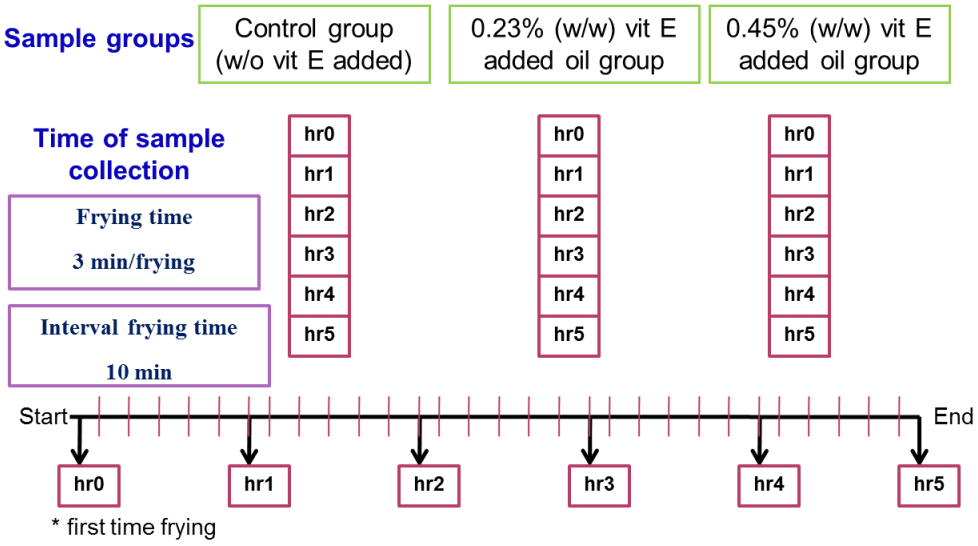


**Figure 8** Sample groups and time of sample collection

### 3.3.3 Fat extraction

Ultrasonic extraction with n-hexane as solvent and the appropriate condition; 60°C, 40% ultrasonic intensity level for 120 minutes which reported by Soonpan, 2010 was used. Five grams of Chinese fried dough were be sampling to fat extraction. The round bottom flask containing 5 g of tiny Chinese fried dough sample and 30 ml n-hexane were placed in the ultrasonic bath under the above mention condition. After extraction, the extract was filled through Whatman no.42 filter paper. The filtrate was evaporated by rotary evaporator and dried in vacuum desiccator for 60 minutes.

### 3.3.4 Determination of *trans* fatty acid content of Chinese fried dough

After fat extraction, the attenuated total reflection fourier transform infrared spectrometry (Perkin Elma Spectrum One ATR-FTIR, USA) was used to determine *trans* fatty acid content. The parameter was set up according to the manufacturer's recommendation for using a zinc selenide ATR cell which maintained

at 65±2°C for fully melted sample and other parameters were set as 1050-900 $cm^{-1}$ spectral range, 4 $cm^{-1}$ resolution and 64 scans.

The single-beam spectrum collected of air was used as reference background while the test sample was collected against that of the reference background and convert into absorbance. The height of the negative second derivative of the *trans* band was used for calculating the *trans* fatty acid content since the sensitive and accuracy were improved. After each sample analysis, the ATR cell was cleaned by acetone and each repeated test sample was scanned for 3 times.

Trielaidin (TE) and Triolein (TO) were mixed to generate five standards covering 0.5% to 50% *trans* fatty acid level by TE amount including 0.0015, 0.0150, 0.0300, 0.0900, 0.1500 g and TO amount including 0.2985, 0.2850, 0.2700, 0.2100, and 0.1500 g respectively according to 2000.10 AOAC method in 2000. Then the standard calibration curve between % *trans* fatty acid content of total fat and peak height *trans* band were plotted. *Trans* fatty acid content was calculated by using the linear regression equation computed from standard calibration curve which plotted between peak height of standards and % *trans* fatty acid of total fat. The sample's peak height *trans* band was replaced into the equation to get *trans* fatty acid content as % of total fat. Mean and standard deviation were reported.

### 3.4 Statistical analysis

The effect of using repeatedly used frying oils and vitamin E added in frying oils on *trans* fatty acid content in Chinese fried dough were calculated by following statistics; Analysis of variance between groups (two way-ANOVA) and Tukey HSD test. Frying time parameter was chosen to explain the effect of using repeated frying oil on *trans* fatty acid content. The difference of two groups such as *trans* fatty acid content ( % total fat) of each frying time or each group were analyzed by using two way-ANOVA and Tukey HSD was used to find which pairs were different

# CHAPTER IV

# RESULTS

## 4.1 Appearance of Chinese fried dough

After Chinese fried dough was fried with 170°C temperature controlled, 3 minutes per frying, continually repeated frying every 10 minutes until 5 hours, each batch of the sample was collected and observed at hr0 (first time frying), hr1, hr2, hr3, hr4 and hr5 respectively. The difference of Chinese fried dough appearance at each hour was found. Especially the Chinese fried dough at hr5, physical appearance becomes darkening in color and more stiffen (Table 2).

## 4.2 Weight of Chinese fried dough

Before frying, dough was weighed about 80 g each frying. Then 80 g dough was separated into 10 equal pieces with 2-tail shape and fried. The weights of dough before frying and Chinese fried dough in control group were observed (Table 3).

## 4.3 Total fat content in Chinese fried dough

Total fat content of each group was reported as range and mean ± standard deviations of triplicate (Table 4). Among 3 groups, the highest amount of average total fat content was found in control group at hr5 (11.85±0.33g/100g food) and the lowest was found in 0.45% (w/w) vitamin E added oil group at hr2 (6.71±0.68g/100g food).

**Table 2** Appearance of Chinese fried dough

| Time | Appearance of Chinese fried dough | Level of color [a] |
|---|---|---|
| hr0[*] |  | + |
| hr1 |  | ++ |
| hr2 |  | ++ |
| hr3 |  | +++ |
| hr4 |  | ++++ |
| hr5 |  | +++++ |

[*] hr0 = first time frying
[a] More + is mean more darkening in color

**Table 3** Weight of Chinese fried dough in the control group

| Time | Weight of dough (g) | Weight of Chinese fried dough (g) | Weight of each Chinese fried dough piece (g) | Difference weight of Chinese fried dough before and after frying (g) |
|---|---|---|---|---|
| hr0* | 81.18 | 88.32 | 8.83 | 7.14 |
| hr1 | 80.65 | 87.72 | 8.77 | 7.07 |
| hr2 | 81.24 | 88.07 | 8.81 | 6.83 |
| hr3 | 80.25 | 86.18 | 8.62 | 5.93 |
| hr4 | 79.78 | 85.77 | 8.56 | 5.99 |
| hr5 | 80.60 | 86.24 | 8.62 | 5.64 |

* hr0 = first time frying

**Table 4** Total fat content of Chinese fried dough

| Time | Total fat content (g/100g food) | | |
|---|---|---|---|
| | **Control group** | **0.23% (w/w) vitamin E added oil group** | **0.45% (w/w) vitamin E added oil group** |
| hr0* | 11.20±0.61 (10.55-12.41) | 7.98±1.17 (5.88-9.92) | 7.36±0.22 (6.91-7.59) |
| hr1 | 9.20±0.33 (8.55-9.54) | 8.39±1.12 (6.29-10.13) | 6.98±0.65 (6.01-8.22) |
| hr2 | 9.41±0.54 (8.34-10.05) | 9.06±0.95 (7.34-10.63) | 6.71±0.39 (6.21-7.48) |
| hr3 | 10.09±0.57 (9.47-11.22) | 8.68±0.81 (7.53-10.25) | 6.88±0.45 (6.33-7.76) |
| hr4 | 10.55±0.89 (8.94-12.02) | 9.54±1.42 (8.08-12.39) | 7.01±0.53 (5.95-7.57) |
| hr5 | 11.84±0.19 (11.48-12.13) | 8.89±1.67 (7.24-12.12) | 7.79±0.11 (7.60-7.99) |

Data are presented as mean ± standard error (range) of triplicate extraction
* hr0 = first time frying

## 4.4 Total *trans* fatty acid content of Chinese fried dough

The total *trans* fatty acid content was determined by attenuated total reflection Fourier transform infrared spectrometry (ATR-FTIR), which followed by AOAC Official Method 2000.10.(AOAC, 2000). Then the height of the negative second derivative of the *trans* fatty acid absorption band at wave number 966 $cm^{-1}$ was measured. The height of the negative second derivative of *trans* fatty acid absorption band of Chinese fried dough for control group, 0.23% (w/w) vitamin E added oil group and 0.45% (w/w) vitamin E added oil group are shown in Table 5, 6 and 7 respectively. Trielaidin and triolein were mixed to get five standards covering 0.5% to 50% *trans* fatty acid level (Table 8). Then the standard calibration curve was plotted by % *trans* fatty acid of total fat and the peak height at wavelength number 966 $cm^{-1}$ with a correlation coefficient ($r^2$) of 0.9996 (Figure 9).

*Trans* fatty acid content was calculated by using the linear regression equation computed from standard calibration curve which plotted between peak height of standards and % *trans* fatty acid of total fat. The sample's peak height *trans* band was replaced into the equation to get trans fatty acid content as % of total fat. Mean and standard deviation were reported in Table 8.

**Table 5** The height of the negative second derivative of *trans* fatty acid absorption band of Chinese fried dough for control group

| Time | Average peak height (-)(x $10^{-4}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Experiment 1 | | | Experiment 2 | | | Experiment 3 | | |
| | Replicate batch1 | Replicate batch2 | Replicate batch3 | Replicate batch1 | Replicate batch2 | Replicate batch3 | Replicate batch1 | Replicate batch2 | Replicate batch3 |
| hr0* | 6.00 | 6.00 | 6.00 | 7.00 | -** | 6.00 | 7.00 | 7.00 | 6.33 |
| hr1 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 |
| hr2 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 6.00 | 6.33 | 6.00 |
| hr3 | 7.67 | 7.67 | 8.00 | 7.67 | 7.67 | 8.00 | 7.00 | 7.00 | 7.00 |
| hr4 | 7.67 | 8.00 | 8.00 | 7.67 | 8.00 | 8.00 | 7.00 | 8.00 | 8.00 |
| hr5 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |

Data are presented as average peak height of *trans* fatty acid absorption band at wave number 966 $cm^{-1}$ of triplicate* hr0 = first time frying
** Data lost

**Table 6** The height of the negative second derivative of *trans* fatty acid absorption band of Chinese fried dough for 0.23% (w/w) vitamin E added oil group

| Time | Average peak height (-)(x $10^{-4}$) [a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Experiment 1 | | | Experiment 2 | | | Experiment 3 | | |
| | Replicate batch1 | Replicate batch2 | Replicate batch3 | Replicate batch1 | Replicate batch2 | Replicate batch3 | Replicate batch1 | Replicate batch2 | Replicate batch3 |
| hr0* | 6.00 | 7.00 | 7.00 | 6.33 | 6.00 | 6.33 | 8.00 | 9.00 | 9.00 |
| hr1 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 9.00 | 9.33 | 9.00 |
| hr2 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 10.00 | 10.00 | 9.67 |
| hr3 | 7.00 | 7.00 | 7.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |
| hr4 | 7.67 | 7.67 | 8.00 | 8.00 | 8.00 | 8.00 | 9.00 | 9.00 | 9.00 |
| hr5 | 8.00 | 8.00 | 8.00 | 8.67 | 8.67 | 8.33 | 9.00 | 9.00 | 9.00 |

[a] Average peak height of *trans* fatty acid absorption band at wave number 966 $cm^{-1}$ of triplicate* hr0 = first time frying

**Table 7** The height of the negative second derivative of *trans* fatty acid absorption band of Chinese fried dough for 0.45% (w/w) vitamin E added oil group

| Time | Average peak height (-)(x $10^{-4}$) [a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Experiment 1 | | | Experiment 2 | | | Experiment 3 | | |
| | Replicate batch1 | Replicate batch2 | Replicate batch3 | Replicate batch1 | Replicate batch2 | Replicate batch3 | Replicate batch1 | Replicate batch2 | Replicate batch3 |
| hr0[*] | 7.00 | 7.00 | 7.00 | 5.67 | 5.67 | 6.00 | 6.00 | 6.00 | 6.33 |
| hr1 | 7.00 | 7.00 | 7.00 | 7.00 | 6.67 | 6.33 | 7.00 | 7.00 | 6.67 |
| hr2 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 |
| hr3 | 8.00 | 8.00 | 8.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 |
| hr4 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |
| hr5 | 9.00 | 9.00 | 9.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |

[a] Average peak height of *trans* fatty acid absorption band at wave number 966 $cm^{-1}$ of triplicate [*] hr0 = first time frying

