การวิเคราะห์ปริมาณกรดไขมันชนิดทรานส์ในขนมขบเคี้ยวและครีมเทียม
โดยวิธีแอตเทนนูเอเทดโททัลรีเฟลกชันฟูเรียร์ทรานสฟอร์มอินฟราเรดสเปกโทรสโกปี

นางสาววราภรณ์ สุวรรณกูฏ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
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ANALYSIS OF *TRANS* FATTY ACID CONTENT IN SNACKS AND NON-DAIRY CREAMERS BY ATTENUATED TOTAL REFLECTION FOURIER TRANSFORM INFRARED SPECTROSCOPY

Miss Waraporn Suwannakood

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Food Chemistry and Medical Nutrition
Department of Food and Pharmaceutical Chemistry
Faculty of Pharmaceutical Sciences
Chulalongkorn University
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Thesis Title	ANALYSIS OF *TRANS* FATTY ACID CONTENT IN SNACKS AND NON-DAIRY CREAMERS BY ATTENUATED TOTAL REFLELCTION FOURIER TRANSFORM INFRARED SPECTROSCOPY
By	Miss Waraporn Suwannakood
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วราภรณ์ สุวรรณกูฏ : การวิเคราะห์ปริมาณกรดไขมันชนิดทรานส์ในขนมขบเคี้ยวและครีมเทียมโดยวิธีแอตเทนนูเอเทดโททัลรีเฟลกชันฟูเรียทรานสฟอร์มอินฟราเรดสเปกโทรสโกปี (ANALYSIS OF *TRANS* FATTY ACIDS CONTENT IN SNACKS AND NON-DAIRY CREAMERS BY ATTENUATED TOTAL REFLECTION-FOURIER TRANSFORM INFRARED SPECTROSCOPY) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ภญ.ดร.ลินนา ทองยงค์, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ.ภก.ดร.ชำนาญ ภัตรพานิช, 127 หน้า.

การศึกษานี้มีวัตถุประสงค์เพื่อวิเคราะห์ปริมาณกรดไขมันชนิดทรานส์ในขนมขบเคี้ยวจำนวน 6 กลุ่มตัวอย่าง กลุ่มตัวอย่างละ 3 ชนิด ได้แก่ แครกเกอร์ ขนมปังกรอบชนิดแท่ง (ขาไก่) มันฝรั่งทอด ขนมปังอบกรอบ ข้าวโพดอบโดยใช้เครื่องไมโครเวฟ เวเฟอร์ และครีมเทียมจำนวน 12 ยี่ห้อ ด้วยวิธีแอตเทนนูเอเทดโททัลรีเฟลกชันฟูเรียทรานสฟอร์มอินฟราเรดสเปกโทรสโกปี โดยทำการสุ่มตัวอย่างจากห้างสรรพสินค้าในกรุงเทพมหานครระหว่างช่วงเดือนตุลาคม 2552 ถึงเดือนมกราคม 2553 ผลการวิเคราะห์พบว่า ข้าวโพดอบโดยใช้เครื่องไมโครเวฟมีปริมาณกรดไขมันชนิดทรานส์ 1.90 - 22.12 กรัมต่อ100 กรัมอาหาร เวเฟอร์ ไม่สามารถหาค่าได้ - 8.08 กรัมต่อ 100 กรัมอาหาร ขนมปังอบกรอบ 0.01 - 5.09 กรัมต่อ 100 กรัมอาหาร แครกเกอร์ ไม่สามารถหาค่าได้ - 0.01 กรัมต่อ 100 กรัมอาหาร มันฝรั่งทอด และขนมปังกรอบชนิดแท่ง(ขาไก่)ไม่สามารถหาค่าของปริมาณกรดไขมันชนิดทรานส์ได้ การวิเคราะห์ในกลุ่มครีมเทียมจำนวน 12 ยี่ห้อพบกรดไขมันชนิดทรานส์ โดยครีมเทียมที่มีน้ำมันถั่วเหลืองเป็นส่วนประกอบมีปริมาณกรดไขมันชนิดทรานส์ 2.88 - 32.49 กรัมต่อ 100 กรัมอาหาร สำหรับครีมเทียมที่มีน้ำมันจากเมล็ดปาล์มเป็นส่วนประกอบมีปริมาณกรดไขมันชนิดทรานส์ 0.24 - 1.43 กรัมต่อ 100 กรัมอาหาร จากผลการวิจัยพบว่า ข้าวโพดอบโดยใช้เครื่องไมโครเวฟ เวเฟอร์ ขนมปังอบกรอบและครีมเทียมที่มีไขมันถั่วเหลืองเป็นส่วนประกอบ มีปริมาณกรดไขมันชนิดทรานส์สูง ดังนั้นผู้บริโภคควรหลีกเลี่ยงการรับประทานผลิตภัณฑ์ดังกล่าว และตระหนักถึงผลกระทบของกรดไขมันชนิดทรานส์ต่อความเสี่ยงของการเกิดโรคหัวใจและหลอดเลือด

ภาควิชา……………อาหารและเภสัชเคมี.................... ลายมือชื่อนิสิต……………………………………

สาขาวิชา...อาหารเคมีและโภชนศาสตร์ทางการแพทย์..ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์หลัก…............

ปีการศึกษา ....2553……………………………..ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์ร่วม…..............

5176585933: MAJOR FOOD CHEMISTRY AND MEDICAL NUTRITION
KEYWORDS: *TRANS* FATTY ACID / FOURIER TRANSFORM INFRARED SPECTROSCOPY / SNACK / NON-DAIRY CREAMER

WARAPORN SUWANNAKOOD: ANALYSIS OF *TRANS* FATTY ACID CONTENT IN SNACKS AND NON-DAIRY CREAMERS BY ATTENUATED TOTAL REFLECTION FOURIER TRANSFORM INFRARED SPECTROSCOPY. ADVISOR: ASST.PROF.LINNA TONGYONK, D.Sc., CO-ADVISOR: ASST.PROF.CHAMNAN PATARAPANICH, Ph.D., 127 pp.

This study aimed to determine *trans* fatty acid content in six groups of snacks including crackers, stick biscuits (ka-kai), potato chips, biscuits, microwave popcorns and wafers and 12 brands of non-dairy creamers. All samples were randomly selected from supermarket in Bangkok during October 2009 to January 2010. *Trans* fatty acid level was determined by the attenuated total reflection fourier transform infrared spectroscopy (ATR-FTIR) with negative second derivative mode. *Trans* fatty acid content in the microwave popcorn ranged from 1.90 - 22.12 g/100 g food, wafer; not detected - 8.08 g/ 100 g food, biscuit; 0.01 - 5.09 g/100 g food and cracker; not detected - 0.01 g/100 g food. *Trans* fatty acid contents in potato chip and stick biscuit (ka-kai) were not detected. Twelve brands of non-dairy creamers were found to contain *trans* fatty acid. *Trans* fatty acid levels in non-dairy creamers which made from soybean oil ranged from 22.88 - 32.49 g/ 100 g food while the brands which contained palm kernel oil ranged from 0.24 - 1.43 g/100 g food. This finding showed that microwave popcorn, biscuits, wafers, and non-dairy creamers which contained soybean oil were found to have high amount of *trans* fatty acid. Therefore, consumers should avoid to consume of these products in order to reduce the risk of cardiovascular disease.

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

AHA	American Heart Association
ATR-FTIR	attenuated total reflection fourier transform infrared spectroscopy
CHD	coronary heart disease
CLA	conjugated linoleic acid
CRP	c-reactive protein
DGAC	Dietary Guidelines advisory Committee
FAME	fatty acid methyl ester
g	grams
GC	gas chromatography
HDL	high density lipoprotein
ICAM	intercellular adhesion molecule
IL-6	interleukin-6
IR	infrared spectroscopy
ISSFAL	International Society for the Study of Fatty Acids and Lipids
LDL	low density lipoprotein
ml	milliliters
MUFA	monounsaturated fatty acid
NDC	non-dairy creamer
ND	not determine
NO.	number
PUFA	polyunsaturated fatty acid

TE	trielaidin
TFA	*trans* fatty acid
TO	triolein
TNFR	tumor necrosis factor receptor
TNF	tumor necrosis factor
VCAM	vascular cell adhesion molecule
ZnSe	zinc selenide

CHAPTER I

INTRODUCTION

Trans fatty acids (TFA) are unsaturated fatty acids with at least one double bond in the *trans* isomer. They are formed during the partial hydrogenation of unsaturated fats and oils, which are major source of *trans* fatty acids. Whereas low levels of *trans* fatty acids are found in milk and meat of ruminants such as cattle, sheep, goats that come from the biohydrogenation of ruminant bacteria (Gebauer et al., 2007). *Trans* fatty acids were used in food industry because of their better functional properties such as long shelf life, oxidative stability, semisolidity (Kim et al., 2007; Saunders et al., 2008; Ratnayake et al., 2009). They mostly are found in shortening and margarine which are used in preparation of baked products (McCarthy et al., 2008; Saunders et al., 2008) and are also commonly found in deep-fried fast food, packed snack foods, table spread, crackers (Saunders et al., 2008; Adhikari et al., 2010).

Several studies reported the adverse health effects of *trans* fatty acids. The high *trans* fatty acid consumption was associated with increased risk of coronary heart disease (CHD) (Lemaitre et al., 2006; Gebauer et al., 2007; McCarthy et al., 2008). Moreover, *trans* fatty acids influence the blood lipid profile that increase the level of low density lipoprotein (LDL) cholesterol and decrease the level of high density lipoprotein cholesterol (HDL). In addition, high consumption of *trans* fatty acids was involved risk of cancer, systemic inflammation, oxidative stress, body weight and insulin sensitivity (Gebauer et al., 2007; Martin et al., 2007; Saunders et al., 2008). Since high *trans* fatty acid consumption has been associated to a variety of diseases.

So, many countries such as Malaysia (Neo et al, 2007), Japan (Kawabata et al, 2010), New Zealand (Saunders et al., 2008), Italy (Sofi et al., 2009) have increased concerns about *trans* fatty acids and interested to determine *trans* fatty acid content in many kinds of foods.

The official methods for analysis of *trans* fatty acid content base on gas chromatography (GC) and infrared spectroscopy (IR). GC method can evaluate the total *trans* fatty acid content of fat and other fat compositions. This method consists of several steps such as extracting samples, preparing fatty acids methyl esters (FAME) and internal standard and use a long time for separation (up to 1.5 hours) (Kim et al., 2007; Mossoba et al., 2009). They are necessary to use 100 m highly polar fused silica capillary column while IR is a rapid analytical method with easy and accuracy 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).

Several countries are now regulating *trans* fatty acid level in food labels. In the United Stated, products containing less than 0.5 g of *trans* fatty acids per serving can claim 0 g of *trans* fatty acids on food labels (Remig et al., 2010) while in Australia, content of *trans* fatty acids did require to show on the nutrition label (McCarthy et al, 2008). In Thailand, the amount of *trans* fatty acid is not provided on food labels. Because Thai Food and Drug Administration (FDA) has few data of *trans* fatty acid level in foods. However, there are some studies that has evaluated *trans* fatty acid content by attenuated total reflection fourier transform infrared spectroscopy method in Thailand. Nackwichian (2009) analyzed *trans* fatty acids content in selected foods such as shortening, margarine, butter cookies, brownie, sandwich chocolate cookie, cake cream roll, croissant, rich butter bun and crispy pie while Sunphan (2010) analyzed amount of *trans* fatty acid 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 and cheese and butter.

Moreover, there are some other foods that contain *trans* fatty acids such as snacks and non-dairy creamers but there are few reports about their TFA contents. Therefore, the aim of this study was to determine the *trans* fatty acid content by the attenuated total reflection fourier transform infrared spectroscopy (ATR-FTIR) in snacks and non-dairy creamers available in Bangkok, Thailand during October 2009-January 2010. The results could be useful information for providing amount of *trans* fatty acid on food labels regulation and to help the consumers for food purchase decision.

CHAPTER II

LITERATURE REVIEW

2.1 Physical and Chemical Properties of *Trans* Fatty Acids

In natural, the unsaturated fatty acids are *cis* configuration, the two hydrogen atoms bond to the carbon atom on the same sides of the double bond (Kim et al., 2007; Saunders et al., 2008; Ratnayake et al., 2009). In contrast to *trans* configuration. The *trans* isomer, two hydrogen atoms bound to the carbon atom on the opposite sides (Kim et al., 2007; Saunders et al., 2008; Ratnayake et al., 2009). Due to the molecules of *trans* isomer are packed more linear structures and more closely, the melting point and the density of the *trans* configuration are higher than the *cis* configuration (Figure 1) (Adhikari et al., 2010). Therefore, the *cis* double bonds are liquid state whereas the *trans* double bonds are semisolid state at the temperature room.

2.2 The Formation of *Trans* Fatty Acids

2.2.1 The Industrial Hydrogenation

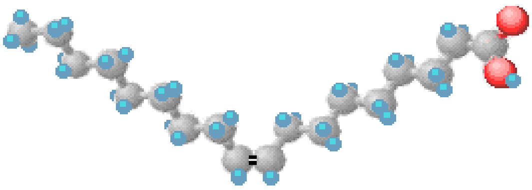
The hydrogenation of the unsaturated fatty acids is added hydrogen atom into liquid oils. During the hydrogenation process, the process parameters, such as hydrogen pressure, stirring speed, reaction time, and the catalyst used (generally nickel) must be critically controlled (Martin et al., 2007; Feldman et al., 1996). For the completed reaction, the saturated fatty acids must be obtained from the process, however, with partial hydrogenation the mixtures of *cis* and *trans* fatty acid will be obtained (Remig et al., 2010). The purpose of the process is to alter the physical properties of fatty acid, the *trans* fatty acid exhibit more oxidative stability which consequently extend its shelf life. Therefore, the *trans* fatty acids are attractive to the food manufacturing. The major sources of the *trans* fatty acids are found in products containing industrially produced partially hydrogenation oils such as bakery products, margarine, shortening, deep - fried fast food, packed snack foods, and crackers (Wagner et al., 2000; McCarthy et al., 2008; Adhikari et al., 2010).

The C18:1t isomers contain approximately 80 to 90 % of total TFA in foods (Gebauer et al., 2007). The elaidic acid (C18:1*t* Δ 9*t*) is the major isomer in partially hydrogenated process and other isomers are0 C16:2*t* isomer, C18:2*t* isomer and C18:3*t* isomer. Table 1 shows the major *trans* fatty acid isomers and *cis* fatty acid isomers (Gebauer et al., 2007).

2.2.2 The Biohydrogenation

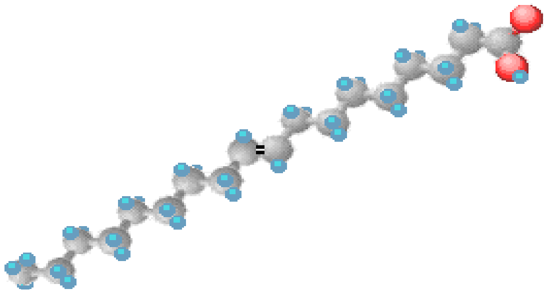
The biohydrogenation by anaerobic bacteria in the ruminants (i.e., sheep, goats, cattle, etc.) such as *Butyrivibrio fibrisolvens* and *Megasphaera esdenii* can isomerized the *cis* double bonds to the *trans* double bonds (Martin et al., 2007; Tsuzuki et al., 2008). The major *trans* isomers, such as vaccenic acid (C18:1*t* Δ 11*t*) is a precursor for conjugated linoleic acid (CLA) (Khanal and Dhiman et al., 2004; Martin et al., 2007; Gebauer et al., 2007; Teegala et al., 2009).

The amounts of *trans* fatty acid (ranged from 3% to 11% of fatty acids) in dairy and meat products depended on feed, climate and breed (Feldman et al, 1996; Gebauer et al., 2007; Teegala et al., 2009).



Cis-9-octadecenoic acid

(Oleic acid)



Trans-9-octadecenoic acid

(Elaidic acid)

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

Table 1 Major *trans* fatty acid isomers (Gebauer et al., 2007)

Common name	Common isomers	Major source
Palmitoleic acid	C16:1	Ruminant meat and milk
Elaidic acid	C18:1Δ9*t*[a]	Partially hydrogenated oils
Vaccenic acid[b]	C18:1Δ11*t*[a]	Partially hydrogenated oils
Linolelaidic acid	C18:2-t9*t*12	Partially hydrogenated oils
Conjugated linoleic acid (CLA)	C18:2-c9*t*11[c] C18:2-*t*10c12	Ruminant meat and milk

[a] C18:1t isomers comprise approximately 80-90% of total TFA in foods

[b] Vaccenic acid constitutes 40-70% of total *trans* 18:1

[c] Predominant isomer of CLA (90%)

2.3 The Health Effects of *Trans* Fatty Acids

In the present, several countries concern about effects of *trans* fatty acids on the human health. The high consumption of *trans* fatty acids have been linked to variety of disease, including coronary heart disease (CHD) (Gebauer et al., 2007; Teegala et al., 2009; Willett et al, 1993.), type II diabetes (Clandinin et al., 2001; Gebauer et al., 2007; Riserus et al., 2006; Lichtenstein et al., 2003.) and cancer (Slattery et al., 2001 and Lisa et al., 2010).

Many studies have shown the positive relation between *trans* fatty acid consumption and coronary heart disease. The mechanism of *trans* fatty acids on CHD is well established (Gebauer et al., 2007; Fernande-San Jaun et al., 2009; Filip et al., 2010.), while the mechanism of *trans* fatty acids on the other diseases are inconclusive (Gebauer et al., 2007).

2.3.1 Coronary Heart Disease (CHD)

The cardiovascular diseases (CVD) are the first of chronic disease that lead to public health problems in Thailand which are ranked in 1 of 3 of the main causes of death in population. In 2000 and 2001, Bureau of Policy and Strategy of the Ministry of Public Health reported that about 30.90 and 30.29 Thai peoples per 100,000 people, respectively die from the CVD (Ekpalakorn, 2003).

The risk factors of coronary heart disease (CHD) include hypertension, dyslipidemia, smoking, diabetes (Root and Anderson, 2004). Numerous studies were found the relationship between dietary fat and development of CHD. Saturated fatty acids and *trans* fatty acids consumption was related to increase risk of coronary heart disease (Hayes, 2000). Because a high intake of saturated fatty acids and *trans* fatty acids affect on lipid profile which lead to dislipedimia.

Epidemiologic studies have shown the association between consumption of TFA and risk of CHD (Gebauer et al., 2007; Jakobsen et al., 2008; Teegala et al., 2009; Kummerow, 2009; Hunter et al., 2010). Troisi et al (1992) reported that *trans* fatty acid consumption was directly related to total lipid profile in serum. Zutphen Elderly Study reported that 2% increase in baseline TFA intake was associated in a 29% increase in risk of ten year incidence of CHD (RR = 1.29; 95% CI 1.09-1.52) while decrease 2% of energy from TFA intake can significantly reduce risk of CHD (Oomen et al., 2001). In a prospective cohort studies, each 2% increase in energy intake from *trans* fatty acids was associated with a 23% increase incidence of myocardial infarction and CHD death (Mozaffarian, 2006).