**Table 8** Composition of oleic acid and elaidic acid in standard mixture.

| Triolein (g) | Trielaidin (g) | Total (g) | % *trans* fat of total fat |
|---|---|---|---|
| 0.3002 | 0.0022 | 0.3024 | 0.73 |
| 0.2837 | 0.0183 | 0.3020 | 6.06 |
| 0.2699 | 0.0326 | 0.3025 | 10.80 |
| 0.2122 | 0.0903 | 0.3025 | 29.85 |
| 0.1567 | 0.1501 | 0.3068 | 48.92 |

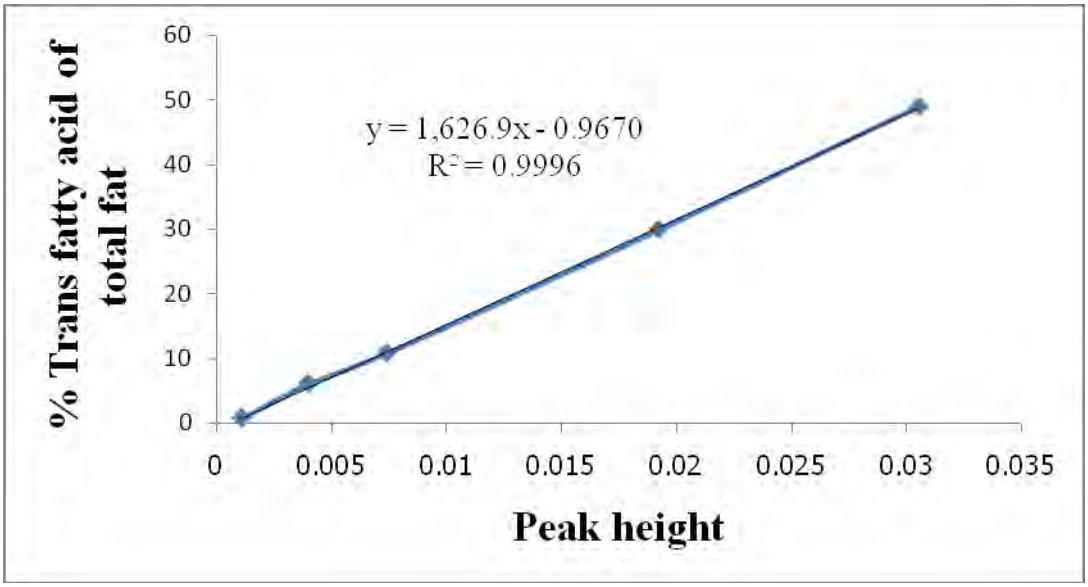


**Figure 9** Standard calibration curve of triolein and trielaidin in standard mixture.

Following Table 9, *trans* fatty acid content was expressed as % of total fat (range and mean ± standard deviations of triplicate). Among these groups, the highest amount of average *trans* fatty acid content was found in the 0.23% (w/w) vitamin E added oil group at hr5 (0.42±0.09 % of total fat) and the lowest was found in the control group at hr0 (0.07±0.07 % of total fat).

Frying time parameter was chosen to explain the effect of using repeated frying oil on *trans* fatty acid content. By using two-way ANOVA in the control group, the frying time affected *trans* fatty acid content ($p$-value < 0.001). While using Tukey HSD test found significant difference of *trans* fatty acid content (% of total fat) between hr0 (first time frying) and hr4, hr0 and hr5 and hr1 and hr5 ($p$-value=0.005, < 0.001 and 0.022 respectively). On the other hand, the significant difference of *trans* fatty acid content (% of total fat) between groups were not found.

**Table 9** *Trans* fatty acid content in Chinese fried dough.

| Time | ***Trans* fatty acid content (% of total fat)** | | |
|---|---|---|---|
| | **Control group** | **0.23% (w/w) vitamin E added oil group** | **0.45% (w/w) vitamin E added oil group** |
| hr0* | 0.07±0.04 (0.01-0.14) [a, c] | 0.20±0.12 (0.05-0.44)[NS] | 0.07±0.05 (ND-0.17)[NS] |
| hr1 | 0.17±0.00 (0.17-0.17) [a, d] | 0.29±0.12 (0.17-0.52)[NS] | 0.14±0.14 (0.12-0.17)[NS] |
| hr2 | 0.20±0.11 (0.03-0.41) [a, d] | 0.33±0.16 (0.17-0.64)[NS] | 0.17±0.00 (0.17-0.17)[NS] |
| hr3 | 0.27±0.05 (0.17-0.33) [a, d] | 0.28±0.05 (0.17-0.33)[NS] | 0.22±0.05 (0.17-0.33)[NS] |
| hr4 | 0.31±0.01 (0.28-0.33) [b, d] | 0.38±0.06 (0.30-0.50)[NS] | 0.33±0.00 (0.33-0.33)[NS] |
| hr5 | 0.37±0.04 (0.33-0.46) [c, e] | 0.42±0.05 (0.33-0.50)[NS] | 0.39±0.06 (0.33-0.50)[NS] |

ND = Non quantified at the level of traces

* hr0 = first time frying

Data are presented as mean ± standard error (range) of triplicate extraction.

The values at the time points not sharing a common letter are significantly different ($P < 0.05$).

# CHAPTER V

# DISCUSSION

Deep fat frying is the most common and age-old method used for cooking and it is popular worldwide. In general market, the oils tend to be used repeatedly for frying due to expenses reduction. When the frying oil was repeated used again and again it might lead to change in the physical appearances of both fried food and oil. The food become darkening and the repeated frying oil will be more viscous and darkening as well. In this study, the condition of sample preparation were set to similar to the peddler's normal behavior that using repeated frying oil without renew oil added. The frying condition was adapted from the research of Wananuwat et al. (2011) that collected the information from paddlers. Interval time between each frying was controlled. Like this study, the appearance of Chinese fried dough and repeated frying oil at the beginning and the end of frying were difference. Since frying can bring the chemical change to frying oil and fried food such as oxidation, pyrolysis, polymerization, hydrolysis and isomerization (Ariyapitipan, 2011).

Many studies have reported that *trans* fatty acid content depends on the quality of fat and the processes to its subjected, such as hydrogenation, heat treatment, deodorization and aeration (Gercˇar, 2003; Quı´lez et al., 2006; Weber et al., 2008; Ngadi et al., 2009; Malheiro et al., 2009). Therefore, *trans* fatty acid content in fried food might be increased because of *trans* isomerization that made configuration changed from *cis* to *trans* form. In this study, the amount of *trans* fatty acid was significantly increase from first time frying to at hour5. Like a previous study of

Tsuzki et al (2008) that reported *trans* fatty acid content increased gradually when 1.0 g of representative oil containing triolein, trilinolein and trilinolenin were fried at 180°C. These results support the hypothesis that frying process may lead to an increase of *trans* fatty acid content in fried food. Because, when oil is heated during processing, *cis* double bonds occuring naturally can change to *trans* isomerization (Wagner and Auer, 2000).

From the hypothesis that *trans* fatty acid in fried food came from *trans* isomerization, there are some studies try to use antioxidant to reuduce *trans* fatty acid formation. Zambonin et al. (2008) confirmed this hypothesis, while demonstrating that antioxidants like vitamin A and E show anti-isomerising effects. In a previous study (Gamel, Kiritsakis, and Petrakis, 1999), adding phenollic extracts from rosemary leaves with synthetic antioxidant, butylated hydroxyanisole (BHA) in amount 100 ppm or adding only the phenollic extracts in amount 200 ppm, showed the decreasing of *trans* fatty acid content in frying oil. In addition, adding 2% (w/w) pharmaceutical grade vitamin E in frying oil could decrease *trans* fatty acid formation in frying oil (Tsuzuki et al., 2008). Therefore, in this study food grade vitamin E was used and *trans* fatty acid content from food instead of frying oil were analyzed. The result implied that adding 0.23% (w/w) and 0.45% (w/w) vitamin E as 50% USP tocopheryl acetate in frying oil. It could not prevent the formation of *trans* fatty acid in Chinese fried dough. It may be that the amount of vitamin E in active form that was calculated from 50% USP tocopheryl acetate might be too low to inhibit or reduce the *trans* fatty acid formation. Moreover, vitamin E added in this study were 50% USP tocopheryl acetate that other components were consisted so the result of vitamin E adding is different from the previous study (Tsuzuki et al., 2008).

Although, the government of some countries requires the level of *trans* fatty acid on nutrition label but there are many non-label foods such as fried food. In the present, the repeated usage of frying oil are concerned because *trans* fatty acid could be produced in this process of cooking. In Thailand, Thai Food and Drug Administration (FDA) has already set punishment of using repeated frying oil which contained polar substance more than 25% (w/w) but there are no labeling regulation of *trans* fatty acid level.

Moreover, the an increase in Chinese fried dough weights in this study have shown that oil absorption by food during the frying process occurred. These can consequently increase not only total fat but also *trans* fat levels in fried foods. Therefore, the consumers should concern about sources and health effects of *trans* fatty acid occurred in fried food.

# CHAPTER VI

# CONCLUSION

In this study the factors affecting *trans* fatty acid in fried food were investigated by using Chinese fried dough (or Pa-tong-ko in Thai), one of the most well-known fried food in Thailand, to be a representative of fried food. *Trans* fatty acid content were detected by using attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) method with negative second derivative mode. This study focused on 2 factors affecting *trans* fatty acid content in fried food. First, effect of using repeated frying oil to *trans* fatty acid content occurred by frying time observed. The second point of this study was the effect of adding vitamin E in frying oil on *trans* fatty acid content in fried food. The result implied that using repeated frying oil might increase *trans* fatty acid content in fried food. On the other hand, adding food grade vitamin E in the levels of 0.23 % and 0.45 % in frying oil cannot reduce *trans* fatty acid content in fried food due to the amount and form of vitamin E added.

However, this is only primary information that implied from only one kind of fried food, further study should research more. More kinds of fried food should be studied to improve database of *trans* fatty acid effecter in fried food and the amount and form of vitamin E added should be adapted.

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# APPENDICES

# Appendix A

- Results of fat extraction
- Results of average *trans* fatty acid content
- Recipe of Chinese fried dough

**Fat extraction of Chinese fried dough in control group (the experiment1)**

| Time | Replicate batch | Sample weight (g) | Fat weight (g) | Total Fat (g/100 g food) | Average |
|---|---|---|---|---|---|
| hr0 | 1 | 5.3098 | 0.5616 | 10.5767 | 10.5507 |
| | 2 | 5.2269 | 0.5562 | 10.6411 | |
| | 3 | 5.0976 | 0.5319 | 10.4343 | |
| hr1 | 1 | 5.5400 | 0.4505 | 8.1318 | 8.5495 |
| | 2 | 5.3019 | 0.4755 | 8.9685 | |
| | 3 | 5.2080 | 0.4452 | 8.5484 | |
| hr2 | 1 | 5.1637 | 0.5039 | 9.7585 | 10.0544 |
| | 2 | 5.2100 | 0.4656 | 8.9367 | |
| | 3 | 5.4255 | 0.6222 | 11.4681 | |
| hr3 | 1 | 5.2589 | 0.4259 | 8.0987 | 9.4703 |
| | 2 | 5.2946 | 0.5450 | 10.2935 | |
| | 3 | 5.3829 | 0.5393 | 10.0188 | |
| hr4 | 1 | 5.1696 | 0.6736 | 13.0300 | 12.0224 |
| | 2 | 5.2697 | 0.6749 | 12.8072 | |
| | 3 | 5.0283 | 0.5144 | 10.2301 | |
| hr5 | 1 | 5.1877 | 0.6074 | 11.7085 | 11.4794 |
| | 2 | 5.1914 | 0.5624 | 10.8333 | |
| | 3 | 5.2722 | 0.6272 | 11.8964 | |

**Fat extraction of Chinese fried dough in control group (the experiment2)**

| Time | Replicate batch | Sample weight (g) | Fat weight (g) | Total Fat (g/100 g food) | Average |
|---|---|---|---|---|---|
| hr0 | 1 | 5.2643 | 0.6916 | 13.1375 | 12.4110 |
| | 2 | 5.3708 | 0.6394 | 11.9051 | |
| | 3 | 5.2402 | 0.6388 | 12.1904 | |
| hr1 | 1 | 5.1744 | 0.5054 | 9.7673 | 9.5215 |
| | 2 | 5.0115 | 0.4676 | 9.3305 | |
| | 3 | 5.1286 | 0.4855 | 9.4665 | |
| hr2 | 1 | 5.0545 | 0.4185 | 8.2798 | 8.3441 |
| | 2 | 5.2965 | 0.5058 | 9.5497 | |
| | 3 | 5.1632 | 0.3719 | 7.2029 | |
| hr3 | 1 | 5.1944 | 0.5526 | 10.6384 | 9.5836 |
| | 2 | 5.2064 | 0.4359 | 8.3724 | |
| | 3 | 5.2392 | 0.5103 | 9.7400 | |
| hr4 | 1 | 5.2879 | 0.3732 | 7.0576 | 8.9444 |
| | 2 | 5.3059 | 0.6070 | 11.4401 | |
| | 3 | 5.2054 | 0.4339 | 8.3356 | |
| hr5 | 1 | 5.1520 | 0.6193 | 12.0206 | 11.9239 |
| | 2 | 5.4707 | 0.6300 | 11.5159 | |
| | 3 | 5.4899 | 0.6717 | 12.2352 | |

**Fat extraction of Chinese fried dough in control group (the experiment3)**

| Time | Replicate batch | Sample weight (g) | Fat weight (g) | Total Fat (g/100 g food) | Average |
|---|---|---|---|---|---|
| hr0 | 1 | 5.3861 | 0.6111 | 11.3459 | 10.6258 |
| | 2 | 5.3534 | 0.5062 | 9.4557 | |
| | 3 | 5.2799 | 0.5848 | 11.0760 | |
| hr1 | 1 | 5.2382 | 0.4752 | 9.0718 | 9.5430 |
| | 2 | 5.1479 | 0.4752 | 9.2309 | |
| | 3 | 5.3223 | 0.5496 | 10.3264 | |
| hr2 | 1 | 5.0300 | 0.5831 | 11.5924 | 9.8481 |
| | 2 | 5.0988 | 0.4758 | 9.3316 | |
| | 3 | 5.2911 | 0.4561 | 8.6201 | |
| hr3 | 1 | 5.2095 | 0.6022 | 11.5597 | 11.2247 |
| | 2 | 5.1886 | 0.5798 | 11.1745 | |
| | 3 | 5.1015 | 0.5581 | 10.9399 | |
| hr4 | 1 | 5.0930 | 0.5995 | 11.7711 | 10.6799 |
| | 2 | 5.0813 | 0.5071 | 9.9797 | |
| | 3 | 5.1706 | 0.5320 | 10.2889 | |
| hr5 | 1 | 5.1983 | 0.5004 | 9.6262 | 12.1339 |
| | 2 | 5.3385 | 0.6487 | 12.1514 | |
| | 3 | 5.1121 | 0.7476 | 14.6241 | |

**Fat extraction of Chinese fried dough in 0.23% (w/w) vitamin E added oil group (the experiment1)**

| Time | Replicate batch | Sample weight (g) | Fat weight (g) | Total Fat (g/100 g food) | Average |
|---|---|---|---|---|---|
| hr0 | 1 | 5.1896 | 0.4564 | 8.7945 | 9.9164 |
| | 2 | 5.1285 | 0.4640 | 9.0475 | |
| | 3 | 5.1263 | 0.6104 | 11.9072 | |
| hr1 | 1 | 5.0889 | 0.4604 | 9.0471 | 8.7373 |
| | 2 | 5.0703 | 0.4189 | 8.2618 | |
| | 3 | 5.1444 | 0.4580 | 8.9029 | |
| hr2 | 1 | 5.1778 | 0.5848 | 11.2944 | 10.6304 |
| | 2 | 5.0845 | 0.4690 | 9.2241 | |
| | 3 | 5.2775 | 0.6002 | 11.3728 | |
| hr3 | 1 | 5.0347 | 0.5247 | 10.4217 | 10.2536 |
| | 2 | 5.1143 | 0.5174 | 10.1167 | |
| | 3 | 5.1563 | 0.5271 | 10.2224 | |
| hr4 | 1 | 5.2925 | 0.7093 | 13.4020 | 12.3893 |
| | 2 | 5.1371 | 0.6587 | 12.8224 | |
| | 3 | 5.3393 | 0.5843 | 10.9434 | |
| hr5 | 1 | 5.0862 | 0.6083 | 11.9598 | 12.1228 |
| | 2 | 5.2564 | 0.5756 | 10.9505 | |
| | 3 | 5.1344 | 0.6910 | 13.4582 | |

**Fat extraction of Chinese fried dough in 0.23% (w/w) vitamin E added oil group (the experiment2)**

| Time | Replicate batch | Sample weight (g) | Fat weight (g) | Total Fat (g/100 g food) | Average |
|---|---|---|---|---|---|
| hr0 | 1 | 5.1853 | 0.4878 | 9.4074 | 8.1383 |
| | 2 | 5.2338 | 0.3753 | 7.1707 | |
| | 3 | 5.3899 | 0.4224 | 7.8369 | |
| hr1 | 1 | 5.0413 | 0.5025 | 9.9677 | 10.1291 |
| | 2 | 5.0898 | 0.5049 | 9.9198 | |
| | 3 | 5.3373 | 0.5604 | 10.4997 | |
| hr2 | 1 | 5.2020 | 0.4475 | 8.6025 | 9.2086 |
| | 2 | 5.1502 | 0.4729 | 9.1822 | |
| | 3 | 5.0828 | 0.5002 | 9.8410 | |
| hr3 | 1 | 5.1425 | 0.5092 | 9.9018 | 8.2462 |
| | 2 | 5.0728 | 0.3251 | 6.4087 | |
| | 3 | 5.2218 | 0.4401 | 8.4281 | |
| hr4 | 1 | 5.1104 | 0.5034 | 9.8505 | 8.0838 |
| | 2 | 5.0643 | 0.3751 | 7.4067 | |
| | 3 | 5.0800 | 0.3553 | 6.9941 | |
| hr5 | 1 | 5.1793 | 0.4121 | 7.9567 | 7.2385 |
| | 2 | 5.1245 | 0.3886 | 7.5832 | |
| | 3 | 5.2060 | 0.3215 | 6.1756 | |

**Fat extraction of Chinese fried dough in 0.23% (w/w) vitamin E added oil group (the experiment3)**

| Time | Replicate batch | Sample weight (g) | Fat weight (g) | Total Fat (g/100 g food) | Average |
|---|---|---|---|---|---|
| hr0 | 1 | 5.0380 | 0.3197 | 6.3458 | 5.8827 |
| | 2 | 5.2892 | 0.2952 | 5.5812 | |
| | 3 | 5.0707 | 0.2901 | 5.7211 | |
| hr1 | 1 | 5.0774 | 0.2925 | 5.7608 | 6.2920 |
| | 2 | 5.1601 | 0.3470 | 6.7247 | |
| | 3 | 5.0654 | 0.3237 | 6.3904 | |
| hr2 | 1 | 5.0712 | 0.4150 | 8.1835 | 7.3404 |
| | 2 | 5.0362 | 0.3729 | 7.4044 | |
| | 3 | 5.0860 | 0.3272 | 6.4333 | |
| hr3 | 1 | 5.0990 | 0.4243 | 8.3212 | 7.5258 |
| | 2 | 5.2676 | 0.3876 | 7.3582 | |
| | 3 | 5.1711 | 0.3567 | 6.8980 | |
| hr4 | 1 | 5.1925 | 0.4087 | 7.8710 | 8.1576 |
| | 2 | 5.0402 | 0.4671 | 9.2675 | |
| | 3 | 5.1961 | 0.3811 | 7.3343 | |
| hr5 | 1 | 5.0631 | 0.4580 | 9.0458 | 7.3035 |
| | 2 | 5.1067 | 0.3402 | 6.6618 | |
| | 3 | 5.1734 | 0.3209 | 6.2029 | |

**Fat extraction of Chinese fried dough in 0.45% (w/w) vitamin E added oil group (the experiment1)**

| Time | Replicate batch | Sample weight (g) | Fat weight (g) | Total Fat (g/100 g food) | Average |
|---|---|---|---|---|---|
| hr0 | 1 | 5.0248 | 0.3980 | 7.9207 | 7.5739 |
| | 2 | 5.1243 | 0.2880 | 5.6203 | |
| | 3 | 5.0878 | 0.4671 | 9.1803 | |
| hr1 | 1 | 5.1910 | 0.3724 | 7.1740 | 6.7177 |
| | 2 | 5.1093 | 0.3133 | 6.1320 | |
| | 3 | 5.1116 | 0.3500 | 6.8472 | |
| hr2 | 1 | 5.0746 | 0.4063 | 8.0065 | 6.4467 |
| | 2 | 5.3086 | 0.3167 | 5.9658 | |
| | 3 | 5.3057 | 0.2848 | 5.3678 | |
| hr3 | 1 | 5.0404 | 0.2023 | 4.0136 | 6.5450 |
| | 2 | 5.1360 | 0.3267 | 6.3610 | |
| | 3 | 5.1618 | 0.4780 | 9.2603 | |
| hr4 | 1 | 5.1816 | 0.3105 | 5.9924 | 7.5131 |
| | 2 | 5.2070 | 0.3695 | 7.0962 | |
| | 3 | 5.0134 | 0.4738 | 9.4507 | |
| hr5 | 1 | 5.0236 | 0.4568 | 9.0931 | 7.6044 |
| | 2 | 5.1095 | 0.3602 | 7.0496 | |
| | 3 | 5.2649 | 0.3512 | 6.6706 | |

**Fat extraction of Chinese fried dough in 0.45% (w/w) vitamin E added oil group**

| Time | Replicate batch | Sample weight (g) | Fat weight (g) | Total Fat (g/100 g food) | Average |
|---|---|---|---|---|---|
| hr0 | 1 | 5.0465 | 0.3509 | 6.9533 | 6.9096 |
| | 2 | 5.1118 | 0.3184 | 6.2287 | |
| | 3 | 5.3320 | 0.4024 | 7.5469 | |
| hr1 | 1 | 5.1968 | 0.4512 | 8.6823 | 8.2203 |
| | 2 | 5.0831 | 0.3612 | 7.1059 | |
| | 3 | 5.1190 | 0.4542 | 8.8728 | |
| hr2 | 1 | 5.0555 | 0.3706 | 7.3306 | 7.4806 |
| | 2 | 5.0652 | 0.3762 | 7.4271 | |
| | 3 | 5.0339 | 0.3868 | 7.6839 | |
| hr3 | 1 | 5.1142 | 0.3916 | 7.6571 | 7.7556 |
| | 2 | 5.0030 | 0.3526 | 7.0478 | |
| | 3 | 5.0958 | 0.4363 | 8.5620 | |
| hr4 | 1 | 5.2422 | 0.3569 | 6.8082 | 7.5693 |
| | 2 | 5.0101 | 0.4173 | 8.3292 | |
| | 3 | 5.2374 | 0.3965 | 7.5706 | |
| hr5 | 1 | 5.1225 | 0.4999 | 9.7589 | 7.9881 |
| | 2 | 5.0622 | 0.4236 | 8.3679 | |
| | 3 | 5.2729 | 0.3078 | 5.8374 | |

**(the experiment2)**

**Fat extraction of Chinese fried dough in 0.45% (w/w) vitamin E added oil group (the experiment3)**

| Time | Replicate batch | Sample weight (g) | Fat weight (g) | Total Fat (g/100 g food) | Average |
|---|---|---|---|---|---|
| hr0 | 1 | 5.2315 | 0.4168 | 7.9671 | 7.5914 |
| | 2 | 5.2715 | 0.4011 | 7.6088 | |
| | 3 | 5.2332 | 0.3767 | 7.1983 | |
| hr1 | 1 | 5.1420 | 0.3517 | 6.8398 | 6.0119 |
| | 2 | 5.1189 | 0.2524 | 4.9307 | |
| | 3 | 5.0564 | 0.3168 | 6.2653 | |
| hr2 | 1 | 5.0390 | 0.3202 | 6.3544 | 6.2065 |
| | 2 | 5.2333 | 0.2759 | 5.2720 | |
| | 3 | 5.2223 | 0.3652 | 6.9931 | |
| hr3 | 1 | 5.0691 | 0.2637 | 5.2021 | 6.3338 |
| | 2 | 5.0623 | 0.3324 | 6.5662 | |
| | 3 | 5.1223 | 0.3705 | 7.2331 | |
| hr4 | 1 | 5.1108 | 0.2834 | 5.5451 | 5.9465 |
| | 2 | 5.0884 | 0.2432 | 4.7795 | |
| | 3 | 5.1111 | 0.3841 | 7.5150 | |
| hr5 | 1 | 5.0088 | 0.3816 | 7.6186 | 7.7725 |
| | 2 | 5.1465 | 0.3403 | 6.6123 | |
| | 3 | 5.1526 | 0.4682 | 9.0867 | |