2.3.2 Serum Lipid Profile

Several reports showed that *trans* fatty acid intake was associated with significantly increase LDL cholesterol, triglycerides, LDL cholesterol to HDL cholesterol ratio and at the same time, decrease HCL cholesterol (Hargreaves et al., 1991; Stampfer et al., 1991; Castelli et al., 1992; Shapiro et al., 1997; Mensink et al., 2003; Mozaffarain, 2006; Gebauer et al., 2007; Sun et al., 2007; Sofi et al., 2009; Brouwer et al., 2010; Remig et al., 2010; Wanders et al., 2010). *Trans* fatty acid containing diet can affect plasma lipoproteins in human than saturated fatty acid. Because the effects of *trans* fatty acid on human lipoprotein metabolism depresses HDL cholesterol while raises LDL cholesterol. In contrast, saturated fatty acids that increase LDL cholesterol also raise HDL cholesterol (Sundram et al., 1997; Hay, 2000).

2.3.3 Systemic Inflammation and Endothelial Function

Trans fatty acids have an adverse effect on biomarkers of inflammation, including c-reactive protein (CRP) and interleukin-6 (IL-6) (Mozaffarain et al., 2004; Lopez-Garcia et al., 2005). The Nurses' Health Study reported the positively association between levels of tumor necrosis factor receptor (TNFR) and *trans* fatty acid consumption on healthy woman (p for trend < 0.001), and the association with CRP, IL-6 in higher BMI woman (p-0.03) (Gebauer et al., 2007). The high inflammatory biomarkers level can be the risk of developing to CHD (Garcia et al., 2004). *Trans* fatty acids could impair endothelial function. The high consumption of *trans* fatty acids was associated with high vascular cell adhesion molecule (VCAM), intercellular adhesion molecule (ICAM) and E-selectin (p < 0.01.) (Lopez - Garcia et al., 2005).

However, the mechanisms of *trans* fatty acids with effects on endothelial function are not well established (Gebauer et al., 2007). *Trans* fatty acids can be incorporated into endothelial cell membrane and change the function of membrane. This could be result in endothelial dysfunction (Lopez-Garcia et al., 2005).

2.3.4 Insulin Sensitivity and Type 2 Diabetes

Trans fatty acid intake was related with increased risk of type 2 diabetes and may reduced insulin sensitivity (Salmeron et al., 2001). There is a 39-40% increase in risk when a 2% raise in energy intake from *trans* fatty acids (95% CI: 1.15-.67; p < .001) (Clandinin et al., 2001; Gebauer et al., 2007). In addition, some studies did not found the relation between *trans* fatty acids and risk of type 2 diabetes (Louheranta et al., 1999; Lichtenstein et al., 2001; Riserus., 2006). Louheranta (1999) reported that there was no significant difference in insulin sensitivity between a monounsaturated fatty acid containing diet and *trans* fatty acid containing diet in healthy adults. In addition, other studies showed no difference in serum fatty acid profiles and insulin sensitivity when compared amount of *trans* fatty acid in food (3-7% of total energy) in hypercholesterolemic overweight adults (Lichtenstein et al., 2001; Riserus., 2006).

2.3.5 Cancer

Trans fatty acids have been hypothesized to be carcinogenic. Few studies reported the association between *trans* fatty acids consumption and cancer. The high consumption of *trans* fatty acids may increase risk of colorectal cancer (Slattery et al., 2001, Lisa et al., 2010) and prostate cancer (Chavarro et al., 2008). The Nurses' Health Study reported that *trans* fatty acids intake during the premenopausal year was increased risk of postmenopausal breast cancer (Kim et al., 2006; Chajes et al., 2008).

2.3.6 The Other Health Effects of *Trans* Fatty Acids

2.3.6.1 Body weight

Few studies have shown the association between *trans* fatty acid intake and weight gain (Dorfman et al., 2009). Koh et al (2003) reported in cohort study that more than 16,000 men were measured abdominal circumference over 9 years and each 2% increase in energy intake from *trans* fatty acids was associated with a 2.7 cm increase in abdominal circumference ($p \leq 0.001$) In more than 41,000 woman were measured weight over 8 years, high *trans* fatty acid consumption were associated with increase body weight (Field et al., 2007). In monkeys, *trans* fatty acids containing diet affected to their bodyweight higher than MUFA containing diet. The results showed that *trans* fatty acids containing diet increased 7.2% of their body weight while MUFA containing diet increased 1.8% of their body weight (Kavanagh et al., 2007).

2.3.6.2 Alzheimer's Disease

A diet high in saturated fatty acids and *trans* fatty acids may be associated with cognitive decline (Morris et al., 2004; Morris et al., 2006; Devore et al., 2009). Moreover, the high dietary intake of copper in conjunction with a diet high in saturated fatty acid and *trans* fatty acid may be associated with accelerated cognitive decline (Morris et al., 2006).

2.4 The Consumption of *Trans* Fatty Acids

Trans fatty acid consumption has been varies in difference countries. It depended on the lifestyle such as eating habits and socioeconomic status of the population (Fernandez-San Juan, 2009). The average *trans* fatty acid intake in European countries was 1.6 to 5.4 g /day (Table 2) (Fernandez-San Juan, 2009). The highest intake was found in the Iceland with 5.4 g /day (Fernandez-San Juan, 2009) and lowest intake was found in Portugal and Italy with 1.6 g /day. The lower consumption of *trans* fatty acids was found in the Mediterranean countries, such as Finland where olive oil was generally used (Filip et al., 2010). In Asia countries, Korea and Japan were found lower *trans* fatty acid intake (less than 1-2 grams per day) (Fernandez-San Juan, 2009; Filip et al., 2010).

Table 2 Average consumption of *trans* fatty acids in European countries (Fernandez-San Juan, 2009)

Country	**Average consumption of *trans* fatty acid (g/day)**
Iceland	5.4
Netherlands	4.3
Belgium	4.1
Norway	4.0
United Kingdom	2.8
France	2.3
Denmark	2.6
Germany	2.2
Spain	2.1
Portugal	1.6
Italy	1.6

2.5 The Regulation and the Nutrition Label of *Trans* Fatty Acids

Several countries concern about health effect of *trans* fatty acids. Therefore, the expert committees have a recommendation for limiting dietary *trans* fatty acid intake (Table 3) (Gebauer et al., 2007). The regulation for limiting *trans* fatty acid content is differences in each country (Table 4) (Gebauer et al., 2007). *Trans* fatty acid content on the nutrition label is necessary in some countries such as the United States. If *trans* fatty acid level of the product is less than or equal to 0.5 g per serving, the manufactures can show 0 g of *trans* fatty acids on the nutrition label, In Denmark, *trans* fatty acid content is not require to show on nutrition label but the *trans* fatty acids content from partially hydrogenated oils should be limited to 2% of the total amount of fat or oil in food (Moss et al., 2006; Gebauer et al., 2007). In Canada, the Minister of Health limits the amount of TFA content at 2% of total fat content in all vegetable oils and soft, spreadable margarines (Gebauer et al., 2007). In Australia, there is no regulation to control *trans* fatty acid level, so the government established National Collaboration on *Trans* Fat for reducing *trans* fatty acids level in food (Gebauer et al., 2007).

In Thailand, *trans* fatty acids level are not required to show on nutrition label. Therefore, it is important to educate consumers for health effect of *trans* fatty acids and aware of the products which showed 0 g of *trans* fatty acids because they are not necessarily *trans* fat free.

Table 3 The recommendation for limiting dietary *trans* fatty acid intake (Gebauer et al., 2007)

	Year of Recommendation	**Recommendation**
Institute of Medicine (IOM), USA	2002	As low as possible.
Dietary Guidelines Advisory Committee (DGAC), USA	2005	As low as possible, which is about 1% of energy intake.
Dietary Guidelines for Americans	2005	As low as possible.
WHO/FAO report, Diet, Nutrition, and Chronic Disease	2003	Less than 1% energy from *trans* fatty acids.
International Society for the Study of Fatty Acids and Lipids (ISSFAL)	1999	Maximum level of *trans* fatty acids should be 1% energy from *trans* fatty acids.
Nutrition and Diet for Healthy Lifestyles in Europe, (EURODIET)	2000	Less than 2% energy from *trans* fatty acids.
UK Ministry of Agriculture	1998	Less than 2% energy from *trans* fatty acids.
Netherlands Health Council	2006	Less than 1% energy from *trans* fatty acids.
American Heart Association (AHA)	2006	Less than 1% energy from *trans* fatty acids.
American Diabetes Association	2007	As low as possible.

Table 4 The regulation for limiting *trans* fatty acid content in foods (Gebauer et al., 2007)

Countries	**Limitation of TFA**
United States	*trans* fatty acids content of less than 0.5 g per serving can be show 0 g *trans* fatty acids on the food label.
Canada	2% of total fat in oil and margarines 5% of total fat in other foods The products with less than 0.2 g of *trans* fatty acid level per serving may be labeled *trans* fatty acids free.
Denmark	2% of total fat.
Australia	Reduced *trans* fatty acids in foods.
Switzerland	Followed Denmark’s regulation.
United Kingdom	Started *trans* fatty acids ban and reduced *trans* fatty acids in foods.

2.6 The Method of Lipid Extraction

Lipids are usually defined as food components that are insoluble in water but soluble in organic solvents (Shahidi et al., 1998). The ideal solvent for lipid extraction would completely extract all the lipid components from a food. The efficiency of solvent extraction depends on the polarity of the lipids present compared to the polarity of the solvent. Polar lipids such as glycolipids or phospholipids are more soluble in polar solvents such as alcohols, than in non-polar solvents such as hexane. In contrast, non-polar lipids such as triacylglycerols are more soluble in non-polar solvents than in polar ones (Meloan and Pomeranz, 1994).

The different lipids have different polarities. Therefore, it is impossible to select a single organic solvent to extract them all. Thus the total lipid content determined by solvent extraction depends on the nature of the organic solvent used in carrying out the extraction. The total lipid content determined using one solvent may be different from that determined using another solvent (Meloan and Pomeranz, 1994).

The criteria for solvent selection including, a solvent should be inexpensive, have a relatively low boiling point that it can easily be removed by evaporation, be non-toxic and be nonflammable. Ethyl ether and petroleum ether are the most commonly used solvents, but pentane and hexane are also used for some foods (Nichols, 2003).

Many extraction methods for the determination of fat in food depend on the nature of sample. Roese-Gottlieb (RG) method is a reference method for fat extraction from dairy products. It requires alkaline pretreatment of sample that commonly use ammonium hydroxide before liquid-liquid extraction. Petroleum ether and ethyl ether are used as extraction solvents. During the procedure, if sample forms gelatinous mixture, a small amount of methanol will be added into sample for treat mixture of sample. The other method; a soxhlet method requires a longer time (about 4-24 hours for extraction (Garcia-Olmo et al, 2004).

Ultrasonic extraction, also as sonication, uses ultrasonic vibration to contact between sample and solvent. Ultrasonic irradiation of aqueous solution induces acoustic cavitation into liquid media, when an ultrasonic wave passes though a liquid, the wave's vibrating pressure can break molecules of sample which leads to penetration of solvent into sample.

The ultrasonic method allows extraction of the amount of total fat in a time shorter than that require by soxhlet method. For example, the time of fat extraction from snacks by sonication method (1 hour) was shorter than the conventional soxhlet extraction (8 hour) (Ruiz-Jimenez et al, 2004). In the extaction of cookies, the time was shorter more than five times, from 16 hour to 3 hour which compared with soxhlet extraction (Ruiz-Jimenez et al, 2004).

2.7 The Official Methods for the Determination of *Trans* Fatty acid

2.7.1 Gas Chromotographic (GC) Method

The GC method can be used to determine the total *trans* fatty acid level of fats and can detect the lower level of *trans* fatty acid (Favier et al., 1996). This method consists of several procedures: fat extraction, fatty acid methyl ester (FAME) preparation and an internal standard addition (IS) and requires the specific column for separate FAME components (Mossoba., 2009). Currently, there is 100 m columns for determination of *trans* fatty acid level to give optimum separation of *trans* configuration which is very little overlap of *cis* isomers (Ratnayake., 2004). GC method can analyze individual identification and quantitation of each isomer (Ledoux et al., 2000; Mossoba., 2009).

2.7.2 Infrared Spectroscopic (IR) Method

The Infrared Spectroscopy method was used for the determination of *trans* fatty acid content which, based on the C-H out-of-plane deformation band observed at 966 cm^{-1}. This wave length is unique characteristic of isolated *trans* double bond (Mossoba et al., 2009; Mahesar et al., 2010). This method has been extensively used in fats and oils. However, the accuracy will be reduced if *trans* fatty acid content is below 5%. Therefore, many modifications have been purposed to improve the accuracy of IR method. Such as, attenuated total reflection fourier transform infrared spectroscopy (ATR-FTIR) method.

2.7.2.1 Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) method

ATR-FTIR method was used to detect and determine the amount of isolated *trans* double bonds in fats and oils for rapid (about 5 minutes) measurement of the wavelength number 966 cm^{-1}. ATR uses the phenomenon of total internal reflection. The IR light penetrates a short distance into the melted fat or oil when it is placed on the surface of an internal reflection crystal such as zinc selenide (ZnSe) or diamond (Figure 2) (Mossoba et al., 2009). The advantages of this method than GC method are rapid, use a small amount of sample (about 50 μl), and it is not necessary to prepare the test sample but the results of this method show total *trans* fatty acids of sample whereas GC method show individual identification isomers of *trans* fatty acid. Figure 3 shows the attenuated total reflection -fourier transform infrared (ATR-FTIR) spectrometer.

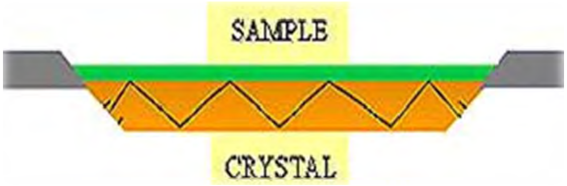


Figure 2 Attenuated total reflection IR cell diagram

Figure 3 Attenuated total reflection -Fourier Transform Infrared (ATR-FTIR) Spectrometer

However, the limitation of ATR-FTIR method is determining *trans* fatty acid content more than 5% of total fats (Mossoba et al., 2009). But a new ATR-FTIR procedure is called negative second derivative (-2D) methodology which improve sensitivity and accuracy by measuring the height of the negative second derivative (-2D) of *trans* absorption band relative to air (Figure 4) (Mossoba et al., 2009). The precision of this method is 0.1% *trans* fatty acids as a percentage of total fat (Milosevic et al., 2004). The negative second derivative IR procedure has been successfully to eliminate interferences from both conjugated and saturated fats

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

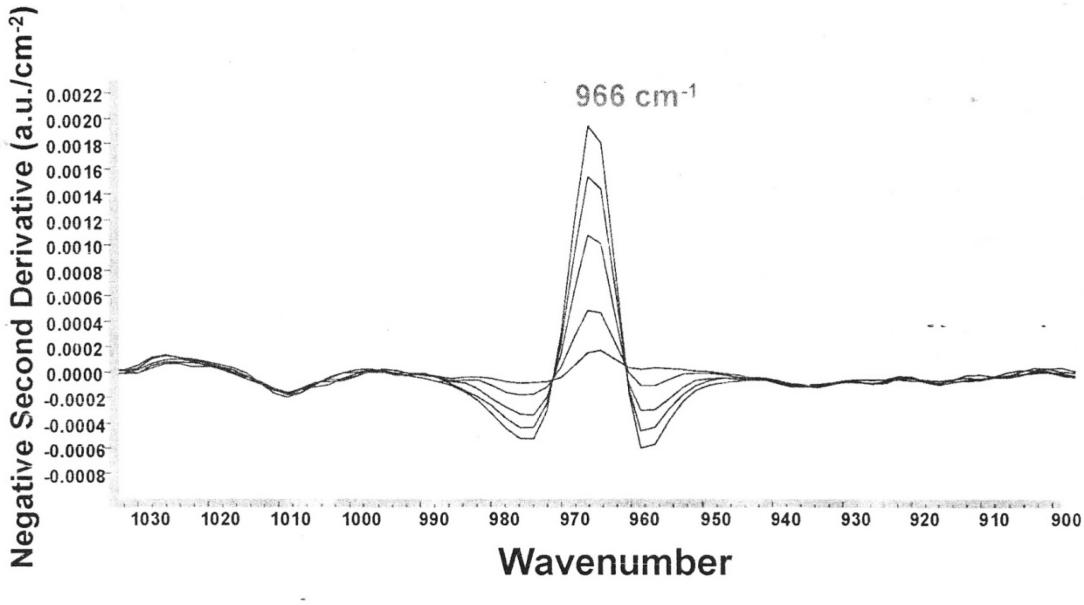


Figure 4 Negative second derivative of ATR-FTIR spectra for neat (without solvent) *trans* fat sample measured relative to open beam (air)

2.8 *Trans* Fatty Acid Content in Some Foods Determined By Gas Chromotographic (GC) Method and Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) method

Since, the *trans* fatty acid labeling regulation was issued in many countries, the food industry reduced *trans* fatty acid by reformulation of food products. Therefore, the method for determination of *trans* fatty acid level should be rapid, accurate, reproducible, and sensitive validated (Mossoba et al., 2009). GC and ATR-FTIR have been used to indentify and quantify total *trans* fatty acid in food products (Mossoba et al., 2009). Table 5 shows a comparison of *trans* fatty acid content in some foods that were analyzed by GC method and ATR-FTIR method (Fu et al., 2008). The amounts of *trans* fatty acids determined by GC method were lower than those of ATR-FTIR method. GC method can analyze individual identification of each isomer such as saturated fatty acids, *cis*-monounsatureated fatty acids, *cis*-polyunsatureated fatty acids and *trans* fatty acids while the result of ATR-FTIR method shows total *trans* fatty acids.

Therefore, it should investigate for appropriated method with the purpose of analysis and the results application.

Table 5 *Trans* fatty acid content in some foods determined by GC method and ATR-FTIR method (Fu et al., 2008)

Products	***Trans* fatty acids content (% total fat)**	
	GC Method	**ATR-FTIR Method**
	Range	**Mean ± SD**
Cake (n=2)		
Chocolate cake	0.89 – 1.35	2.42 ± 0.14
Egg flavor cake		4.02 ± 0.32
Muffin (n=2)		
Butter grape muffin	2.62 – 2.86	5.32 ± 0.23
Butter muffin		5.34 ± 0.46
Wafer (n=2)		
Chocolate wafer	19.68 – 20.02	26.00 ± 0.79
Milk wafer		27.90 ± 0.09
Chips (n=4)		
Potato chips (natural)	ND – 3.52	0.91 ± 0.11
Onion crisps		0.92 ± 0.08
Pizza chips		2.44 ± 0.16
Shrimp chips		1.33 ± 0.07
Cracker (n=3)		
Crisp cracker	ND	< 0.05
Moring tea cracker		< 0.05
Sesame cracker		< 0.05

ND = not detected

CHAPTER III

MATERIALS AND METHODS

3.1 Instruments

An ultrasonic bath (Transsonic Digital S, Elma, Germany) and Rohrig tube 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. *Trans* fatty acid was determined by the Fourier transform infrared spectrometer (Perkin Elma Spectrum One FTIR, USA) and a zinc selenide crystal (ZnSe trough plate 45º, Perkin Elma, USA) attenuated total reflection infrared cell.

3.2 Chemicals

Fatty acid standards were trielaidin [1,2,3 tris(*trans*-9-octadecanoate)] and triolein [1,2,3 tris(*cis*-9-octadecanoate)] with purity ≥ 99%. They were purchased from Sigma Aldrich (St. Louis, MO, USA). N-hexane, petroleum ether and ethyl ether were purchased from Lab Scan LTD., (Dublin, Ireland). Anhydrous sodium sulfate was obtained from Merck (Darmstadt, Germany).