**_Trans_ fatty acid content of Chinese fried dough in control group (the experiment1)**

| Time | Replicate batch | Average peak height (-)(x $10^{-4}$) | _Trans_ fatty acid content (% of total fat) [a] | Average _trans_ fatty acid content (% of total fat) |
|---|---|---|---|---|
| hr0 | 1 | 6.00 | 0.0091 | 0.01 |
| | 2 | 6.00 | 0.0091 | |
| | 3 | 6.00 | 0.0091 | |
| hr1 | 1 | 7.00 | 0.1718 | 0.17 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr2 | 1 | 7.00 | 0.1718 | 0.17 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr3 | 1 | 7.67 | 0.2803 | 0.30 |
| | 2 | 7.67 | 0.2803 | |
| | 3 | 8.00 | 0.2803 | |
| hr4 | 1 | 7.67 | 0.2803 | 0.32 |
| | 2 | 8.00 | 0.3345 | |
| | 3 | 8.00 | 0.3345 | |
| hr5 | 1 | 8.00 | 0.3345 | 0.33 |
| | 2 | 8.00 | 0.3345 | |
| | 3 | 8.00 | 0.3345 | |

[a] Calculated from the linear regression equation (y=1,626.9x-0.9670)

**_Trans_ fatty acid content of Chinese fried dough in control group (the experiment2)**

| Time | Replicate batch | Average peak height (-)(x $10^{-4}$) | _Trans_ fatty acid content (% of total fat) [a] | Average _trans_ fatty acid content (% of total fat) |
|---|---|---|---|---|
| hr0 | 1 | 7.00 | 0.1176 | 0.06 |
| | 2 | - | - | |
| | 3 | 6.00 | 0.0091 | |
| hr1 | 1 | 7.00 | 0.1718 | 0.17 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr2 | 1 | 8.67 | 0.4430 | 0.41 |
| | 2 | 8.00 | 0.3345 | |
| | 3 | 8.67 | 0.4430 | |
| hr3 | 1 | 8.00 | 0.3345 | 0.33 |
| | 2 | 8.00 | 0.3345 | |
| | 3 | 8.00 | 0.3345 | |
| hr4 | 1 | 8.00 | 0.3345 | 0.33 |
| | 2 | 8.00 | 0.3345 | |
| | 3 | 8.00 | 0.3345 | |
| hr5 | 1 | 9.00 | 0.4972 | 0.46 |
| | 2 | 8.67 | 0.4430 | |
| | 3 | 8.67 | 0.4430 | |

[a] Calculated from the linear regression equation (y=1,626.9x-0.9670)

**_Trans_ fatty acid content of Chinese fried dough in control group (the experiment3)**

| Time | Replicate batch | Average peak height (-)(x $10^{-4}$) | _Trans_ fatty acid content (% of total fat) [a] | Average _trans_ fatty acid content (% of total fat) |
|---|---|---|---|---|
| hr0 | 1 | 7.00 | 0.1718 | 0.14 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 6.33 | 0.0634 | |
| hr1 | 1 | 7.00 | 0.1718 | 0.17 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr2 | 1 | 6.00 | 0.0091 | 0.03 |
| | 2 | 6.33 | 0.0634 | |
| | 3 | 6.00 | 0.0091 | |
| hr3 | 1 | 7.00 | 0.1718 | 0.17 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr4 | 1 | 7.00 | 0.1718 | 0.28 |
| | 2 | 8.00 | 0.3345 | |
| | 3 | 8.00 | 0.3345 | |
| hr5 | 1 | 8.00 | 0.3345 | 0.33 |
| | 2 | 8.00 | 0.3345 | |
| | 3 | 8.00 | 0.3345 | |

[a] Calculated from the linear regression equation (y=1,626.9x-0.9670)

**_Trans_ fatty acid content of Chinese fried dough in 0.23% (w/w) vitamin E added oil group (the experiment1)**

| Time | Replicate batch | Average peak height (-)(x $10^{-4}$) | _Trans_ fatty acid content (% of total fat) [a] | Average _trans_ fatty acid content (% of total fat) |
|---|---|---|---|---|
| hr0 | 1 | 6.00 | 0.0091 | 0.12 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr1 | 1 | 7.00 | 0.1718 | 0.17 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr2 | 1 | 7.00 | 0.1718 | 0.17 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr3 | 1 | 7.00 | 0.1718 | 0.17 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr4 | 1 | 7.67 | 0.2803 | 0.30 |
| | 2 | 7.67 | 0.2803 | |
| | 3 | 8.00 | 0.3345 | |
| hr5 | 1 | 8.00 | 0.3345 | 0.33 |
| | 2 | 8.00 | 0.3345 | |
| | 3 | 8.00 | 0.3345 | |

[a] Calculated from the linear regression equation (y=1,626.9x-0.9670)

**_Trans_ fatty acid content of Chinese fried dough in 0.23% (w/w) vitamin E added oil group (the experiment2)**

| Time | Replicate batch | Average peak height (-)(x $10^{-4}$) | _Trans_ fatty acid content (% of total fat) [a] | Average _trans_ fatty acid content (% of total fat) |
|---|---|---|---|---|
| hr0 | 1 | 6.33 | 0.0634 | 0.05 |
| | 2 | 6.00 | 0.0091 | |
| | 3 | 6.33 | 0.0634 | |
| hr1 | 1 | 7.00 | 0.1718 | 0.17 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr2 | 1 | 7.00 | 0.1718 | 0.17 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr3 | 1 | 8.00 | 0.3345 | 0.33 |
| | 2 | 8.00 | 0.3345 | |
| | 3 | 8.00 | 0.3345 | |
| hr4 | 1 | 8.00 | 0.3345 | 0.33 |
| | 2 | 8.00 | 0.3345 | |
| | 3 | 8.00 | 0.3345 | |
| hr5 | 1 | 8.67 | 0.4430 | 0.42 |
| | 2 | 8.67 | 0.4430 | |
| | 3 | 8.33 | 0.3888 | |

[a] Calculated from the linear regression equation (y=1,626.9x-0.9670)

**_Trans_ fatty acid content of Chinese fried dough in 0.23% (w/w) vitamin E added oil group (the experiment3)**

| Time | Replicate batch | Average peak height (-)(x $10^{-4}$) | _Trans_ fatty acid content (% of total fat) [a] | Average _trans_ fatty acid content (% of total fat) |
|---|---|---|---|---|
| hr0 | 1 | 8.00 | 0.3345 | 0.44 |
| | 2 | 9.00 | 0.4972 | |
| | 3 | 9.00 | 0.4972 | |
| hr1 | 1 | 9.00 | 0.4972 | 0.52 |
| | 2 | 9.33 | 0.5514 | |
| | 3 | 9.00 | 0.4972 | |
| hr2 | 1 | 10.00 | 0.6599 | 0.64 |
| | 2 | 10.00 | 0.6599 | |
| | 3 | 9.67 | 0.6599 | |
| hr3 | 1 | 8.00 | 0.3345 | 0.33 |
| | 2 | 8.00 | 0.3345 | |
| | 3 | 8.00 | 0.3345 | |
| hr4 | 1 | 9.00 | 0.4972 | 0.50 |
| | 2 | 9.00 | 0.4972 | |
| | 3 | 9.00 | 0.4972 | |
| hr5 | 1 | 9.00 | 0.4972 | 0.50 |
| | 2 | 9.00 | 0.4972 | |
| | 3 | 9.00 | 0.4972 | |

[a] Calculated from the linear regression equation (y=1,626.9x-0.9670)

**_Trans_ fatty acid content of Chinese fried dough in 0.45% (w/w) vitamin E added oil group (the experiment1)**

| Time | Replicate batch | Average peak height (-)(x $10^{-4}$) | _Trans_ fatty acid content (% of total fat) [a] | Average _trans_ fatty acid content (% of total fat) |
|---|---|---|---|---|
| hr0 | 1 | 7.00 | 0.1718 | 0.17 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr1 | 1 | 7.00 | 0.1718 | 0.17 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr2 | 1 | 7.00 | 0.1718 | 0.17 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr3 | 1 | 8.00 | 0.3345 | 0.33 |
| | 2 | 8.00 | 0.3345 | |
| | 3 | 8.00 | 0.3345 | |
| hr4 | 1 | 8.00 | 0.3345 | 0.33 |
| | 2 | 8.00 | 0.3345 | |
| | 3 | 8.00 | 0.3345 | |
| hr5 | 1 | 9.00 | 0.4972 | 0.50 |
| | 2 | 9.00 | 0.4972 | |
| | 3 | 9.00 | 0.4972 | |

[a] Calculated from the linear regression equation (y=1,626.9x-0.9670)

**_Trans_ fatty acid content of Chinese fried dough in 0.45% (w/w) vitamin E added oil group (the experiment2)**

| Time | Replicate batch | Average peak height (-)(x $10^{-4}$) | _Trans_ fatty acid content (% of total fat) [a] | Average _trans_ fatty acid content (% of total fat) |
|---|---|---|---|---|
| hr0 | 1 | 5.67 | -0.0451 | -0.03 |
| | 2 | 5.67 | -0.0451 | |
| | 3 | 6.00 | 0.0091 | |
| hr1 | 1 | 7.00 | 0.1718 | 0.12 |
| | 2 | 6.67 | 0.1176 | |
| | 3 | 6.33 | 0.0634 | |
| hr2 | 1 | 7.00 | 0.1718 | 0.17 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr3 | 1 | 7.00 | 0.1718 | 0.17 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr4 | 1 | 8.00 | 0.3345 | 0.33 |
| | 2 | 8.00 | 0.3345 | |
| | 3 | 8.00 | 0.3345 | |
| hr5 | 1 | 8.00 | 0.3345 | 0.33 |
| | 2 | 8.00 | 0.3345 | |
| | 3 | 8.00 | 0.3345 | |

[a] Calculated from the linear regression equation (y=1,626.9x-0.9670)

**_Trans_ fatty acid content of Chinese fried dough in 0.45% (w/w) vitamin E added oil group (the experiment3)**

| Time | Replicate batch | Average peak height (-)(x $10^{-4}$) | _Trans_ fatty acid content (% of total fat) [a] | Average _trans_ fatty acid content (% of total fat) |
|---|---|---|---|---|
| hr0 | 1 | 6.00 | 0.0091 | 0.03 |
| | 2 | 6.00 | 0.0091 | |
| | 3 | 6.33 | 0.0634 | |
| hr1 | 1 | 7.00 | 0.1718 | 0.14 |
| | 2 | 6.67 | 0.1176 | |
| | 3 | 6.67 | 0.1176 | |
| hr2 | 1 | 7.00 | 0.1718 | 0.17 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr3 | 1 | 7.00 | 0.1718 | 0.17 |
| | 2 | 7.00 | 0.1718 | |
| | 3 | 7.00 | 0.1718 | |
| hr4 | 1 | 8.00 | 0.3345 | 0.33 |
| | 2 | 8.00 | 0.3345 | |
| | 3 | 8.00 | 0.3345 | |
| hr5 | 1 | 8.00 | 0.3345 | 0.33 |
| | 2 | 8.00 | 0.3345 | |
| | 3 | 8.00 | 0.3345 | |

[a] Calculated from the linear regression equation (y=1,626.9x-0.9670)

**Recipe of Chinese fried dough**

| Ingredients | Type | Trademark | Amount |
|---|---|---|---|
| 1. Instant Patonggo mix powder | Wheat powder | Uncle Barns® | 2,000 grams |
| 2. Water | - | - | 7 cups |
| 3. Vegetable oil | Soybean oil | A-ngun® | 4 tablespoons |
| 4. Yeast | Instant yeast | Nurmaya® | 4 teaspoons |

# Appendix B

Statistical analysis

**Descriptive Statistics**

TFA (% of total fat)

| group | time | Mean | Std. Error | N |
|---|---|---|---|---|
| control | hr0 | .0700 | .03786 | 3 |
| | hr1 | .1700 | .00000 | 3 |
| | hr2 | .2033 | .11096 | 3 |
| | hr3 | .2667 | .04910 | 3 |
| | hr4 | .3100 | .01528 | 3 |
| | hr5 | .3733 | .04333 | 3 |
| 0.23% vit E | hr0 | .2033 | .12005 | 3 |
| | hr1 | .2867 | .11667 | 3 |
| | hr2 | .3267 | .15667 | 3 |
| | hr3 | .2767 | .05333 | 3 |
| | hr4 | .3767 | .06227 | 3 |
| | hr5 | .4167 | .04910 | 3 |
| 0.45% vit E | hr0 | .0667 | .05239 | 3 |
| | hr1 | .1433 | .01453 | 3 |
| | hr2 | .1700 | .00000 | 3 |
| | hr3 | .2233 | .05333 | 3 |
| | hr4 | .3300 | .00000 | 3 |
| | hr5 | .3867 | .05667 | 3 |

**Tests of Between-Subjects Effects**

TFA (% of total fat)

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | .572[a] | 17 | .034 | 2.268 | .019 |
| Intercept | 3.527 | 1 | 3.527 | 237.812 | .000 |
| group | .095 | 2 | .047 | 3.202 | .052 |
| time | .445 | 5 | .089 | 6.000 | .000 |
| group * time | .032 | 10 | .003 | .216 | .993 |
| Error | .534 | 36 | .015 | | |
| Total | 4.632 | 54 | | | |
| Corrected Total | 1.106 | 53 | | | |

a. R Squared = .517 (Adjusted R Squared = .289)

**Multiple Comparisons Between Groups**

TFA (% of total fat)

Tukey HSD

| (I) group | (J) group | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| control | 60g vitE | -.0822 | .04059 | .121 | -.1814 | .0170 |
| | 120g vitE | .0122 | .04059 | .951 | -.0870 | .1114 |
| 60 g vitE | control | .0822 | .04059 | .121 | -.0170 | .1814 |
| | 120g vitE | .0944 | .04059 | .065 | -.0048 | .1937 |
| 120 g vitE | control | -.0122 | .04059 | .951 | -.1114 | .0870 |
| | 60g vitE | -.0944 | .04059 | .065 | -.1937 | .0048 |

Based on observed means.