3.3 Methods

3.3.1 Samples Collection

The samples were six groups of snacks including crackers, stick biscuits (ka-Kai), potato chips, biscuits, microwave popcorns and wafers. Each group was collected three brands. Twelve brands of non-dairy creamers were selected from available brands in supermarket. All samples were purchased from supermarket in Bangkok, Thailand during October 2009 to January 2010.

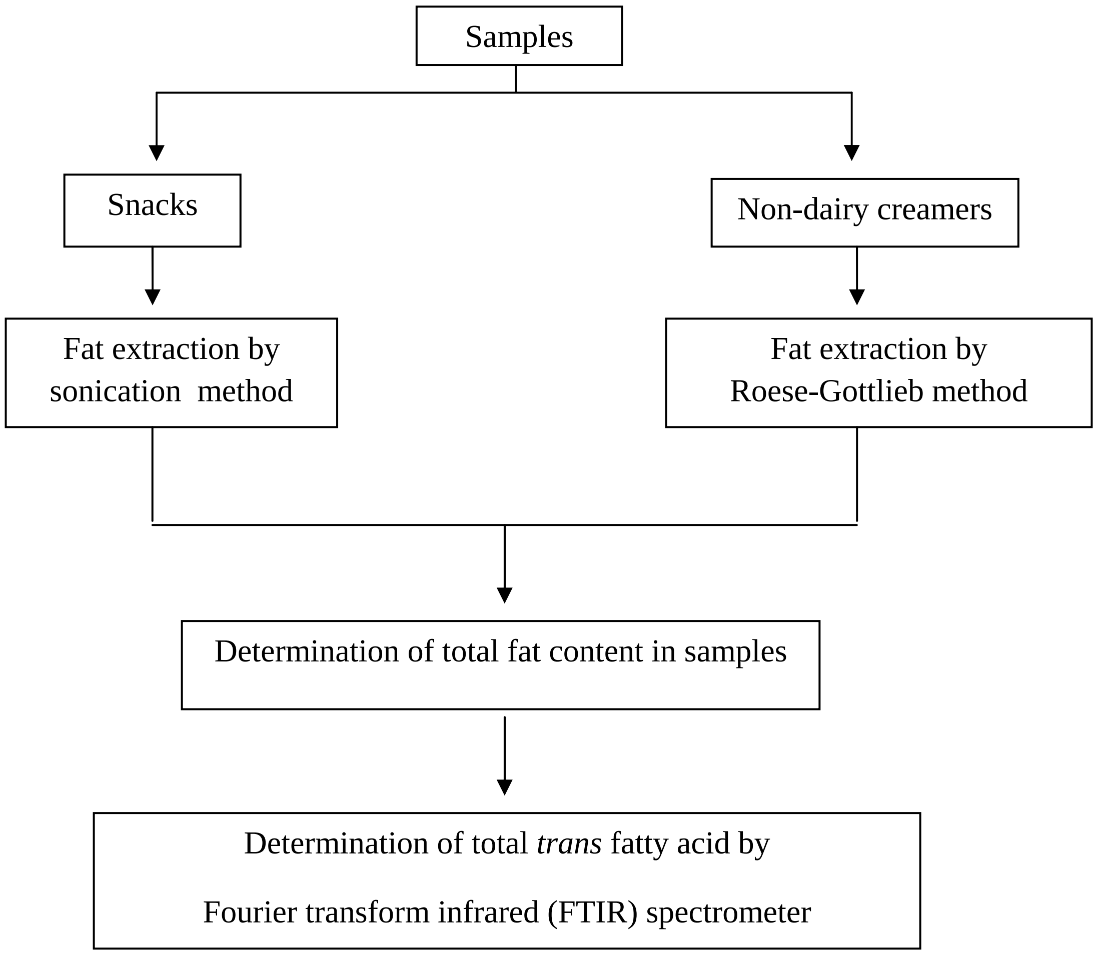


Figure 5 The procedure of experiment

3.3.2 The Procedure of Experiment

The procedure of experiment to determine total *trans* fatty acid contents in the food samples is shown in Figure 3. In this study, *trans* fatty acid contents in the food samples were analyzed in snacks and non-dairy creamers. Fat in snacks and non-dairy creamers were extracted by sonication and Roese-Gottlieb methods, respectively. Then, total fat content in the samples were determined. After that, total *trans* fatty acids were analyzed by Fourier transform infrared (FTIR) spectrometer.

3.3.3 Fat Extraction

3.3.3.1 Extraction of snacks

The snack samples were crushed into small pieces and stored in polyethylene bag at 4° C until use. Total fat contents were extracted using the sonication method. Five grams of the small pieces of each snack sample were weighed and placed into 250 ml round bottom flask with 60 ml of n-hexane and put into the ultrasonic bath. The sample-solvent mixture was extracted at appropriate condition. The condition was 120 minutes for extraction time, n-hexane for extraction solvent and 40% ultrasonic intensity level. After extraction, the mixture was filtered through filter paper Whatman no. 42. Then, the filtrate was evaporated by rotary evaporator and the residue (fat) was dried in vacuum desiccators for 60 minute. Then, the fat was weighted and stored at -10° C until analysis.

3.3.3.2 Extraction of non-dairy creamers

The Roese-Gottlieb method (AOAC Method 932.06 for milk powder) (AOAC, 2000) was used to determine the total fat content. One gram of each non-dairy creamer was weighed and filled into 10 ml beaker with 1 ml of distilled water. The sample was melted and mixed through homogenous on water bath. Then, 9 milliliters of distilled water and 1.25 ml of ammonium hydroxide were added into the beaker. The sample mixture was transferred into Rohrig tube and added 10 ml of ethyl alcohol when the mixture was cool.

Twenty-five milliliters of ethyl ether were filled into Rohrig tube and swirled in vertical line gently for 1 minute. After that, twenty-five milliliters of petroleum ether were added and swirled again in the same manner then waited for cool and for phase separation. The sample mixture was waited for separated phase. If the sample mixture formed emulsion, it would be treated with a few milliliters of methanol. After phase of sample mixture was separated, the ethyl ether phase was released into the constant weighted beaker. The lower phase of petroleum ether was extracted twice by 15 ml of ethyl ether and 15 ml of petroleum ether.

The finally extraction, if the volume of ethyl ether phase was lower than the level of the stopcock, distilled water should be added until the volume of solvent was upper. The ethyl ether phase was released into the constant weighted beaker again. The filtrate was evaporated on water bath in the hood. The residue was dried in vacuum desiccators for 60 minute. After extraction, the fat was weighted and stored at -10º C until analysis.

3.4 Determination of Total *Trans* Fatty Acids Content of Snacks and Non-Dairy Creamers

3.4.1 Principle of the Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy

The attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy is the method to measure isolated *trans* double bond. This method is suitable for natural or processed oils and fat containing long chain fatty acids, fatty acid methyl esters (FAME) and triacylglycerols with *trans* fatty acid content ≥ 5% (Mossoba et al., 2009). The determination of this method is based on the C-H out of plane deformation measured at 966^{-1} which is uniquely characteristic of *trans* configuration (Mossoba et al., 2009). Fats and oils are not required to FAME preparation before analysis.

3.4.2 Preparation of Standards

The primary standards were used trielaidin (TE) and triolein (TO). *Trans* fats calibration standards were prepared by weighing accurately to nearest 0.0001 g, (0.3-x) g of TO and x g of TE into 10-ml beaker, where x equals 0.0015, 0.0030, 0.0150, 0.0300, 0.0600, 0.0900, 0.1200, and 0.1500 g in order to prepare 0.5, 1, 5, 10, 20, 30, 40 and 50% *trans* calibration standards, respectively (AOAC method 2000.10) (Appendix B). The symbol of x in this study is the weight of TE.

3.4.3 Preparation of Test Samples

The test samples were solid fats which gently melted and mixed before sampling on stream bath. If the sample appeared cloudy due to the presence of water, it would be treated with anhydrous sodium sulfate until clear and filtered before analysis.

3.4.4 Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy Determination

The ATR-FTIR parameters were set up according to the manufacturer's recommendations for using a zinc selenide ATR cell with the following parameters: the spectral range of 1050 - 900 cm^{-1}, 4 cm^{-1} resolution and 64 scans. The ATR cell was maintained at 65±2 ºC for sample melting. The reference background was used the single beam spectrum collected of air. The volume of sample (50 μl) was transferred by disposable pipette to cover the surface of ATR cell. The single beam spectrum of test sample was collected against that of the reference background, and converted to absorbance. Figure 6 and 7 shows the spectrum of *trans* fatty acid absorption and the spectrum of the negative second derivative of *trans* fatty acid absorption of sample at wavelength number 966 cm^{-1} respectively. After each analysis, the ATR cell was cleaned with acetone before the other samples were repeated.

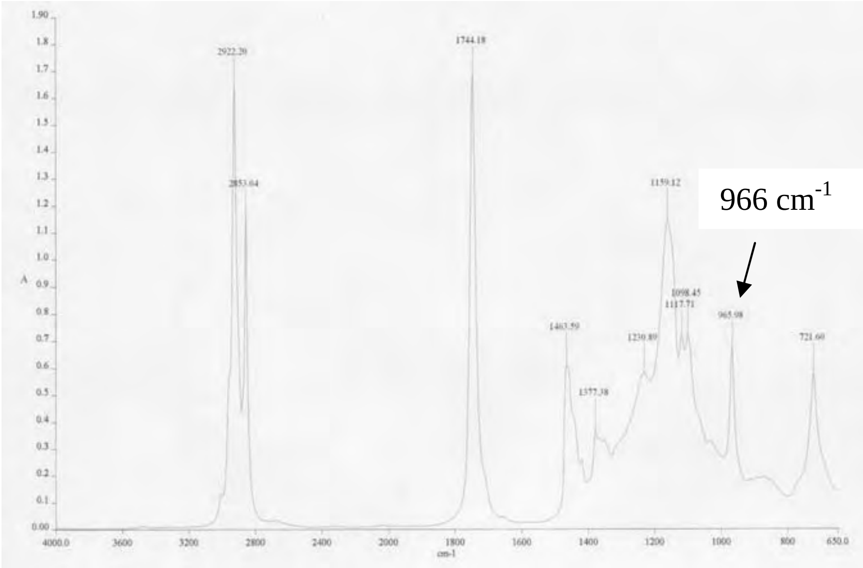


Figure 6 The spectrum of *trans* fatty acid absorption of sample at wavelength number 966 cm^{-1}

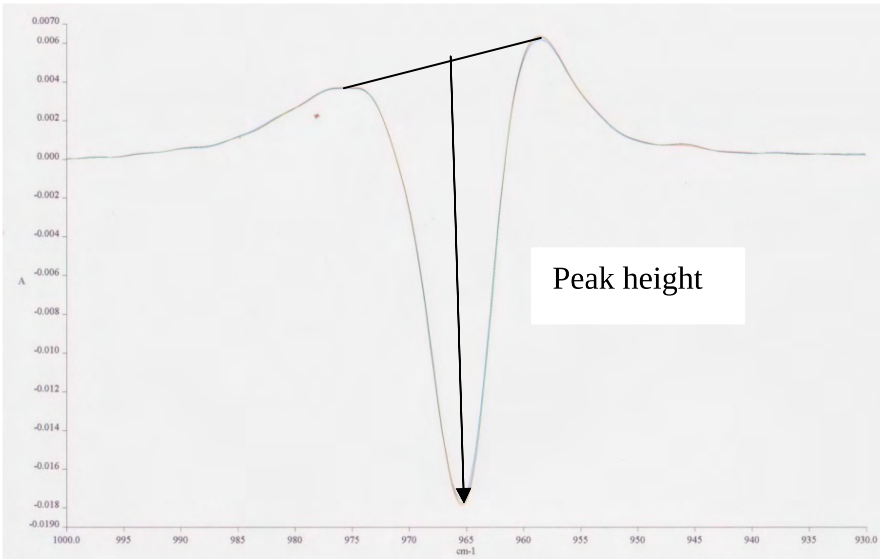


Figure 7 The spectrum of the negative second derivative of *trans* fatty acid absorption of sample at wavelength number 966 cm^{-1}

3.4.5 Calculation of % *Trans* Fatty Acids

Standard calibration curve of *trans* fatty acids content was plotted between peak height of standard *trans* fatty acid versus % trans fatty acid of total fat. The linear regression equation was calculated from standard calibration curve. The % *trans* fatty acids for sample was calculated by substituting the value of the integrated height of the negative second derivative of *trans* fatty acid band in the following equation:

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

CHAPTER IV

RESULTS

4.1 Determination of Total Fat Content of Some Selected Snacks and Non-dairy Creamers

The total fat content of some selected snacks as six groups including crackers, stick biscuits (ka-kai), potato chips, biscuits, microwave popcorn and wafers and twelve brands of non-dairy creamers were analyzed in triplicate and the values expressed as range and mean ± standard deviation (SD) are shown in Table 6. The highest total fat content was found in the stick biscuits (ka-kai) (19.71 – 31.92 g/ 100g food) while the lowest content was found in biscuits (15.25 - 22.98 g/100g food). The range of total fat content in non-dairy creamers which were produced from palm kernel oil was 29.20 - 34.09 g per 100 g food while non-dairy creamers produced from soybean oil range from 23.31 - 35.54 g per 100 g food.

4.2 Total *Trans* Fatty Acid Content of Some Selected Snacks and Non-dairy Creamers

The total *trans* fatty acid content was determined by attenuated total reflection Fourier transform infrared spectrometer (ATR-FTIR), which recommended in AOAC Official Method 2000.10.(AOAC, 2000). The new ATR-FTIR was improved sensitivity and increased accuracy for detection by measuring the height of the negative second derivative of the *trans* fatty acid absorption band at wave number 966 cm^{-1}. The height of the negative second derivative of *trans* fatty acid absorption band of snacks and non-dairy creamers are shown in table 7 and 8 respectively.

Table 6 Total fat content of some selected snacks and non-dairy creamers

Products	Total fat content (g/100g food)	
	Range[a]	Mean ± SD
Cracker (n=3)	17.40-21.29	19.72 ± 2.05
Stick biscuit (n=3)	19.71-31.92	27.45 ± 6.74
Potato chip (n=3)	22.84-26.68	24.92 ± 1.94
Biscuit (n=3)	15.25-22.98	18.07 ± 4.26
Microwave popcorn (n=3)	22.29-27.04	24.95 ± 2.43
Wafer (n=3)	15.62-22.36	18.20 ± 3.64
Non-dairy creamer (n=12)		
Palm kernel oil (n=10)	29.20-34.90	31.42 ± 1.87
Soybean oil (n=2)	23.31-35.54	29.43 ± 8.65

[a] All values were the mean of triplicate extraction

Table 7 The height of the negative second derivative of *trans* fatty acid absorption band of snacks at wavelength number 966 cm^{-1} by ATR – FTIR Method.

Products	Peak height (-)	Average peak height (-)
Cracker(brand A)_1	0.0007	
Cracker(brand A)_2	0.0007	0.0007
Cracker(brand A)_3	0.0007	
Cracker(brand B)_1	0.0007	
Cracker(brand B)_2	0.0007	0.0007
Cracker(brand B)_3	0.0007	
Cracker(brand C)_1	0.0005	
Cracker(brand C)_2	0.0005	0.0005
Cracker(brand C)_3	0.0005	
Stick biscuit (brand A)_1	0.0005	
Stick biscuit (brand A)_2	0.0005	0.0005
Stick biscuit (brand A)_3	0.0005	
Stick biscuit (brand B)_1	0.0006	
Stick biscuit (brand B)_2	0.0006	0.0006
Stick biscuit (brand B)_3	0.0005	
Stick biscuit (brand C)_1	0.0008	
Stick biscuit (brand C)_2	0.0005	0.0006
Stick biscuit (brand C)_3	0.0005	
Potatochip (brand A)_1	0.0006	
Potatochip (brand A)_2	0.0005	0.0005
Potatochip (brand A)_3	0.0005	
Potatochip (brand B)_1	0.0006	
Potatochip (brand B)_2	0.0005	0.0006
Potatochip (brand B)_3	0.0006	
Potatochip (brand C)_1	0.0006	
Potatochip (brand C)_2	0.0006	0.0006
Potatochip (brand C)_3	0.0006	

Table 7 The height of the negative second derivative of *trans* fatty acid absorption band of snacks at wavelength number 966 cm^{-1} by ATR – FTIR Method (continued).

Products	Peak height (-)	Average peak height (-)
Biscuit (brand A)_1	0.0007	
Biscuit (brand A)_2	0.0007	0.0007
Biscuit (brand A)_3	0.0007	
Biscuit (brand B)_1	0.0100	
Biscuit (brand B)_2	0.0078	0.0088
Biscuit (brand B)_3	0.0085	
Biscuit (brand C)_1	0.0008	
Biscuit (brand C)_2	0.0008	0.0008
Biscuit (brand C)_3	0.0008	
M popcorn (brand A)_1	0.0224	
M popcorn (brand A)_2	0.0214	0.0217
M popcorn (brand A)_3	0.0213	
M popcorn (brand B)_1	0.0205	
M popcorn (brand B)_2	0.0191	0.0196
M popcorn (brand B)_3	0.0193	
M popcorn (brand C)_1	0.0023	
M popcorn (brand C)_2	0.0025	0.0024
M popcorn (brand C)_3	0.0023	
Wafer (brand A)_1	0.0007	
Wafer (brand A)_2	0.0005	0.0006
Wafer (brand A)_3	0.0005	
Wafer (brand B)_1	0.0012	
Wafer (brand B)_2	0.0008	0.0009
Wafer (brand B)_3	0.0008	
Wafer (brand C)_1	0.0134	
Wafer (brand C)_2	0.0133	0.0132
Wafer (brand C)_3	0.0130	

M popcorn = Microwave popcorn

Table 8 The height of the negative second derivative of *trans* fatty acid absorption band of non-dairy creamers at wavelength number 966 cm^{-1} by ATR – FTIR Method.

Products	Peak height (-)	Average peak height (-)
NDC (brand A)_1	0.0009	
NDC (brand A)_2	0.0009	0.0009
NDC (brand A)_3	0.0010	
NDC (brand B)_1	0.0010	
NDC (brand B)_2	0.0010	0.0010
NDC (brand B)_3	0.0010	
NDC (brand C)_1	0.0009	
NDC (brand C)_2	0.0009	0.0009
NDC (brand C)_3	0.0010	
NDC (brand D)_1	0.0014	
NDC (brand D)_2	0.0014	0.0013
NDC (brand D)_3	0.0013	
NDC (brand E)_1	0.0246	
NDC (brand E)_2	0.0243	0.0245
NDC (brand E)_3	0.0246	
NDC (brand F)_1	0.0229	
NDC (brand F)_2	0.0227	0.0228
NDC (brand F)_3	0.0230	

Table 8 The height of the negative second derivative of *trans* fatty acid absorption band of non-dairy creamers at wavelength number 966 cm^{-1} by ATR – FTIR Method (continued).

Products	Peak height (-)	Average peak height (-)
NDC (brand G)_1	0.0009	
NDC (brand G)_2	0.0009	0.0009
NDC (brand G)_3	0.0009	
NDC (brand H)_1	0.0018	
NDC (brand H)_2	0.0020	0.0019
NDC (brand H)_3	0.0019	
NDC (brand I)_1	0.0009	
NDC (brand I)_2	0.0009	0.0009
NDC (brand I)_3	0.0009	
NDC (brand J)_1	0.0009	
NDC (brand J)_2	0.0009	0.0009
NDC (brand J)_3	0.0009	
NDC (brand K)_1	0.0011	
NDC (brand K)_2	0.0011	0.0011
NDC (brand K)_3	0.0012	
NDC (brand L)_1	0.0009	
NDC (brand L)_2	0.0010	0.0009
NDC (brand L)_3	0.0009	

The reference standard consisting of trielaidin (TE) diluted in triolein (TO) was used to generate calibration curve. The composition of standard solution is shown in table 15 (Appendix B). The spectrum of the negative second derivative of *trans* fatty acid absorption band of reference standard is shown in Figure 8. Figure 9 shows the standard calibration curve of *trans* fatty acid which plotted the % trans fatty acid versus peak height at wavelength number 966 cm^{-1} with 0.9966 of a correlation coefficient (r^2).

The *trans* fatty acid contents of some snacks and some non-dairy creamers were reported in the percentage of total fat and g/100 g food which calculated by the regression equation of the standard calibration curve of *trans* fatty acid content. The results are shown in Table 9 and 10 respectively. The *trans* fatty acid content in snacks group ranged from not detected to 86.75 % of total fat or non detected to 22.12 g per 100 g food while *trans* fatty acids content in non-dairy creamers ranged from 0.81 to 98.17 % of total fat or 0.24 to 32.49 grams per 100 g food. In snack group, the highest level of the total *trans* fatty acids was found in the microwave popcorn and followed by wafers, biscuits, and crackers, respectively while *trans* fatty acid content in potato chip and stick biscuit were not detected. Table 11 and 12 show *trans* fatty acid content per serving size as recommended by the food manufacturer of snacks and non-dairy creamers respectively. In snack groups, microwave popcorn was found to contain high level of *trans* fatty acids per average serving size (0.57 - 6.63 g), while *trans* fatty acids content in stick biscuits (ka-kai) and potato chips were too low to be detected. In non dairy-creamer groups, the higher level of *trans* fatty acid per serving size was found in 2 brands (0.69 - 0.97g) which produced from soybean oil while the other 10 brands which produced from palm kernel oil were found to contain low level of *trans* fatty acids per serving size (0.01 - 0.04 g).

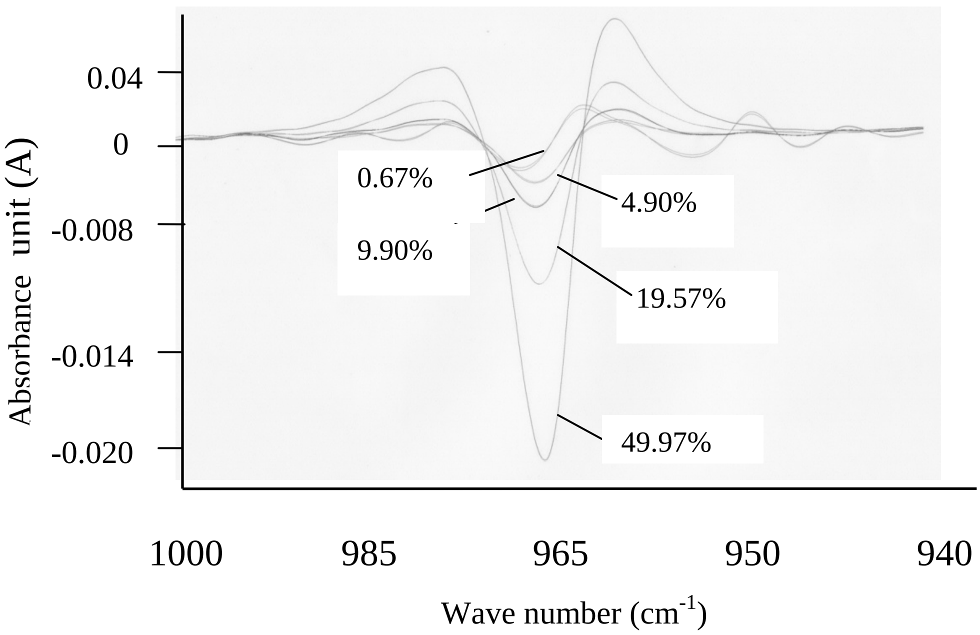


Figure 8 The spectrum of the negative second derivative of *trans* fatty acid absorption of standard mixtures at wavelength number 966 cm^{-1}

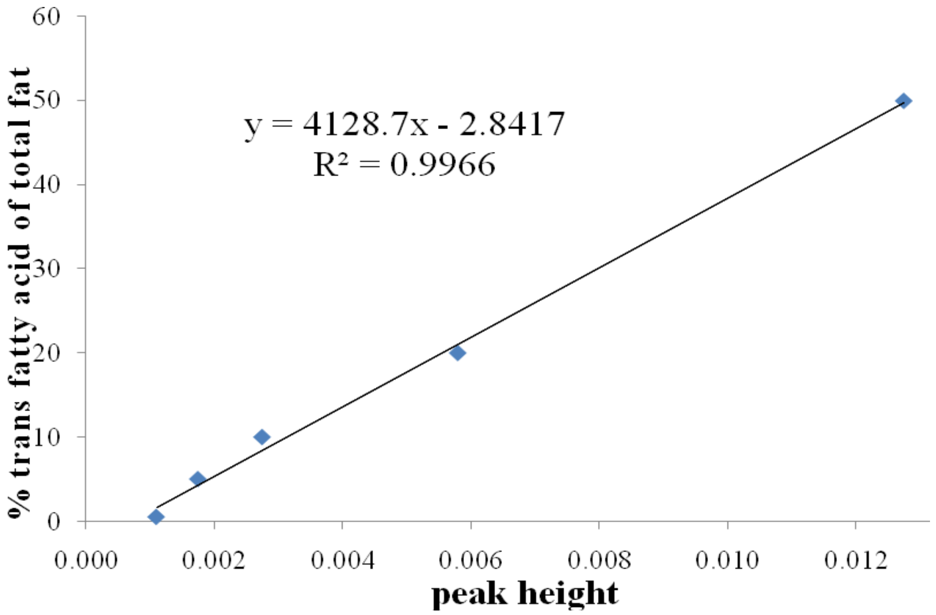


Figure 9 Standard calibration curve of trielaidin (TE) diluted in triolein (TO)

Table 9 The *trans* fatty acid content in some selected snacks.

Products	*Trans* fatty acids content			
	% of total fat	g / 100 g food	Range	
			% of total fat	g / 100 g food
Cracker Brand A	0.05	0.01	ND – 0.05	ND
Cracker Brand B	0.05	0.01		
Cracker Brand C	ND	ND		
Stick biscuit Brand A	ND	ND	ND	ND
Stick biscuit Brand B	ND	ND		
Stick biscuit Brand C	ND	ND		
Potato chip Brand A	ND	ND	ND	ND
Potato chip Brand B	ND	ND		
Potato chip Brand C	ND	ND		
Biscuit Brand A	0.46	0.11	0.05 - 33.35	0.01 - 5.09
Biscuit Brand B	33.35	5.09		
Biscuit Brand C	0.05	0.01		
M popcorn Brand A	78.08	17.40	6.97 - 86.75	1.90 – 22.12
M popcorn Brand B	86.75	22.12		
M popcorn Brand C	6.97	1.90		
Wafer Brand A	ND	ND	ND – 51.75	0 – 8.08
Wafer Brand B	1.01	0.17		
Wafer Brand C	51.75	8.08		

M popcorn = Microwave popcorn

ND = Not detected at the level traces

Table 10 The *trans* fatty acid content in some selected non-dairy creamers.

Products	*Trans* fatty acids content			
	% of total fat	g / 100 g food	Range	
			% of total fat	g / 100 g food
Palm kernel oil group (n=10)				
NDC Brand A	0.94	0.29	0.81 – 4.80	0.24 – 1.43
NDC Brand B	1.22	0.37		
NDC Brand C	0.94	0.29		
NDC Brand D	2.66	0.93		
NDC Brand E	0.87	0.28		
NDC Brand F	4.80	1.43		
NDC Brand G	0.87	0.30		
NDC Brand H	0.81	0.24		
NDC Brand I	1.84	0.59		
NDC Brand J	1.01	0.29		
Soybean oil group (n=2)				
NDC Brand K	91.43	22.88	91.43 – 98.17	22.88 – 32.49
NDC Brand L	98.17	32.49		

NDC = Non-dairy creamer

Table 11 The *trans* fatty acid content per serving size in some selected snacks.

Products	Serving size [a] (g)	*Trans* fatty acids content in serving size of products (g)	Range (g / serving size)
Snack groups			
Cracker Brand A	30	0.02	
Cracker Brand B	30	0.02	0 - 0.02
Cracker Brand C	30	ND	
Stick biscuit Brand A	40	ND	
Stick biscuit Brand B	40	ND	ND
Stick biscuit Brand C	35	ND	
Potato chip Brand A	29	ND	
Potato chip Brand B	30	ND	ND
Potato chip Brand C	30	ND	
Biscuit Brand A	45	0.05	
Biscuit Brand B	45	2.29	0 - 2.29
Biscuit Brand C	45	0	
M popcorn Brand A	30	5.22	
M popcorn Brand B	30	6.63	0.57 - 6.63
M popcorn Brand C	30	0.57	
Wafer Brand A	28	ND	
Wafer Brand B	28	0.05	0 - 2.29
Wafer Brand C	28	2.29	

M popcorn = Microwave popcorn

[a] Serving size as list on nutrition label by manufactures

Table 12 The *trans* fatty acids content per serving size in some selected non-dairy creamers.

Products	Serving size [a] (g)	*Trans* fatty acids content in serving size (g)	Range (g / serving size)
Palm kernel oil group (n=10)			
NDC Brand A	3	0.01	
NDC Brand B	3	0.01	
NDC Brand C	3	0.01	
NDC Brand D	3	0.03	
NDC Brand E	3	0.01	0.01 - 0.04
NDC Brand F	3	0.04	
NDC Brand G	3	0.01	
NDC Brand H	3	0.01	
NDC Brand I	3	0.02	
NDC Brand J	3	0.01	
Soybean oil group (n-2)			
NDC Brand K	3	0.69	0.69 - 0.97
NDC Brand L	3	0.97	

NDC = Non-dairy creamer

CHAPTER V

DISCUSSION

The methods for determination of *trans* fatty acid content are available such as gas chromatography (GC) and infrared (IR) spectroscopy. IR spectroscopy is expressed as total *trans* fatty acid content while GC is expressed as individual amount of *trans* fatty acids isomer. IR spectroscopy is a popular method because the analysis time is about 5 minutes per sample shorter than that of GC method (Ratnayake et al., 2004; Ali et al., 1996). The ATR-FTIR method is suitable for determination of more than 5 % *trans* fatty acid content (Fritsche et al., 1998; Mossoba et al., 2009). However, to improve accuracy and sensitivity, a new ATR-FTIR method that measures the height of the negative second derivative of *trans* fatty acid absorption band relative to air was recently proposed. This method can be used to determine the low level of *trans* fatty acid in food (0.5 to 5%) (Mossaba et al., 2009; Ratnayake et al., 2009). In this study, ATR-FTIR with negative second derivative mode was performed to determine *trans* fatty acid content in some snacks and non-dairy creamers, that were available in Thailand between October 2009 to January 2010.

The finding results showed variation of *trans* fatty acids in some selected snacks. This may be due to the different sources of fat or oil that were used in manufacturing process. In microwave popcorn groups, *trans* fatty acid level of three brands ranged from 1.90 – 22.12 g/ 100 g of food (0.57 – 6.63 g/ serving size). Brand C of microwave popcorn declared "*trans* fat 0 g" on label while analysis of *trans* fatty acids content found 0.57 g/ serving size. Brand A and B of microwave popcorn showed 5.22 g and 6.63 g/ serving size respectively. The ingredient list of microwave popcorn showed soybean oil as an ingredient. The amount of soybean oil was associated with the amount of *trans* fatty acids. The soybean oil may be produced by partial hydrogenation which affect to the high amount of *trans* fatty acids content in microwave popcorn. The difference in production conditions of hydrogenation oil such as temperature, pressure, type and amount of catalyst used in the process can affect the amount of *trans* fatty acid in oil product (McCarthy et al., 2005). The percentage of soybean oil in microwave popcorn brand A and B was 32% and 30 % respectively, and content of *trans* fatty acids in brand A was higher than brand B too. In biscuit groups, brand B showed highest amount of *trans* fatty acid (5.09 g / 100 g food or 2.29 g/ serving size), following by brand A (0.11 g / 100 g food or 0.05 g/ serving size) and brand C (0.01 g / 100 g food or ND g/ serving size). Sixteen percentage of margarine showed on ingredient list which was major source of *trans* fatty acid content in brand B of biscuit groups while 26% of shortening showed on ingredient list of brand A. Margarine and shortening contain partially hydrogenated oil. Narkwichian (2009) reported that *trans* fatty acid level in the margarine which ranged from 1.54 - 1.89 g/ 100 g of food and shortening was 1.84 - 3.37 g/ 100 g of food. While in Canadian foods, content of *trans* fatty acids in margarine was 14.7 - 21.0 g/100g of food and in shortening was 30.6 - 30.9 g/ 100 g of food (Ratnayake et al., 2009). Although the report of *trans* fatty acid level of shortening in Thailand was higher than that of margarine but in present study, the amount of *trans* fatty acid of biscuits brand B which used margarine in the manufacturing process was higher than brand A which used shortening in the manufacturing process. The amount of other component in biscuits such as the amount of milk may affect *trans* fatty acid level. Milk contains a small amount of *trans* fatty acids. Sunphan (2010) reported that *trans* fatty acid level in milk ranged from 0.09 – 0.25 g/ 100 g food. In biscuit brand C used 10% of butter in manufacturing process which have been reported that contained small amount of *trans* fatty acid and brand A did not used margarine or shortening in as an ingredient (not declared in ingredient list). Fu (2008) reported that *trans* fatty acid level of butter ranged from < 0.05 – 5.43% of total fat. Therefore, *trans* fatty acid content in brand C of biscuits was lower than brand B and brand A. In wafer groups, brand C was found the highest content of *trans* fatty acid (8.08 g/ 100 g food). Vegetable oil that was used in formula of brand C might be produced by partial hydrogenation process and so affected the amount of *trans* fatty acid content in brand C. The amount of *trans* fatty acid in brand B and A was 0.17 g/ 100 g of food and not detected respectively. Brand B contained 26% of fat in wafer formulation and did not declare other ingredients which were associated with amount of *trans* fatty acid. It is possible that there were components that contained partial hydrogenated oil in wafer. The difference in *trans* fatty acid level between brand C and brand B was not concluded because the labels did not declare the type of oil and fat that used in the formulas. The difference of type and manufacturing process of oil and fat affected *trans* fatty acid contents. Wafer brand A was imported from the United States and declared “no *trans* fat” on label and also declared coconut oil in the formulation. Coconut oil contained 92% of saturated fatty acids and 8% of unsaturated fatty acids (McGlone et al., 1986). Thus, in the process of coconut oil hydrogenation; amount of unsaturated fatty acid in coconut oil might be transformed into *trans* fatty acids (McGlone et al., 1986). Therefore, brand A of wafer might contain *trans* fatty acids but was not detected and in the United States, the regulation for *trans* fatty acid content allowed the product which contained *trans* fatty acids level not more than 0.5 g per serving size that can declare 0 g of *trans* fatty acids content or no *trans* fat. Moreover, all brands of wafers were filled chocolate cream which might contain partially hydrogenated oil (Fu et.al., 2008). The amount of chocolate cream which filled in wafers was different in different brands. Therefore, the amount of *trans* fatty acid in wafers was varied. *Trans* fatty acid content in crackers was 0.01 g/ 100 g food and potato chips and stick biscuits (ka –kai) were not detected. Most samples of potato chips and stick biscuits (ka –kai) used palm oil in the production. Palm oil contained 82% of saturated fatty acids and 18% of unsaturated fatty acids (McGlone et al., 1986). Because of low level of unsaturated fatty acids, the samples that contained palm oil as ingredient showed low level of *trans* fatty acid. Moreover, palm oil had better fluidity than hydrogenated oil which sprayed on surface of products after baking to provide an attractive glossy finish to the product during the manufacturing process (Fu et al., 2008). In non-dairy creamer products, two brands that made from soybean oil were found higher level of *trans* fatty acids (0.69 g - 0.97 g per serving size) than ten brands made from palm kernel oil (0.01 g - 0.04 g per serving size). This may be explained that soybean oil used in these samples might be the partially hydrogenated products. Although, the samples used palm kernel oil in the manufacturing process showed low level of *trans* fatty acids but the composition of palm kernel oil contains high amounts of saturated fatty acid (Petrauskaite et al, 1998) that can lead to dislipidemia problems.

In the present, several countries concern about the health harmful effects of *trans* fatty acids. Therefore, the government of some countries require to list the level of *trans* fatty acids on nutrition label. Many food manufacturers tried to develop or reformulate their products to reduce *trans* fatty acid content. In Thailand, the declarations of *trans* fatty acid level is not present in nutrition label of foods because of no labeling regulation. Therefore, FDA should educate consumers about sources and health effects of *trans* fatty acids and recommend the manufacturer to declare the correct and complete ingredients and the amount of *trans* fatty acid for increasing purchase decision of consumers.

CHAPTER VI

CONCLUSION

In this study the amount of total *trans* fatty acids in some selected snacks and non-dairy creamers available in Thailand between October 2009 to January 2010 were investigated by attenuated total reflection fourier transform infrared spectroscopy (ATR-FTIR) method with negative second derivative mode. In snack groups, the microwave popcorns were found very high level of total *trans* fatty acids and followed by wafers, biscuits, potato chips and stick-biscuit, respectively. The amounts of *trans* fatty acids in 12 non-dairy creamer brands depend on oil type in manufacturing process. The non-dairy creamer produced from soybean oil showed the higher *trans* fatty acids content than those of non-dairy creamer produced from palm kernel oil. This may be explained that the soybean oil may be produced by partially hydrogenation.

In conclusion, microwave popcorns, biscuits and wafers contained *trans* fatty acids more than 2 grams per serving (as list in nutrition label by manufacturer). Therefore, the consumers should avoid consuming these kinds of food because of their deteriorated effects on health.

Recommended for Further Research

More kinds of food products should be collected in order to improve database of *trans* fatty acids available in food. Moreover, the effect of *trans* fatty acid intake on nutritional status should be observed.