The error term is Mean Square(Error) = .015.

**Multiple Comparisons Between Time**

TFA (% of total fat)

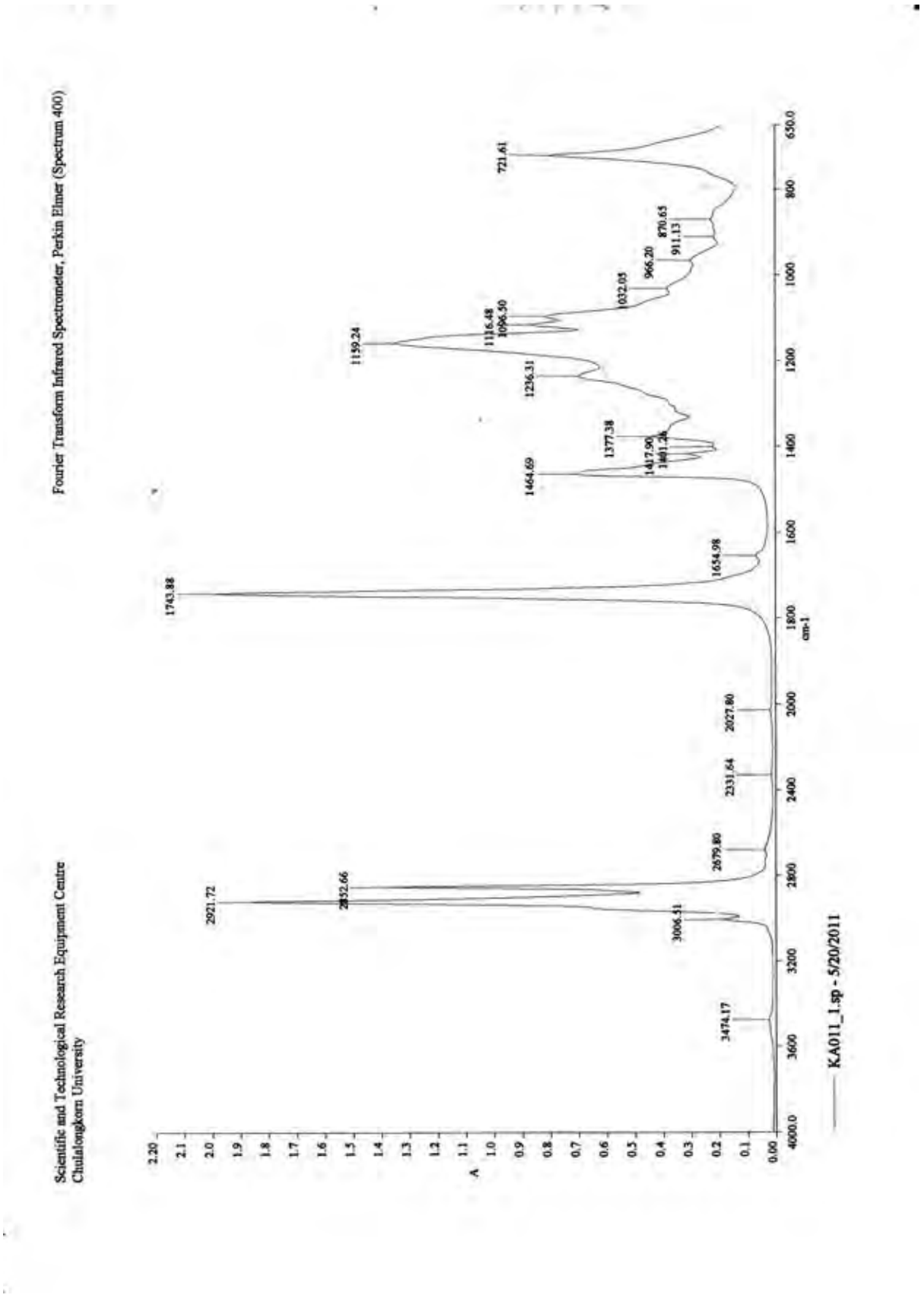
| (I) time | (J) time | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| hr0 | hr1 | -.0867 | .05741 | .660 | -.2594 | .0860 |
| | hr2 | -.1200 | .05741 | .315 | -.2927 | .0527 |
| | hr3 | -.1422 | .05741 | .158 | -.3149 | .0305 |
| | hr4 | -.2256* | .05741 | .005 | -.3983 | -.0528 |
| | hr5 | -.2789* | .05741 | .000 | -.4516 | -.1062 |
| hr1 | hr0 | .0867 | .05741 | .660 | -.0860 | .2594 |
| | hr2 | -.0333 | .05741 | .992 | -.2060 | .1394 |
| | hr3 | -.0556 | .05741 | .925 | -.2283 | .1172 |
| | hr4 | -.1389 | .05741 | .177 | -.3116 | .0338 |
| | hr5 | -.1922* | .05741 | .022 | -.3649 | -.0195 |
| hr2 | hr0 | .1200 | .05741 | .315 | -.0527 | .2927 |
| | hr1 | .0333 | .05741 | .992 | -.1394 | .2060 |
| | hr3 | -.0222 | .05741 | .999 | -.1949 | .1505 |
| | hr4 | -.1056 | .05741 | .455 | -.2783 | .0672 |
| | hr5 | -.1589 | .05741 | .086 | -.3316 | .0138 |
| hr3 | hr0 | .1422 | .05741 | .158 | -.0305 | .3149 |
| | hr1 | .0556 | .05741 | .925 | -.1172 | .2283 |
| | hr2 | .0222 | .05741 | .999 | -.1505 | .1949 |
| | hr4 | -.0833 | .05741 | .696 | -.2560 | .0894 |
| | hr5 | -.1367 | .05741 | .190 | -.3094 | .0360 |
| hr4 | hr0 | .2256* | .05741 | .005 | .0528 | .3983 |
| | hr1 | .1389 | .05741 | .177 | -.0338 | .3116 |
| | hr2 | .1056 | .05741 | .455 | -.0672 | .2783 |
| | hr3 | .0833 | .05741 | .696 | -.0894 | .2560 |
| | hr5 | -.0533 | .05741 | .936 | -.2260 | .1194 |
| hr5 | hr0 | .2789* | .05741 | .000 | .1062 | .4516 |
| | hr1 | .1922* | .05741 | .022 | .0195 | .3649 |
| | hr2 | .1589 | .05741 | .086 | -.0138 | .3316 |
| | hr3 | .1367 | .05741 | .190 | -.0360 | .3094 |
| | hr4 | .0533 | .05741 | .936 | -.1194 | .2260 |

Based on observed means.

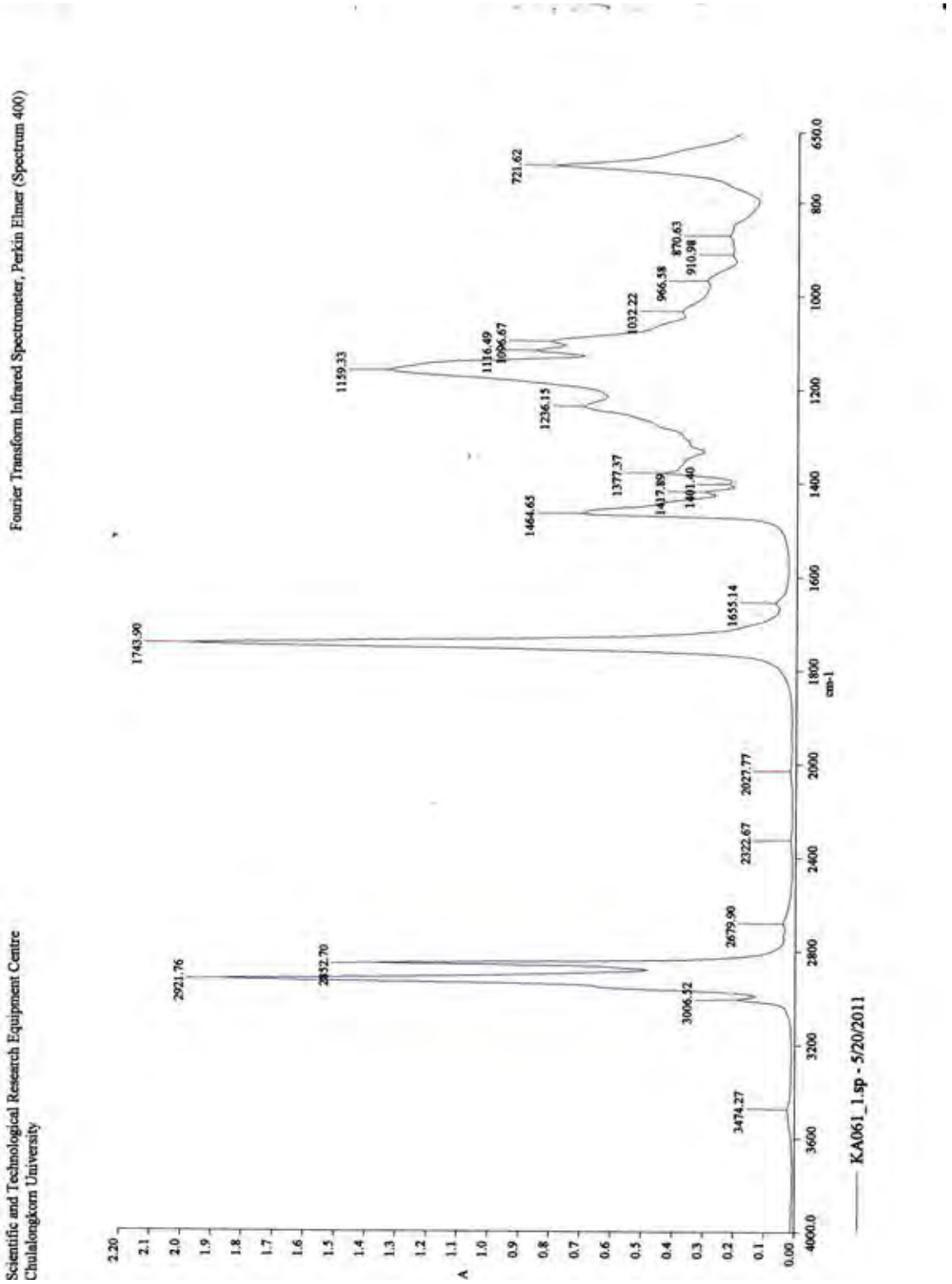
The error term is Mean Square(Error) = .015. *. The mean difference is significant at the .05 level.