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APPENDICES

APPENDIX A

RESULTS OF FAT EXTRACTION

Table 13 Fat content of snacks extracted by n-hexane at 40% ultrasonic intensity for 120 minutes

Sample	Lipid yield (mg/ g sample)			
	beaker 1	**beaker 2**	**beaker 3**	**Average**
Cracker (brand A)	176.71	171.50	173.76	173.99
Cracker (brand B)	215.60	213.52	209.68	212.93
Cracker (brand C)	200.91	207.40	205.40	204.64
Stick biscuit (brand A)	328.99	311.76	316.75	319.17
Stick biscuit (brand B)	195.22	196.52	199.42	197.05
Stick biscuit (brand C)	306.39	309.67	306.18	307.41
Potatochip (brand A)	247.76	258.7	251.29	252.51
Potatochip (brand B)	257.63	270.74	271.92	266.76
Potatochip (brand C)	218.94	228.30	237.95	228.40
Biscuit (brand A)	225.77	230.64	232.88	229.77
Biscuit (brand B)	158.50	150.56	148.31	152.46
Biscuit (brand C)	174.38	143.30	162.31	160.00
Microwave popcorn (brand A)	310.44	312.60	318.20	313.75
Microwave popcorn (brand B)	221.63	228.51	218.51	222.88
Microwave popcorn (brand C)	248.79	265.01	251.47	255.09
Wafer (brand A)	222.90	230.23	217.63	223.59
Wafer (brand B)	173.35	165.56	166.67	168.53
Wafer (brand C)	157.69	153.80	157.06	156.18

Table 14 Fat content of non-dairy creamers extracted by Roese-Gottlieb method

Sample	Lipid yield (mg/ g sample)			
	beaker 1	**beaker 2**	**beaker 3**	**Average**
NDC (brand A)	0.8479	0.8552	1.1137	0.9389
NDC (brand B)	0.9109	0.9153	0.9278	0.9180
NDC (brand C)	1.0814	0.8549	0.8205	0.9189
NDC (brand D)	0.9004	1.1276	1.1139	1.0473
NDC (brand E)	0.6673	0.7090	0.7216	0.6993
NDC (brand F)	1.0664	1.0671	1.0653	1.0663
NDC (brand G)	0.9595	0.9419	0.9858	0.9624
NDC (brand H)	0.8327	0.8760	0.9576	0.8888
NDC (brand I)	0.9568	1.0136	1.0982	1.0229
NDC (brand J)	0.9106	0.9184	0.8685	0.8992
NDC (brand K)	0.9901	0.9817	0.8957	0.9558
NDC (brand L)	0.9023	0.8886	0.8370	0.8760

APPENDIX B

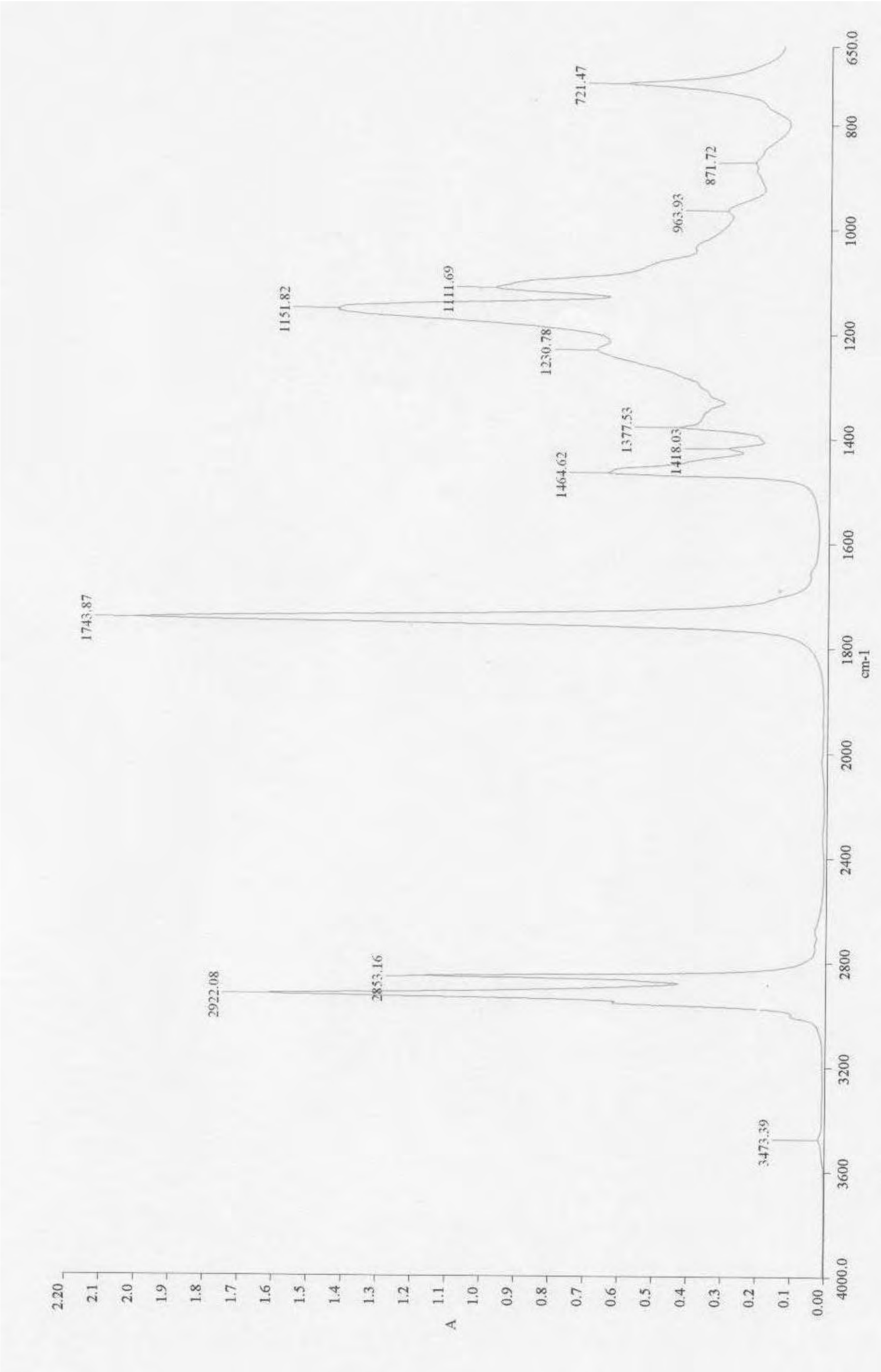
COMPOSTION OF REFERENCE STANDARDS

Table 15 Composition of trielaidin and triolein in reference standard.

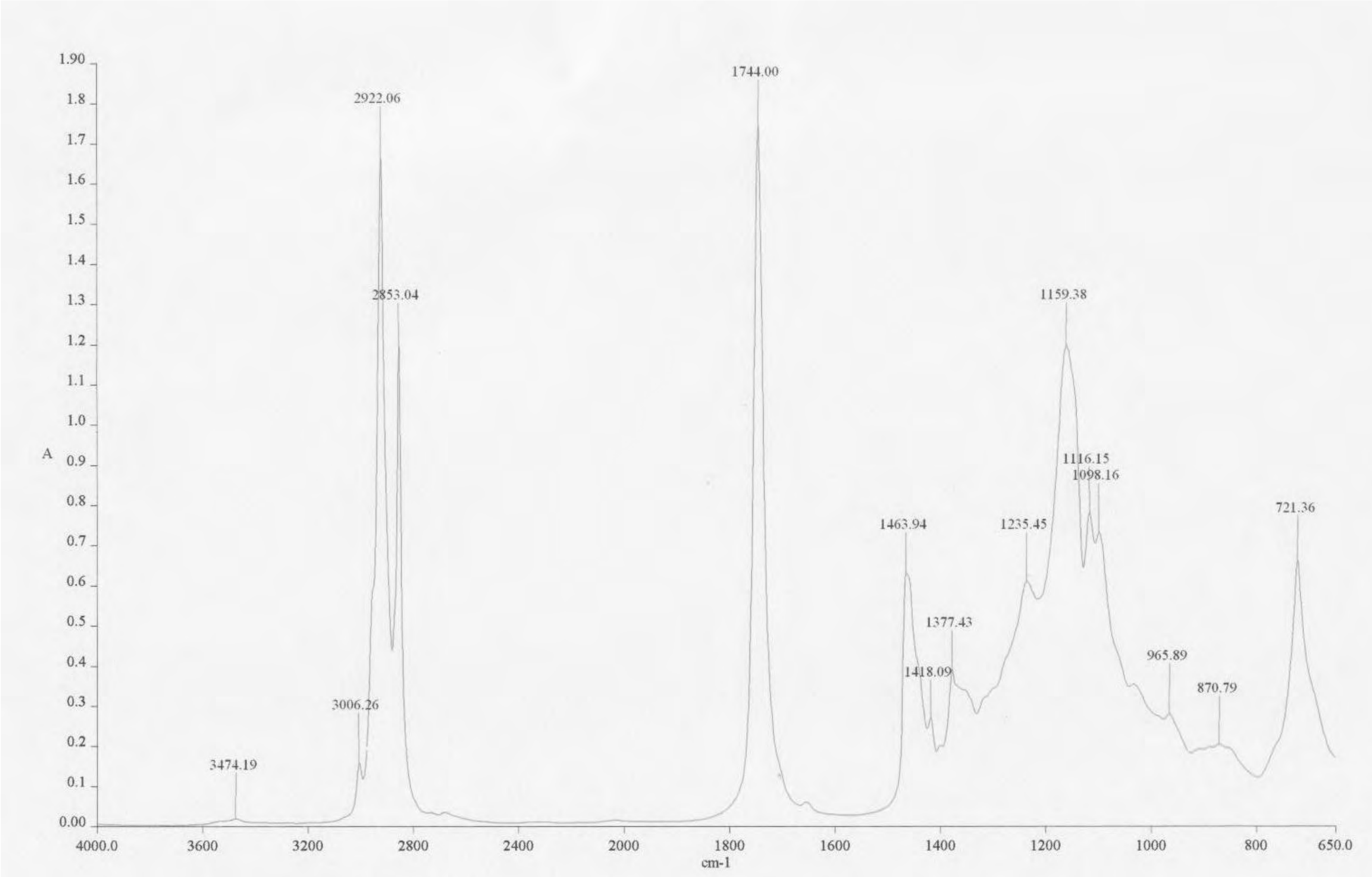
Triolein (g)	Trielaidin (g)	Total (g)	% *trans* fatty acids of total fat
0.2981	0.0020	0.3001	0.67
0.2853	0.1470	0.3000	4.90
0.2709	0.0297	0.3006	9.90
0.2405	0.0596	0.3001	19.57
0.1510	0.1499	0.3009	49.97