# Appendix C

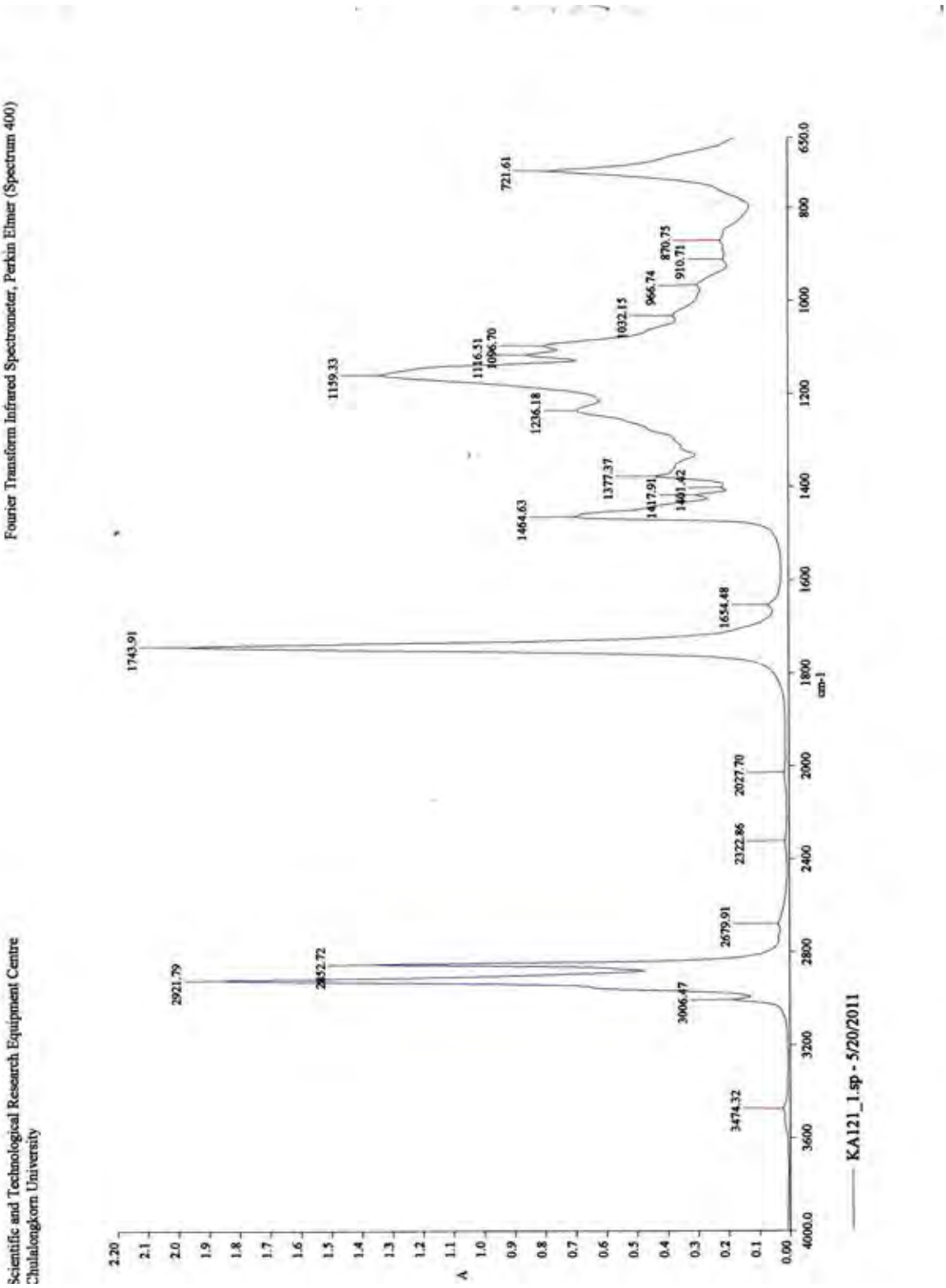
Example of FTIR spectrums



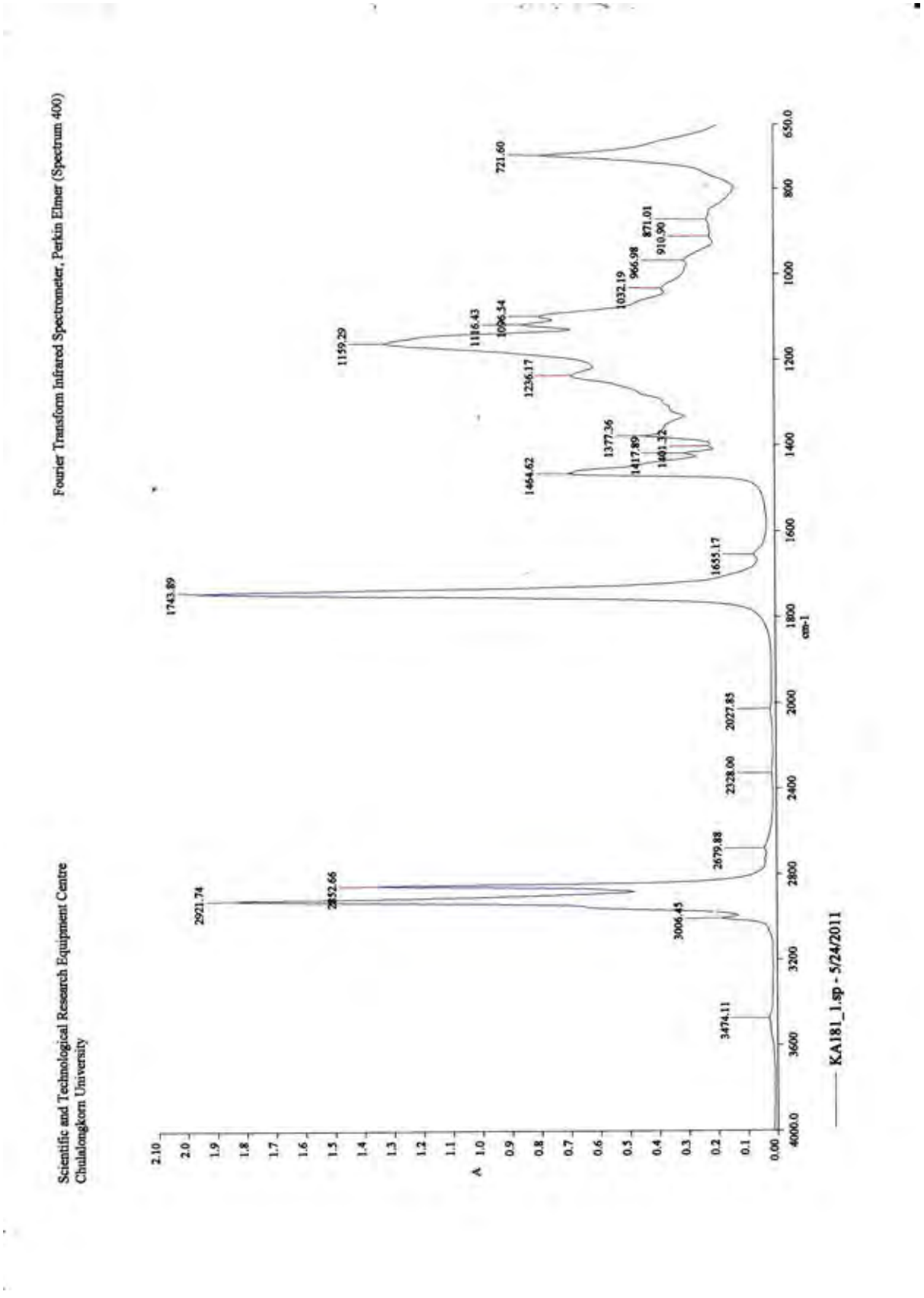

FTIR spectrums of control group at hr0 (the experiment1)



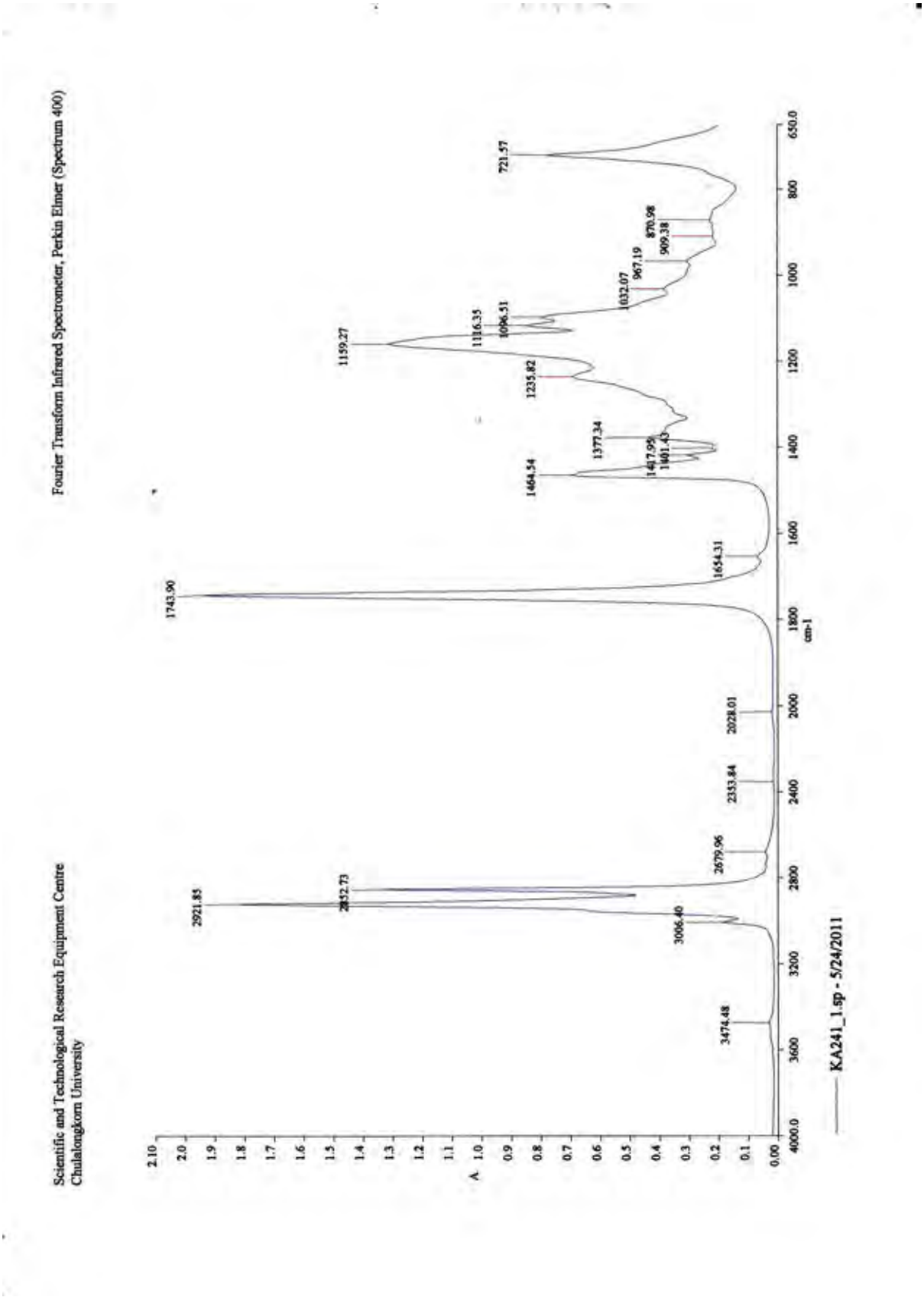

FTIR spectrums of control group at hr1 (the experiment1)