APPENDIX C

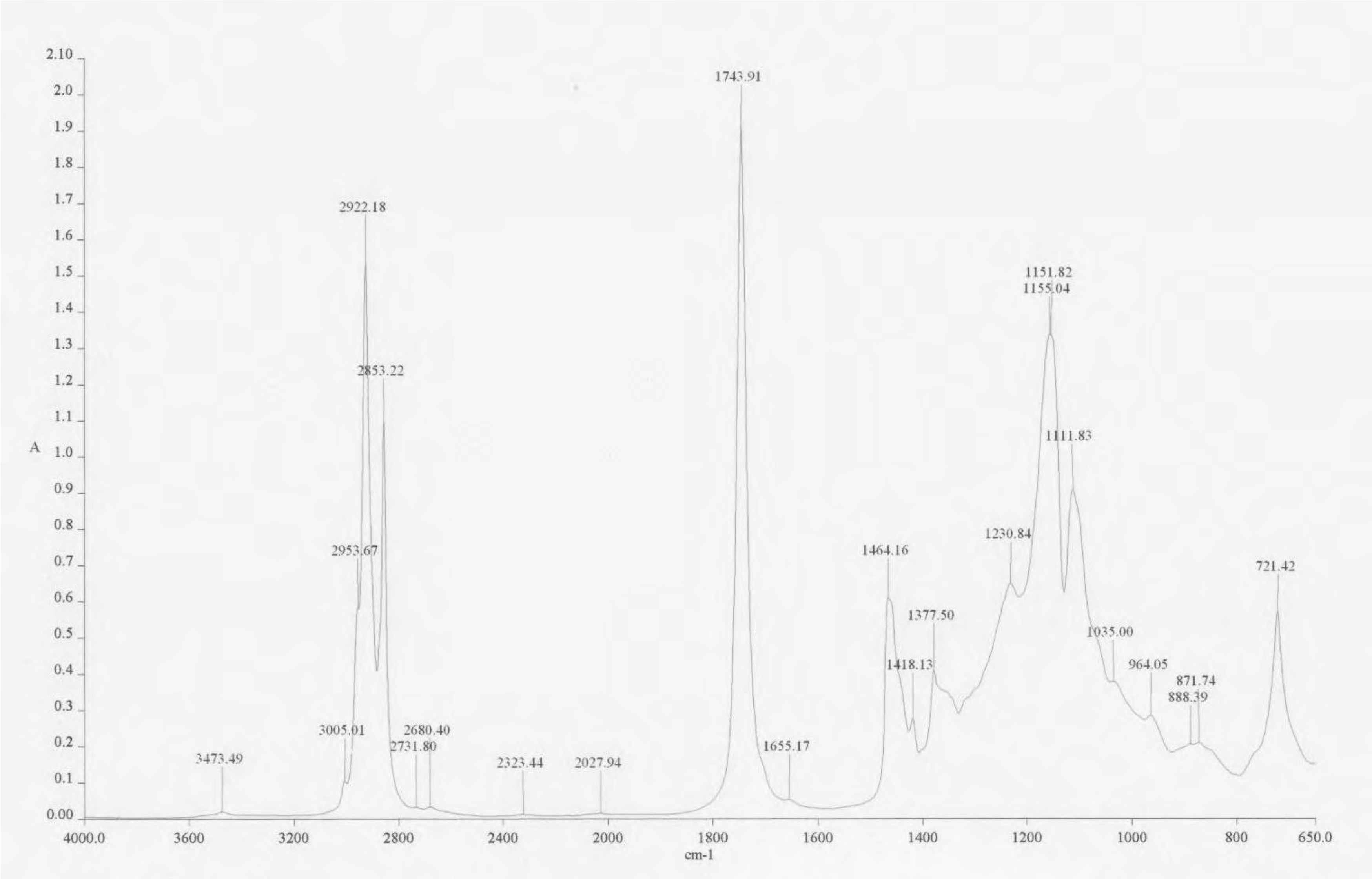
FTIR SPECTRUMS OF SNACKS



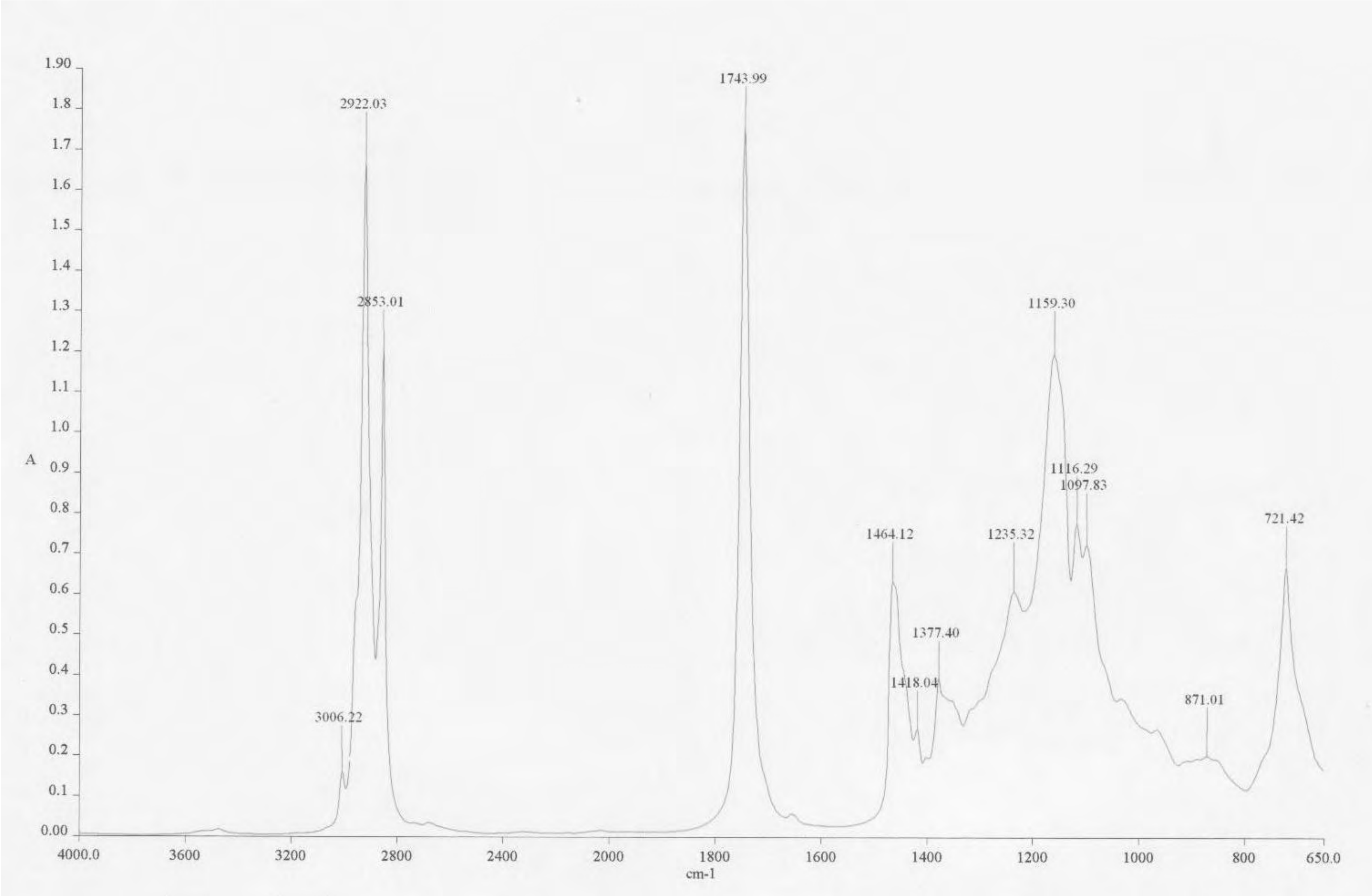
Cracker brand A



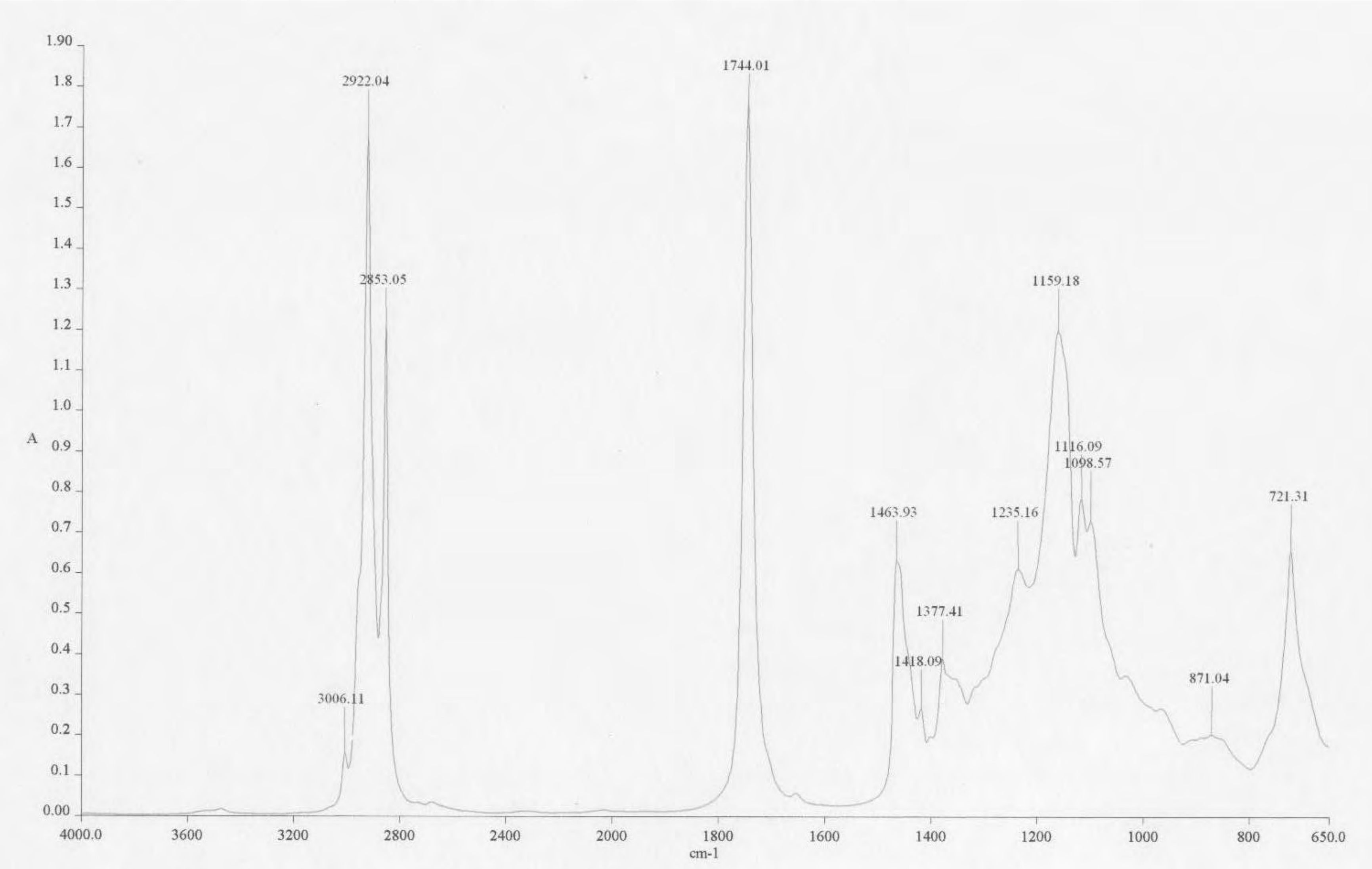
Cracker brand B



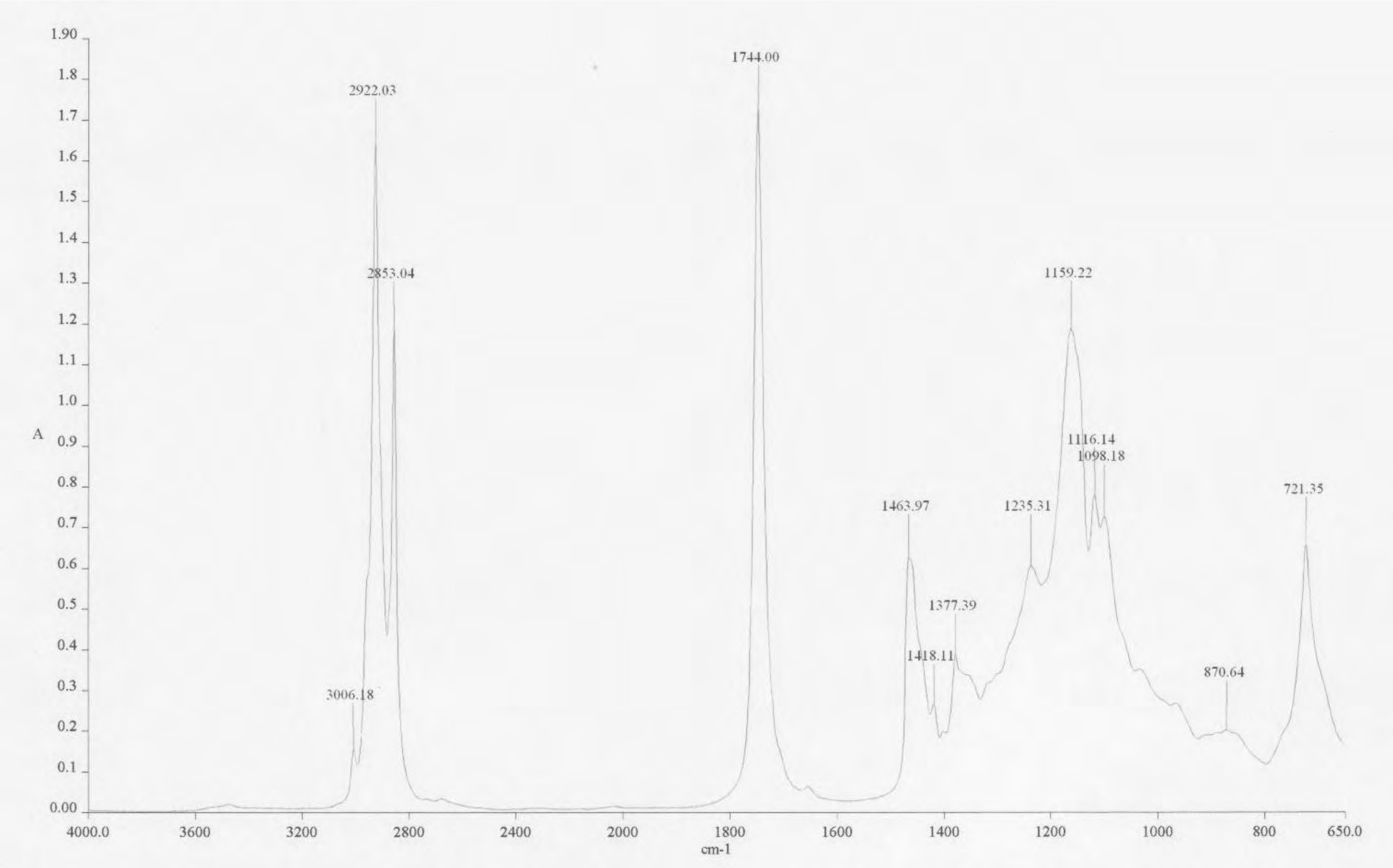
Cracker brand C



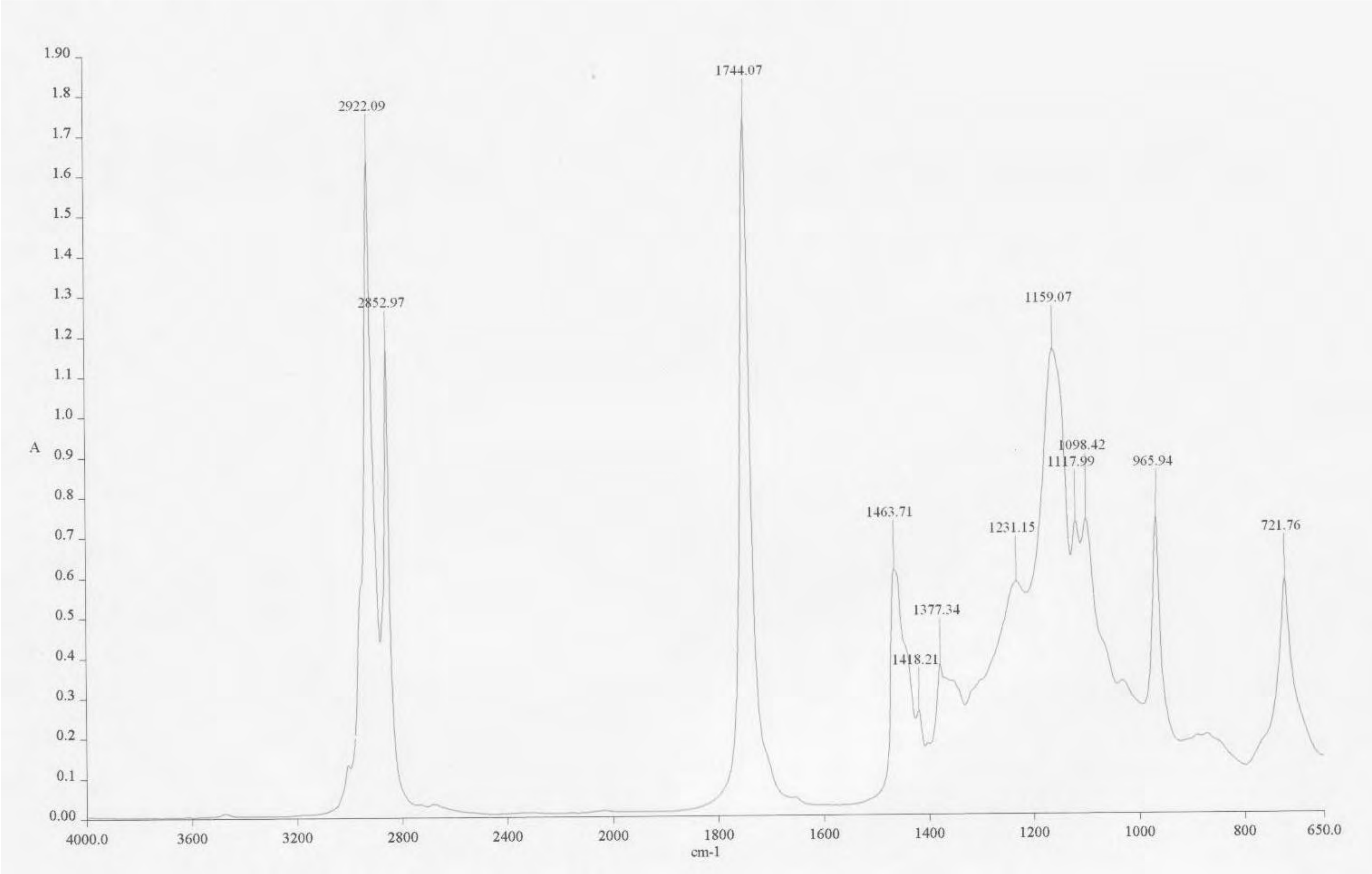
Stick biscuit brand A



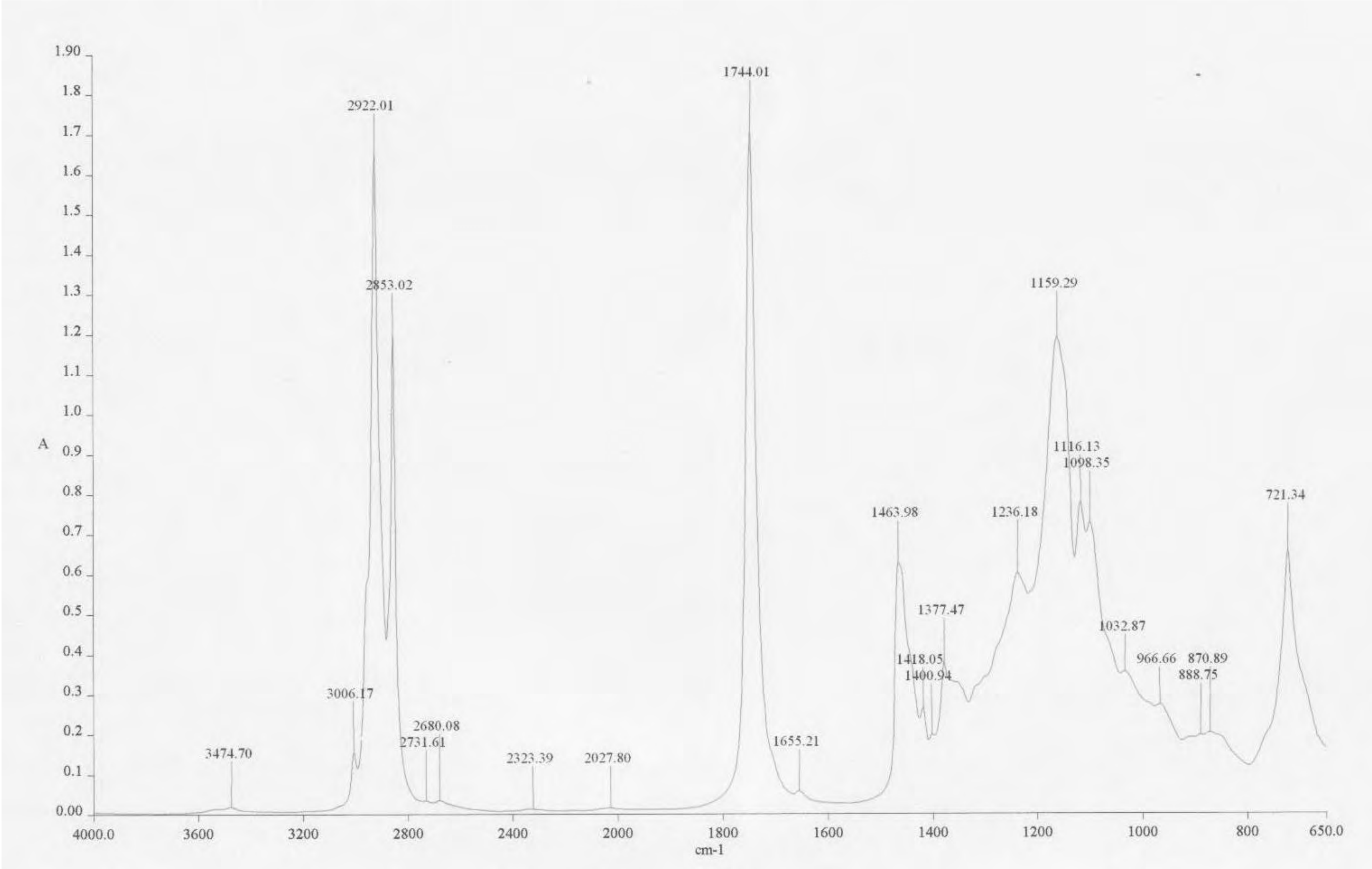
Stick biscuit brand B