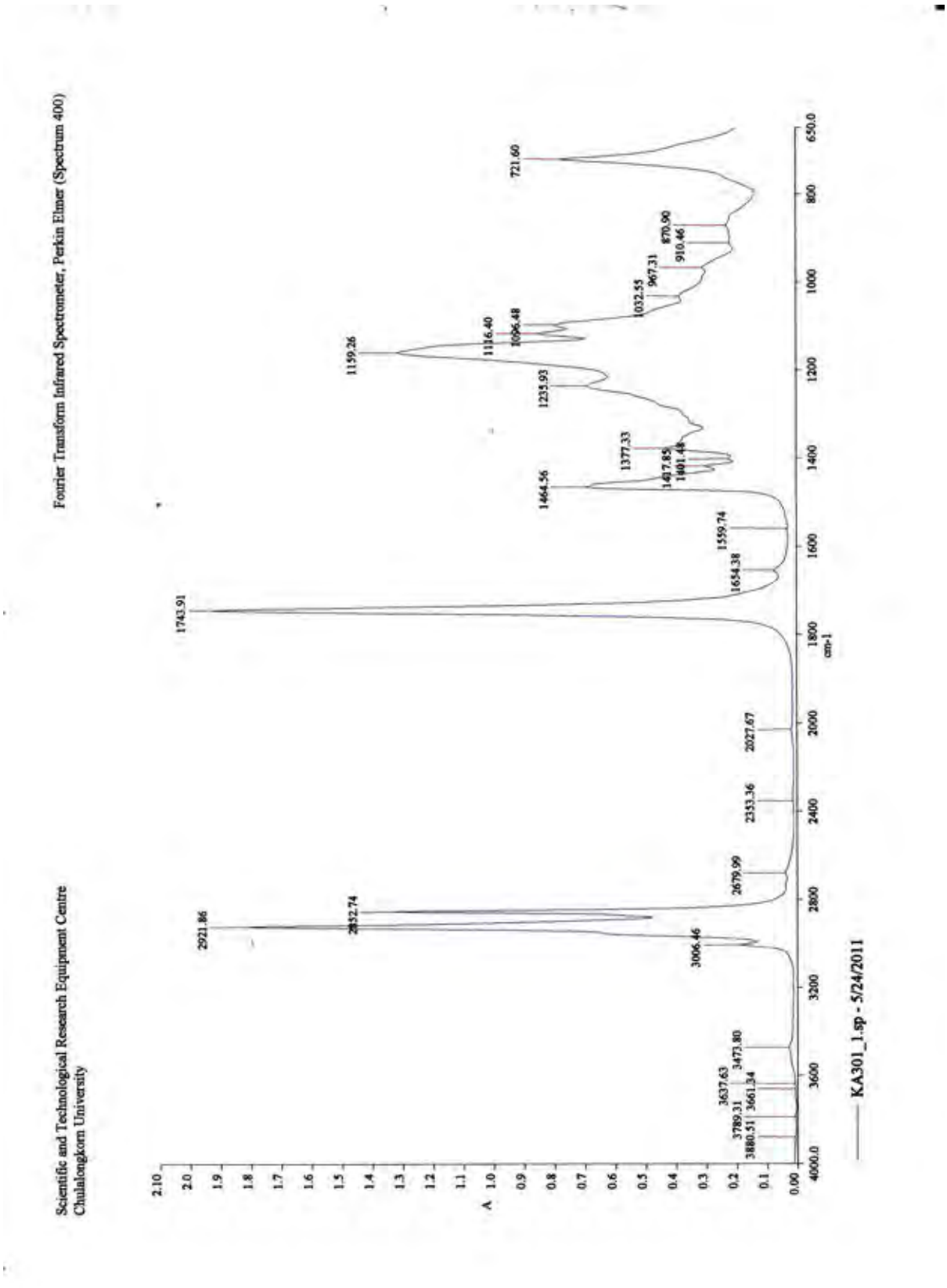

FTIR spectrums of control group at hr2 (the experiment1)




FTIR spectrums of control group at hr3 (the experiment1)



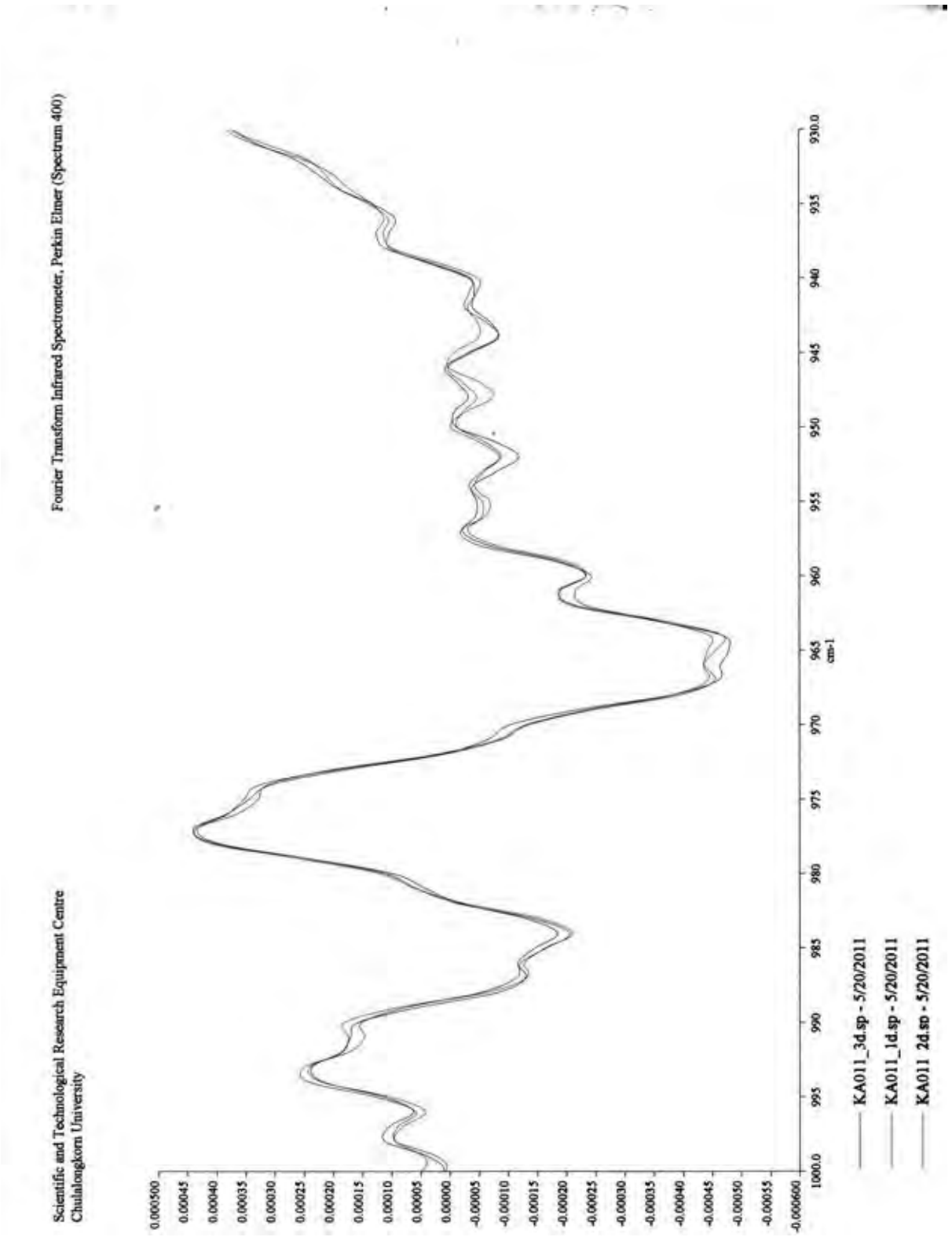

FTIR spectrums of control group at hr4 (the experiment1)



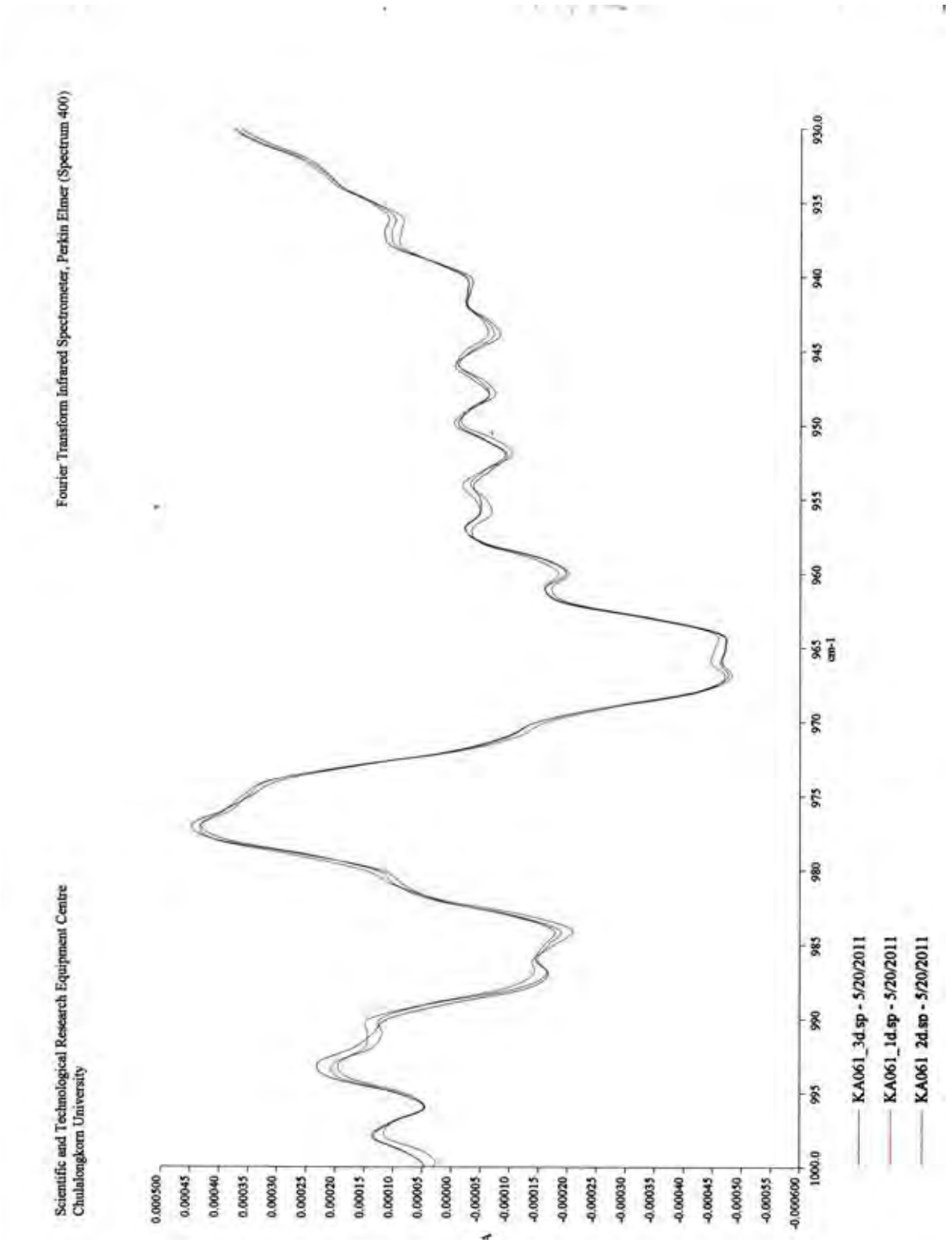

FTIR spectrums of control group at hr5 (the experiment1)

# Appendix D

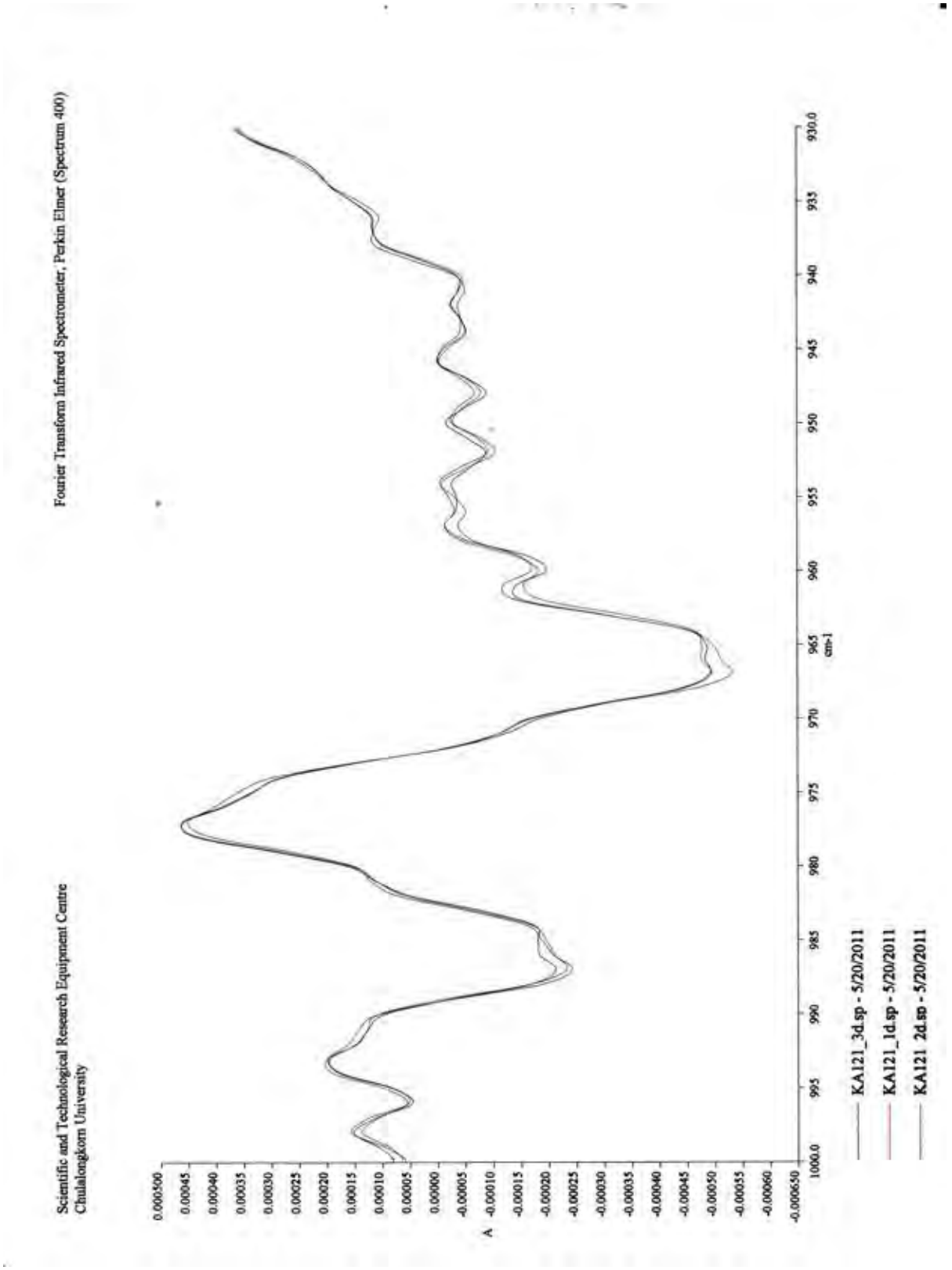
Example of the negative second derivative of control group