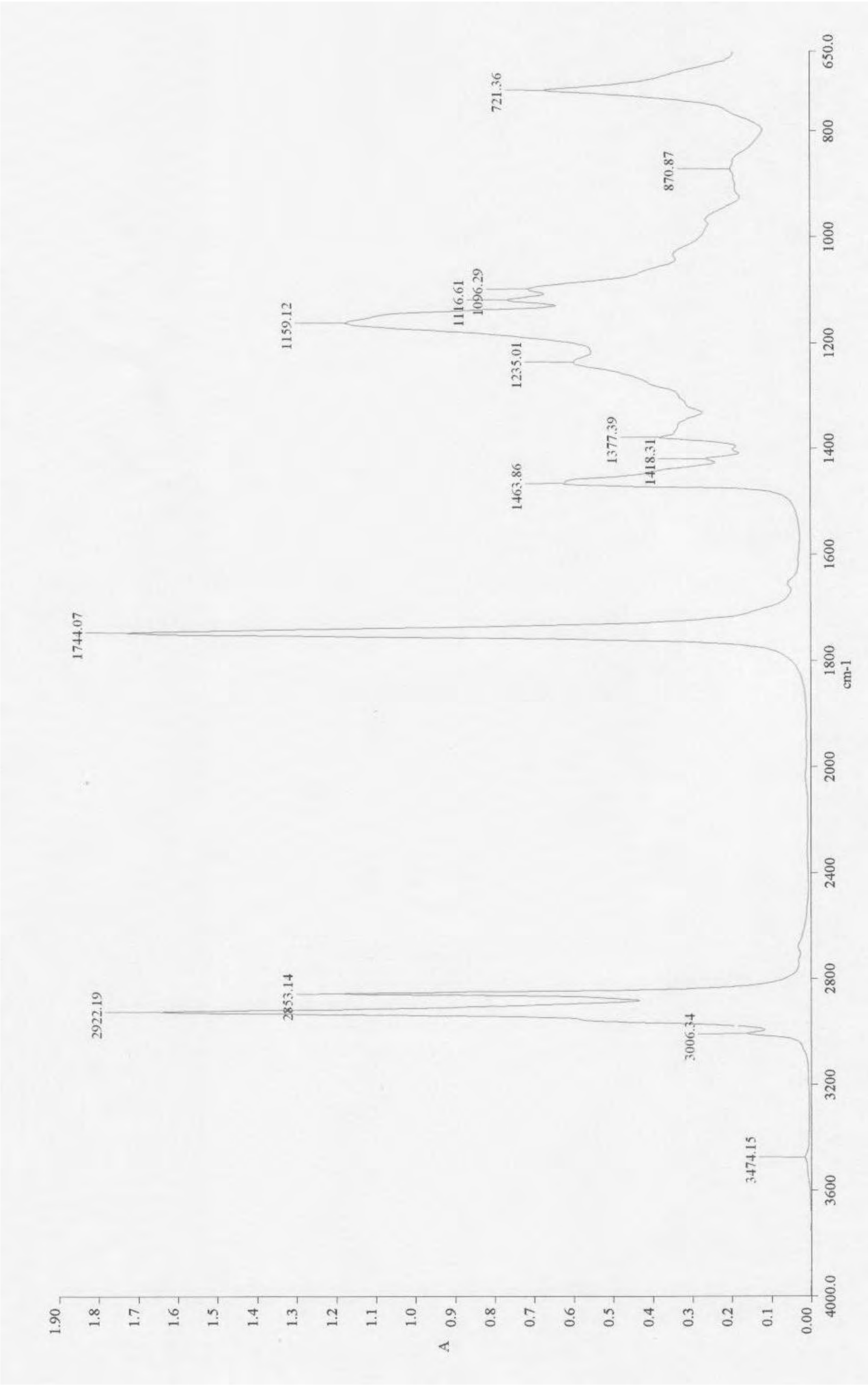
Stick biscuit brand C



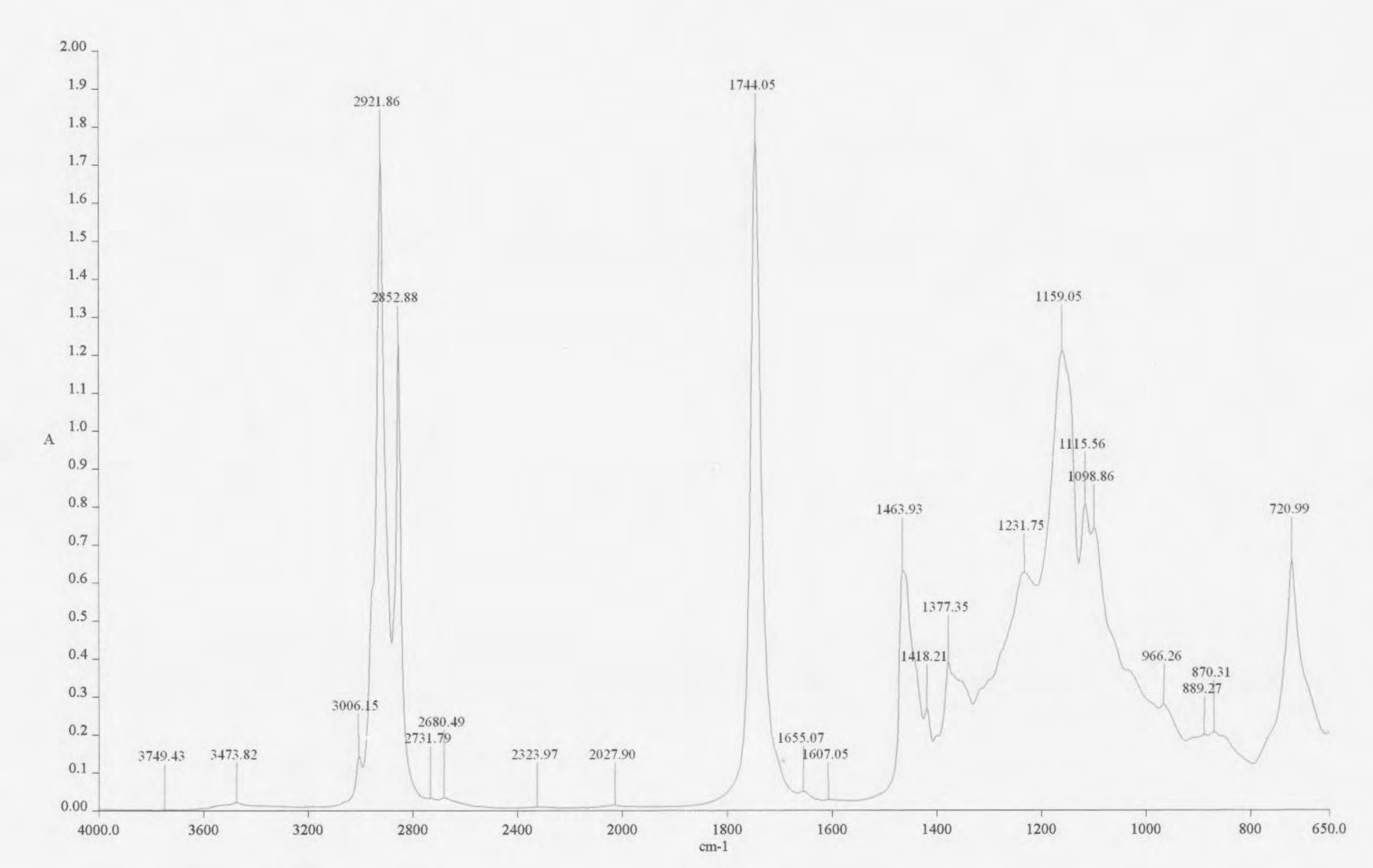
Potato chip brand A



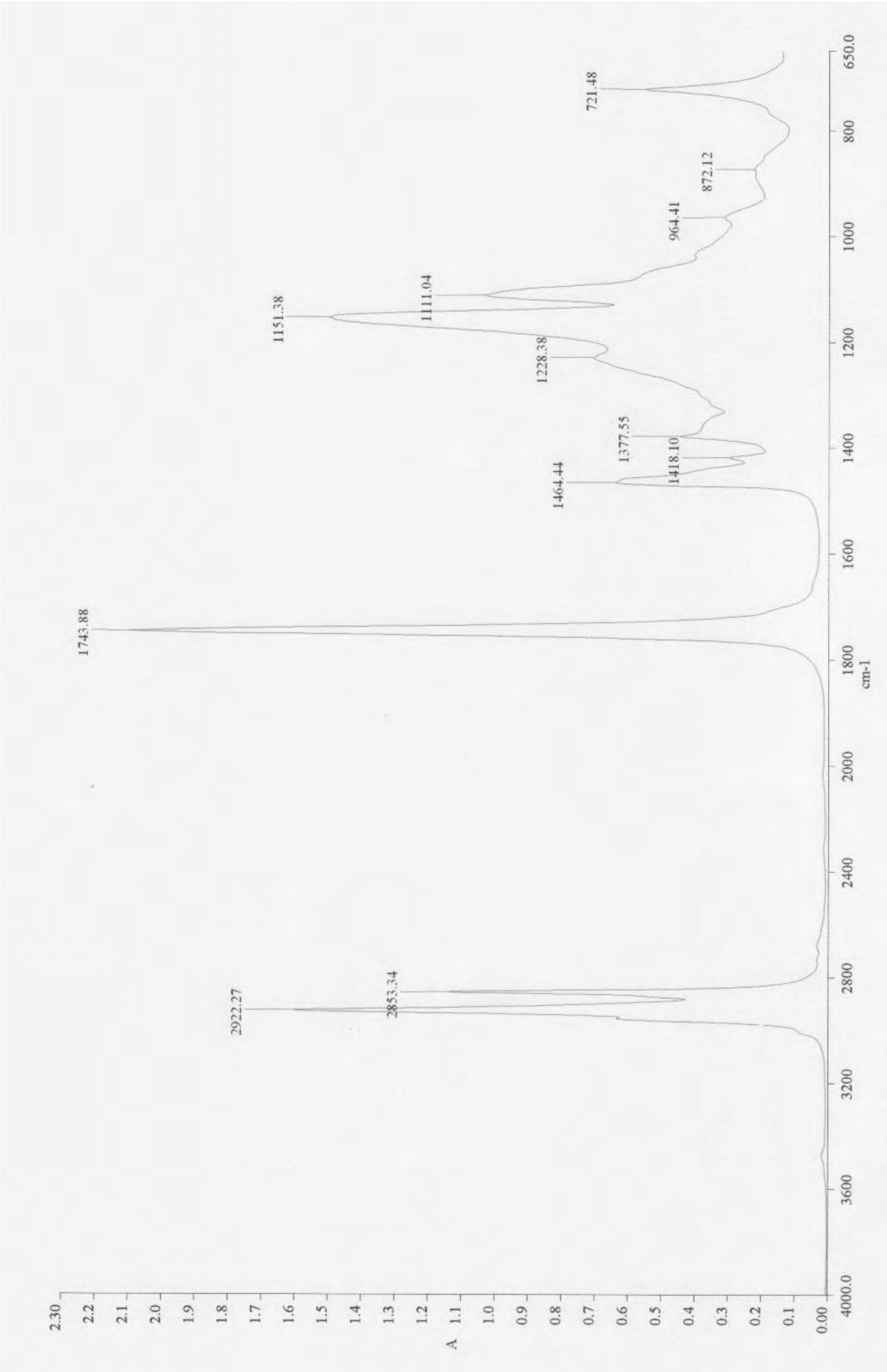
Potato chip brand B



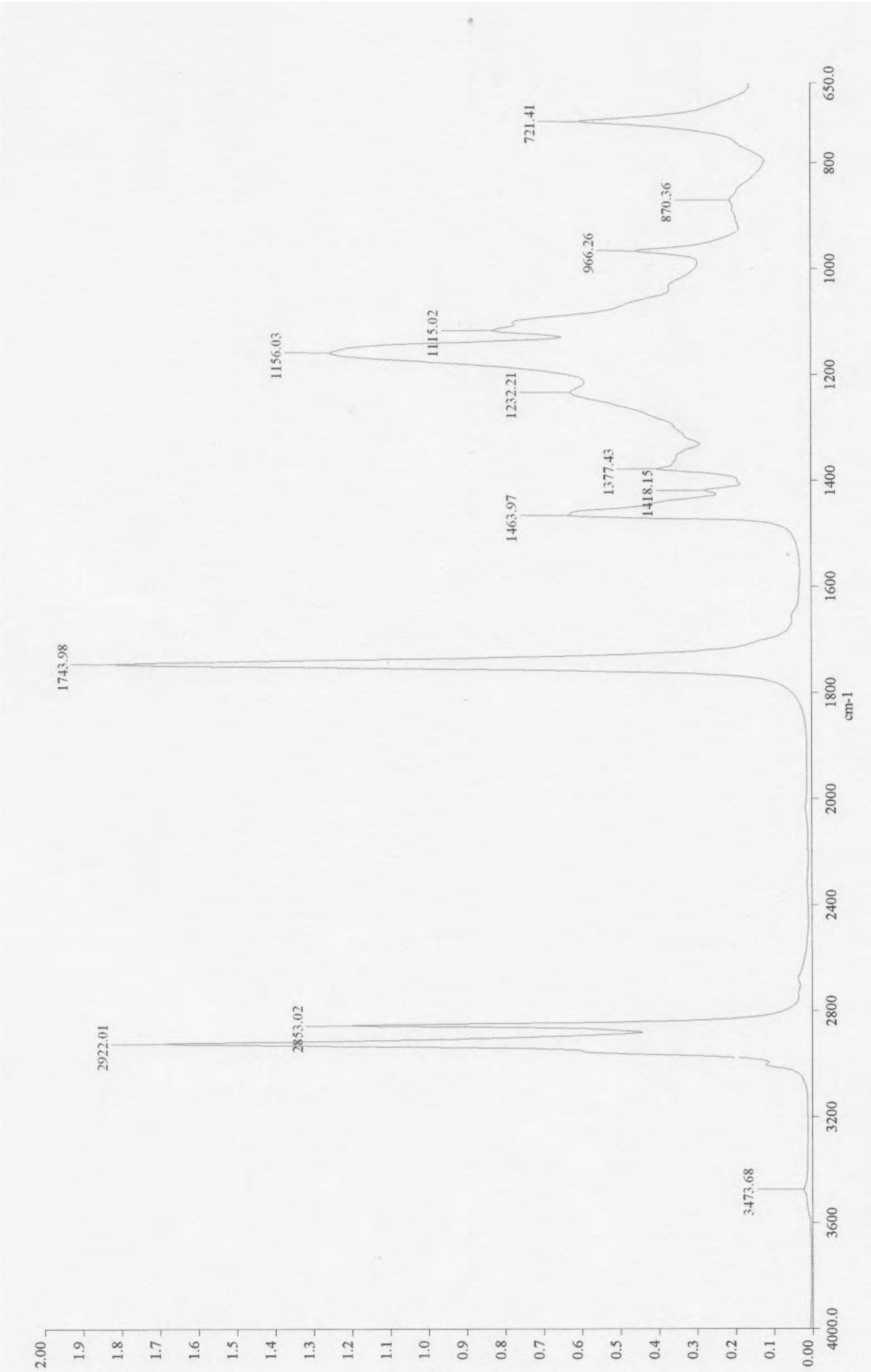
Potato chip brand C



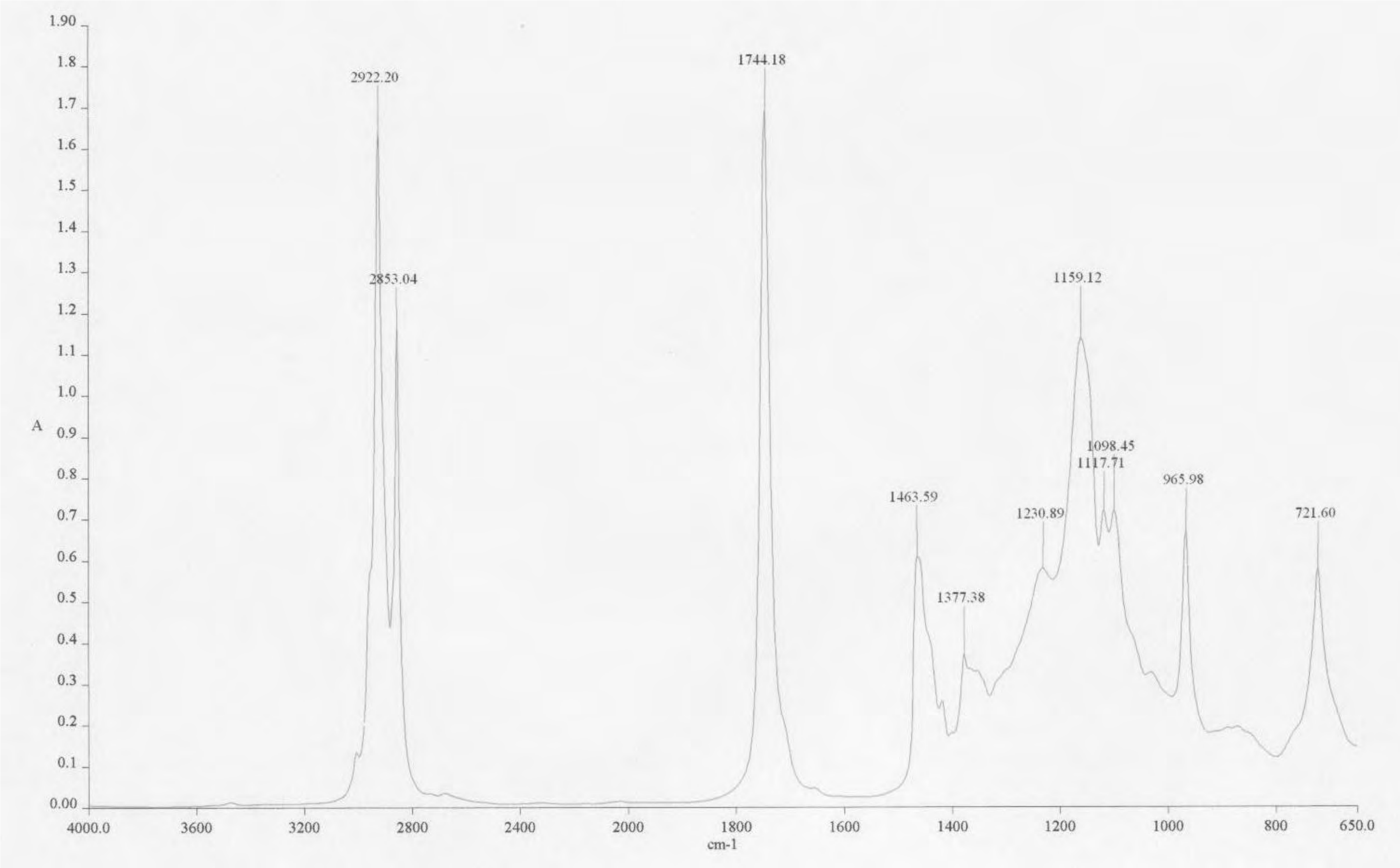
Biscuit brand A



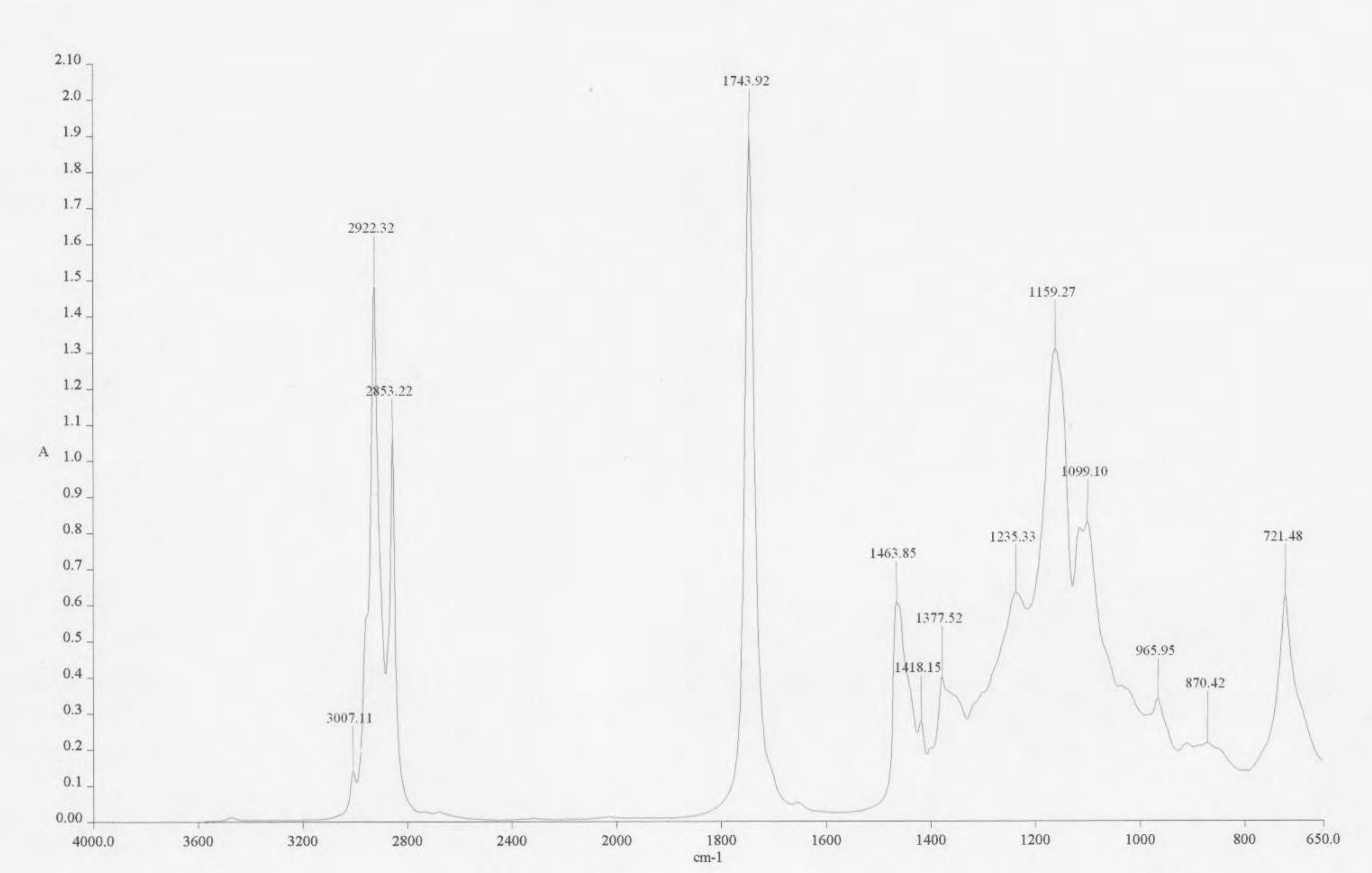
Biscuit brand B