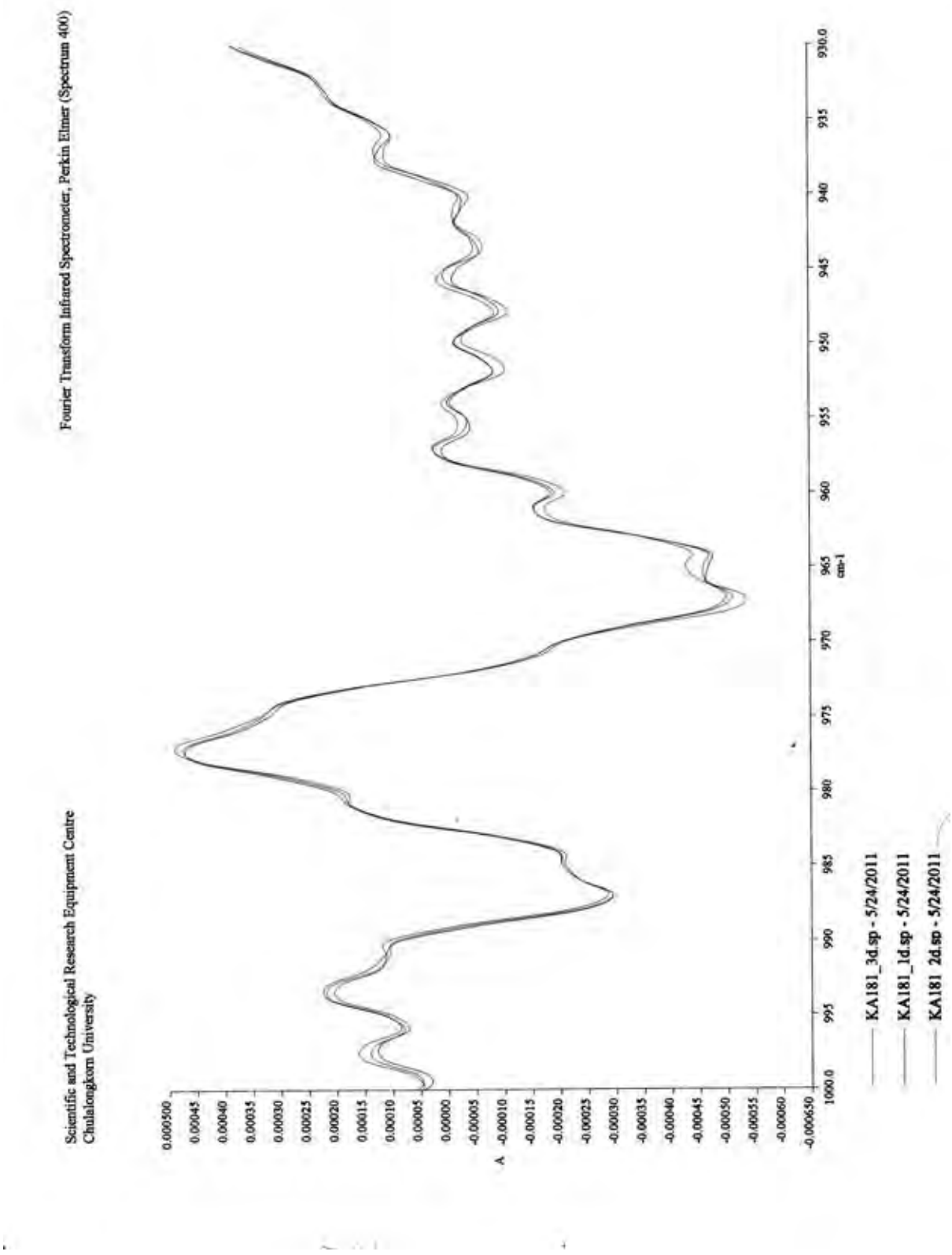

The negative second derivative of control group at hr0 (the experiment1)



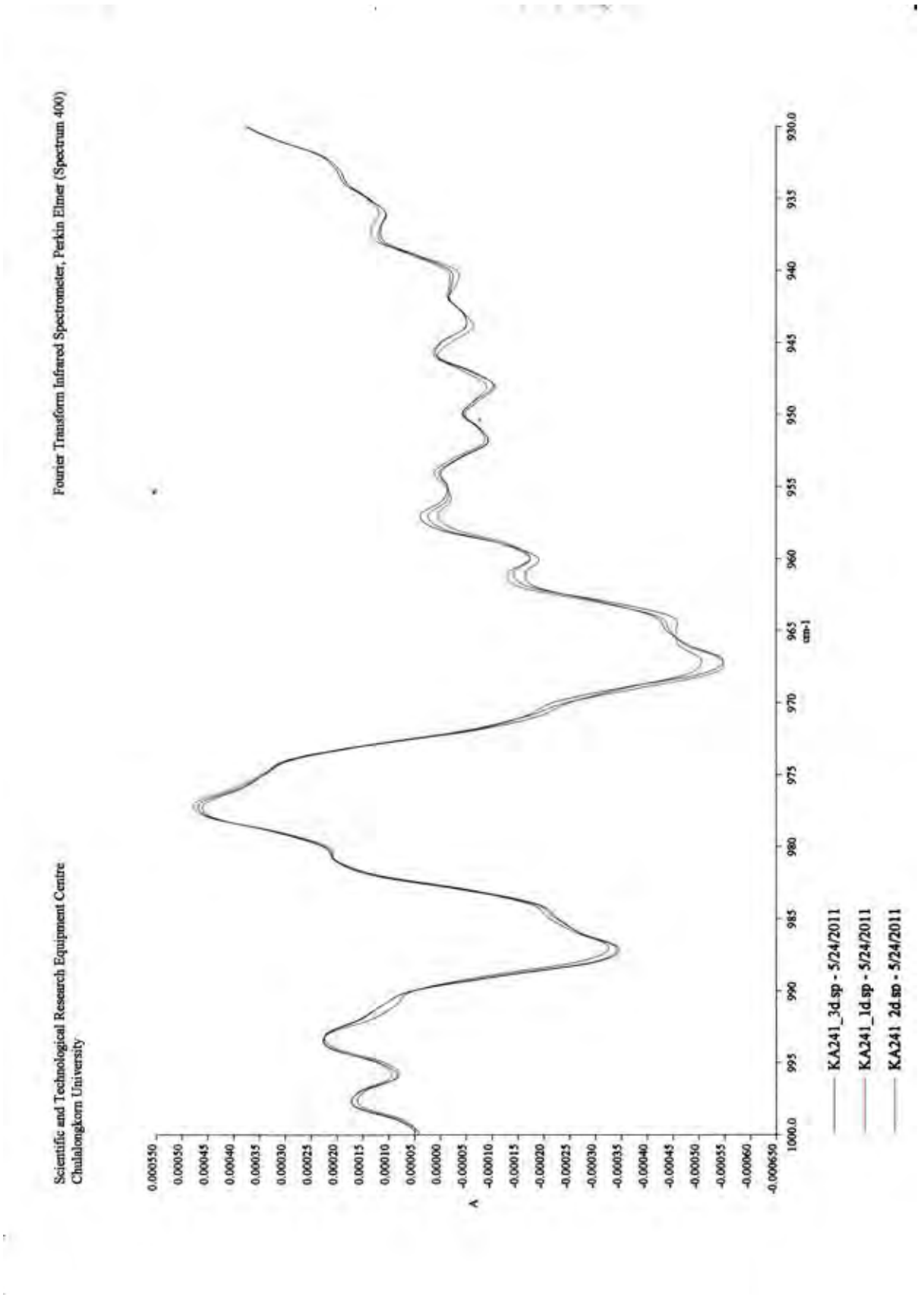

The negative second derivative of control group at hr1 (the experiment1)



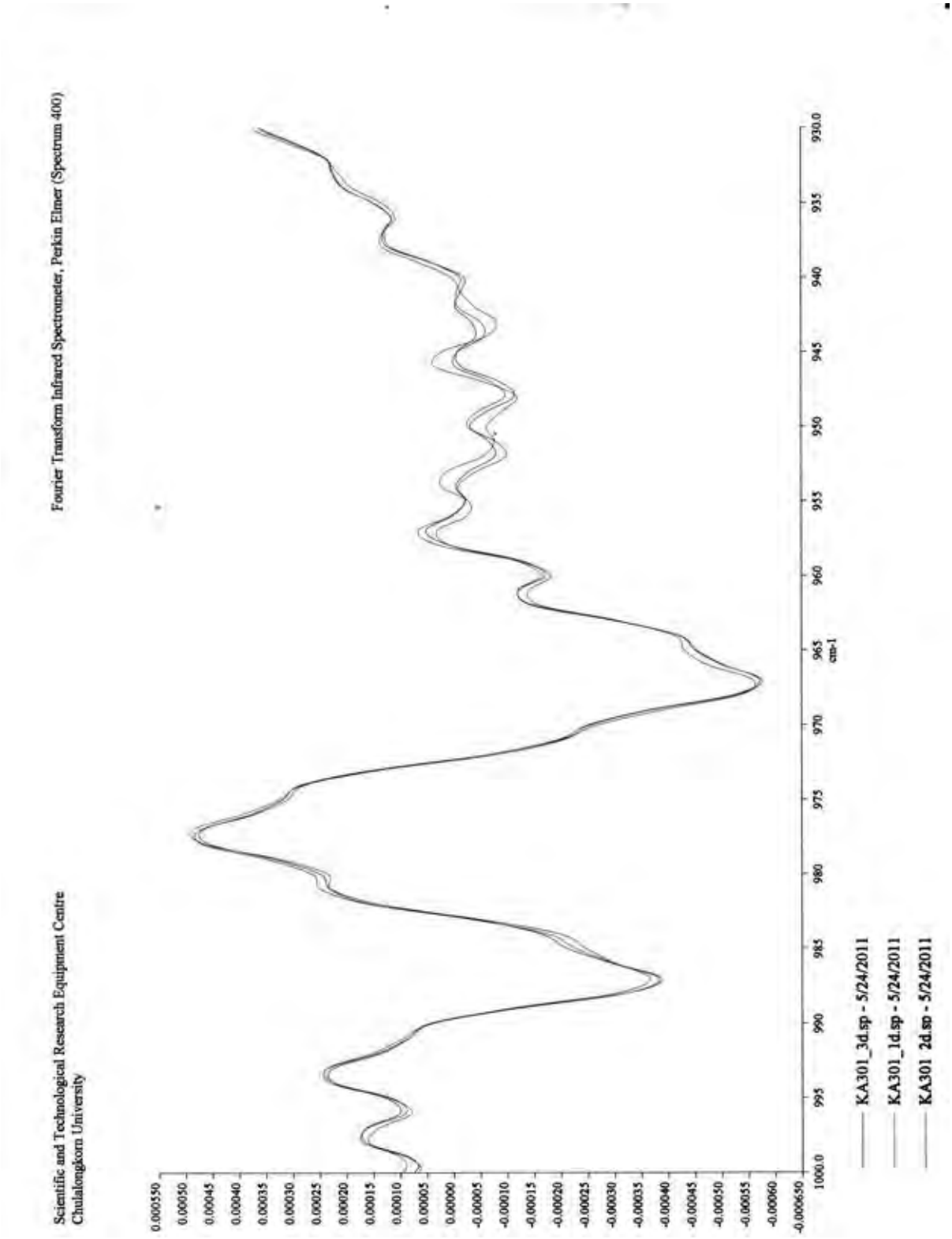

The negative second derivative of control group at hr2 (the experiment1)




The negative second derivative of control group at hr3 (the experiment1)




The negative second derivative of control group at hr4 (the experiment1)




The negative second derivative of control group at hr5 (the experiment1)

# BIOGRAPHY

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