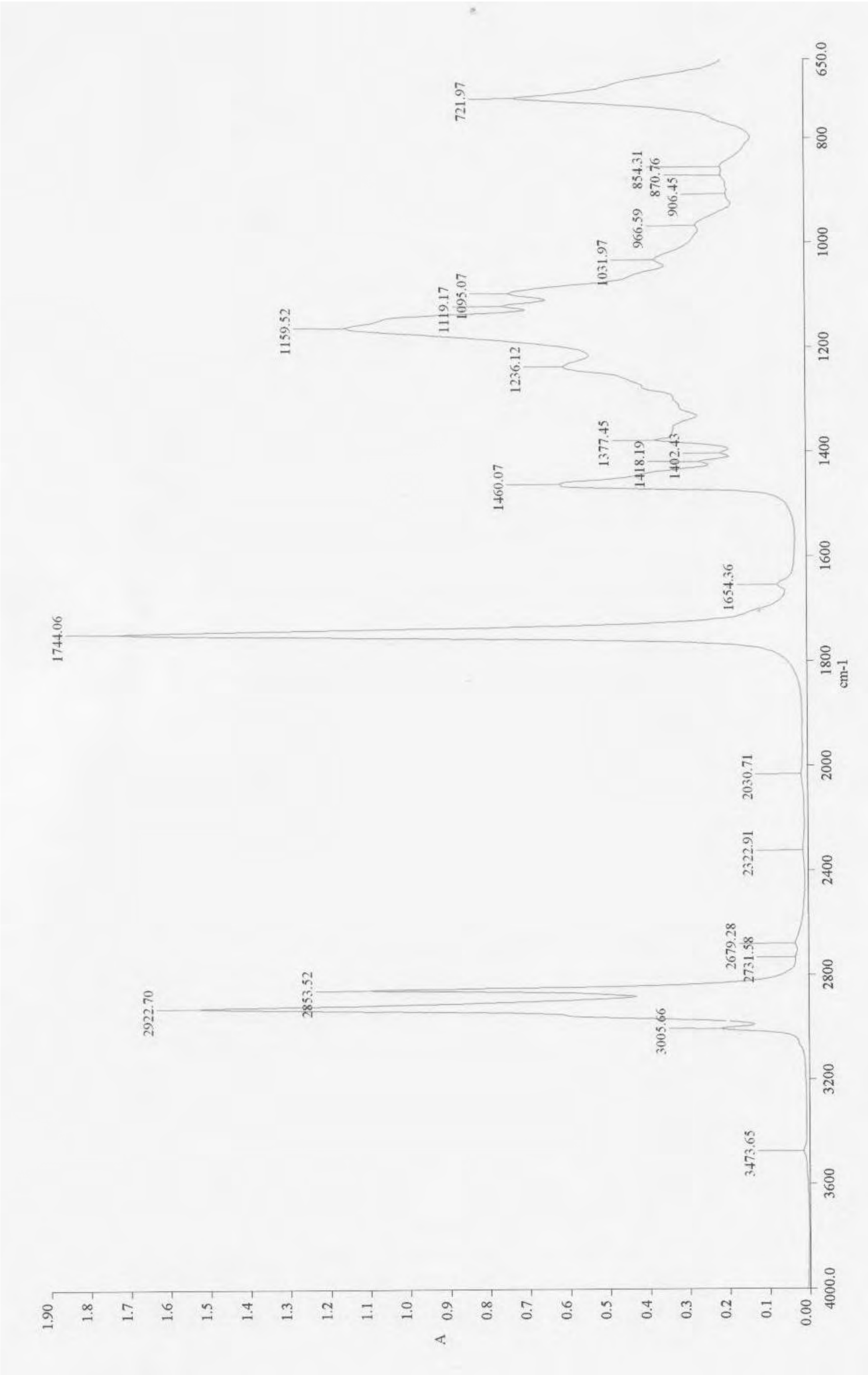
Biscuit brand C



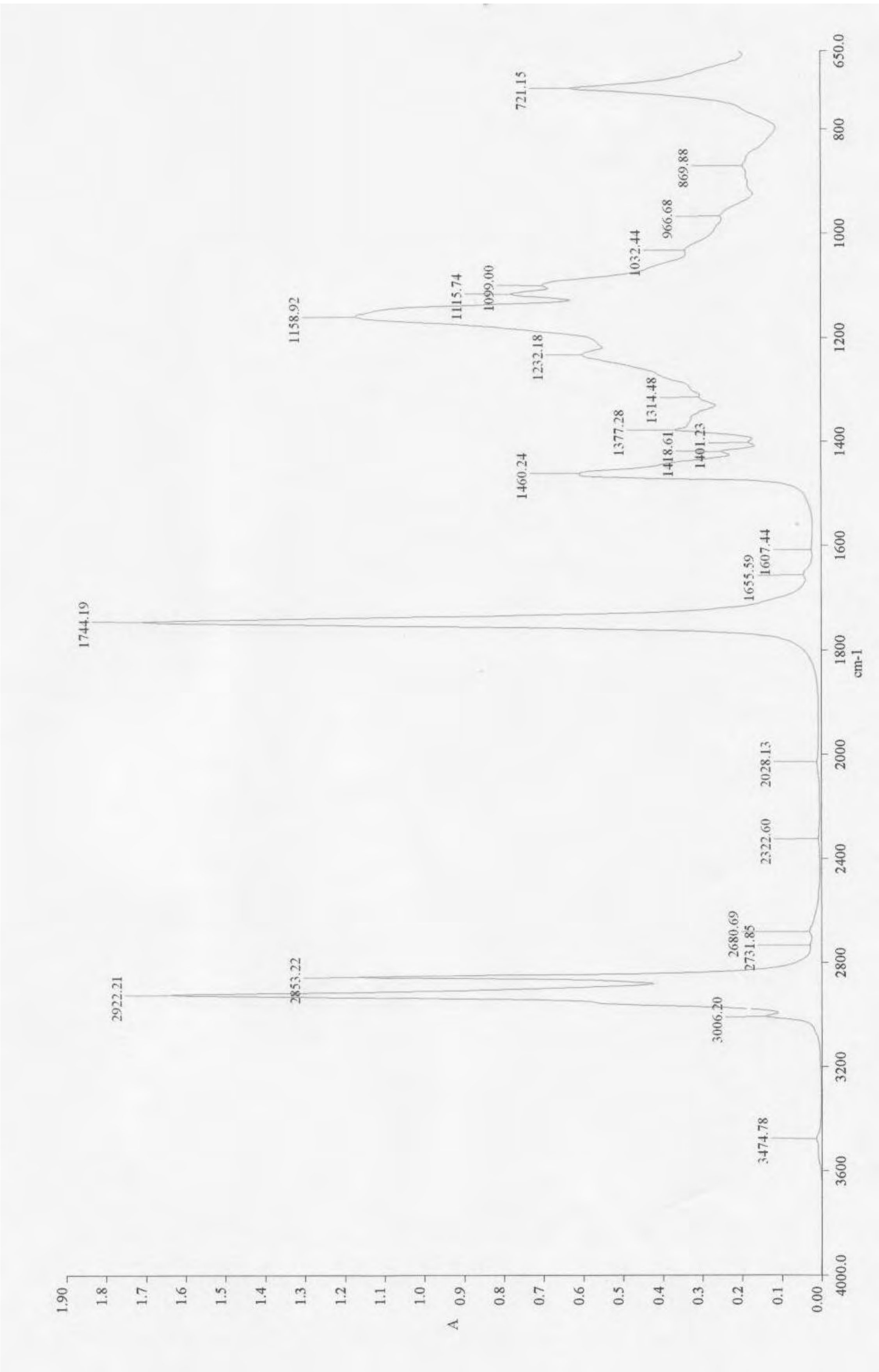
Microwave popcorn brand A



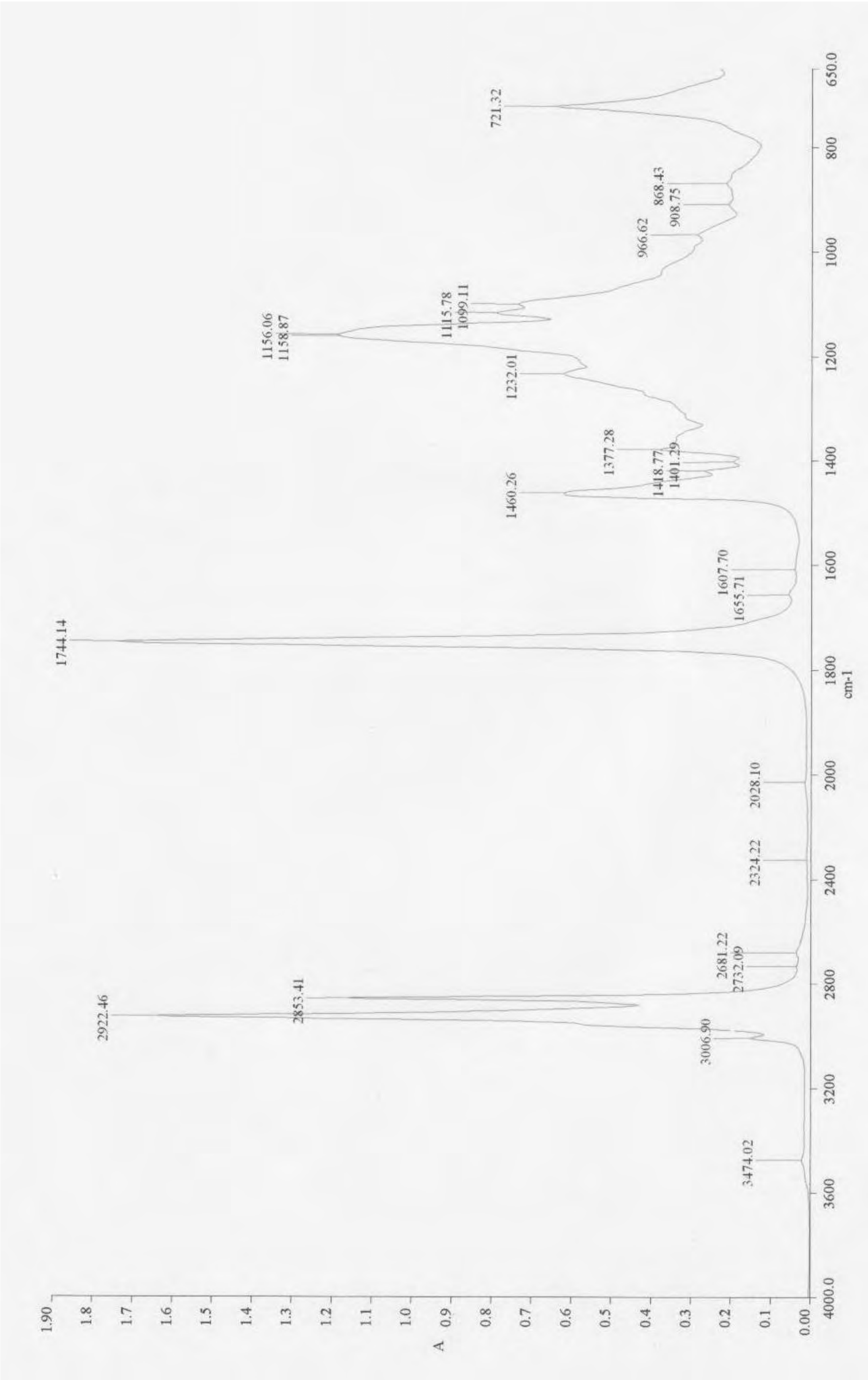
Microwave popcorn brand B



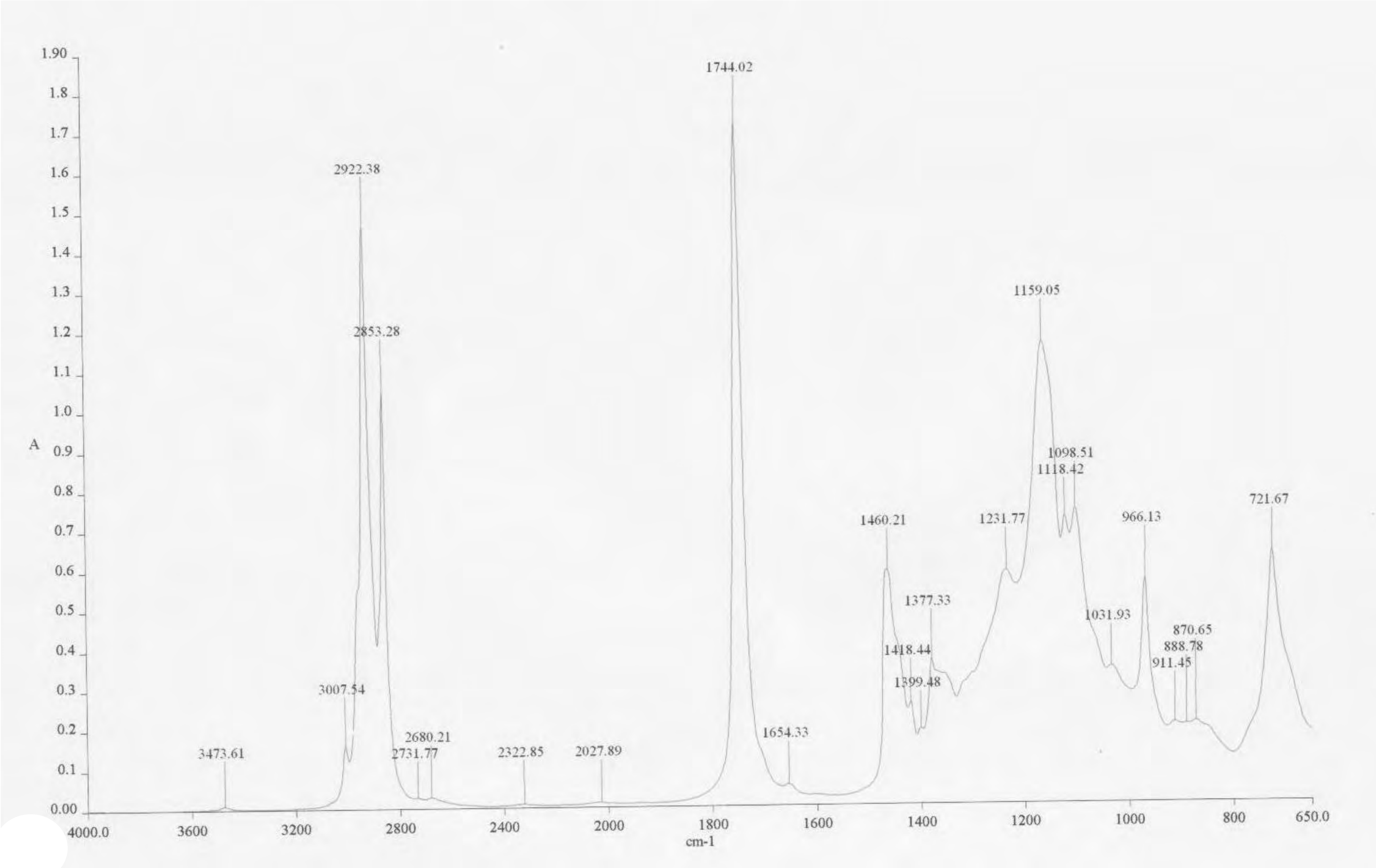
Microwave popcorn brand C



Wafer brand A



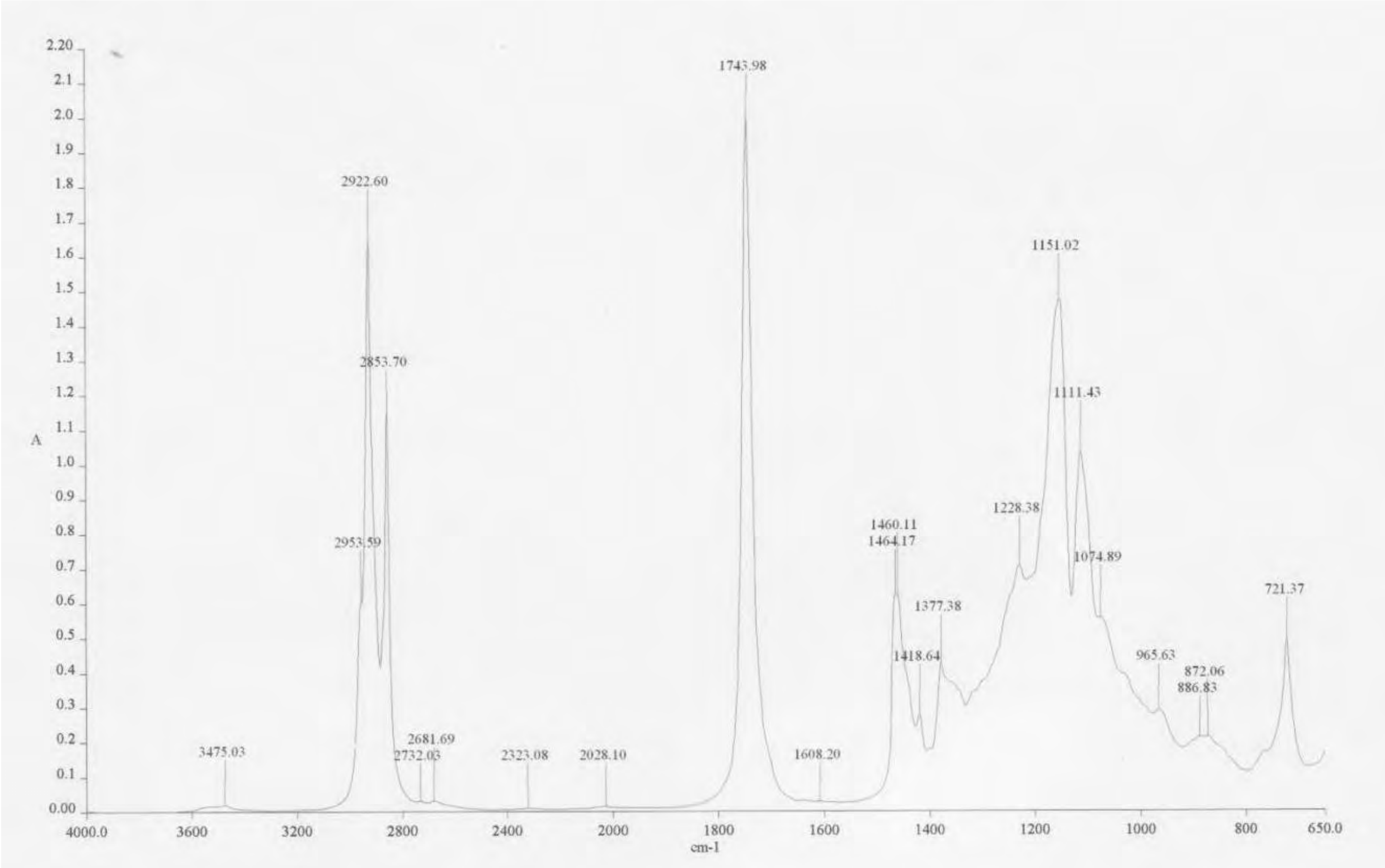
Wafer brand B



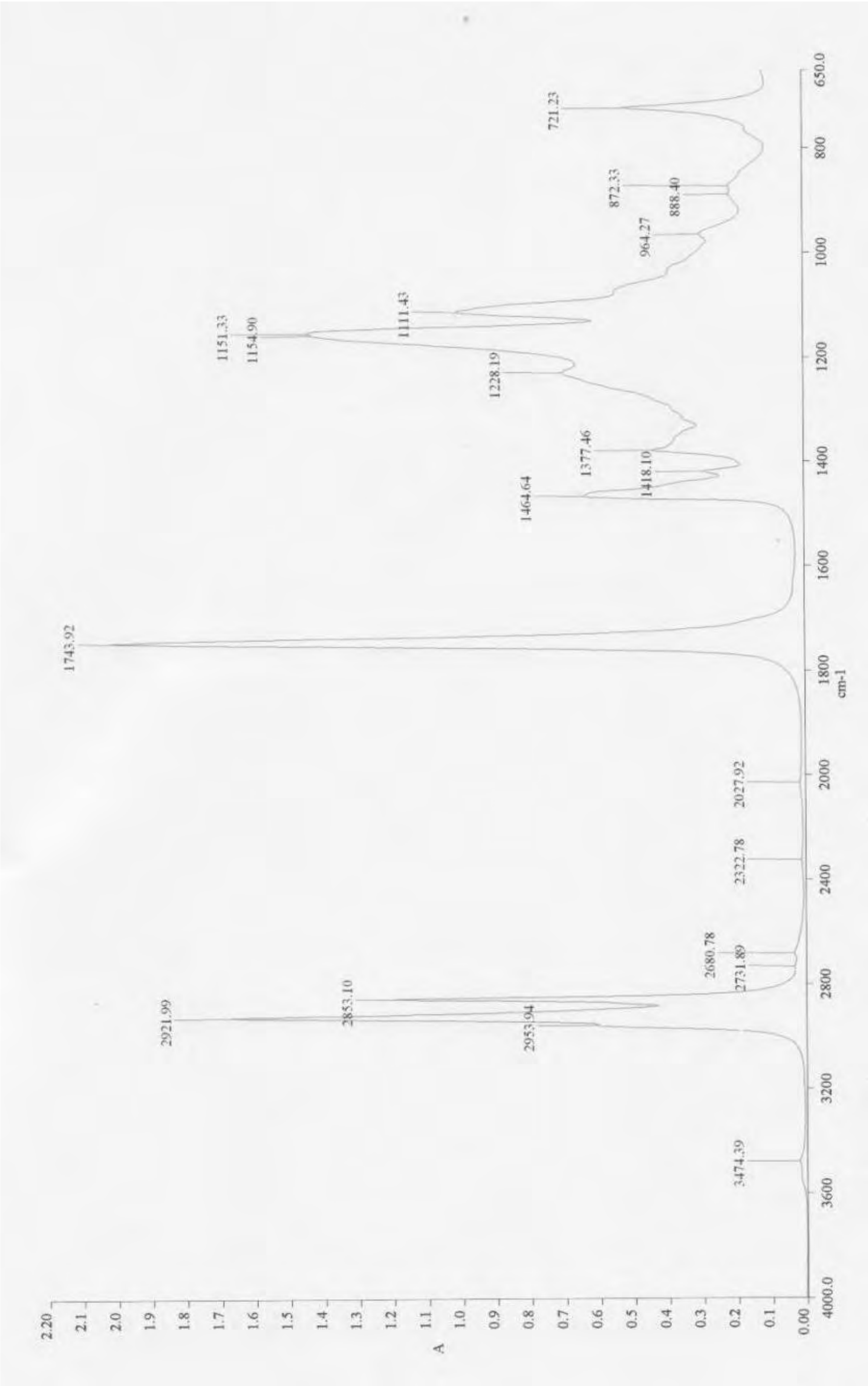
Wafer brand C

APPENDIX D

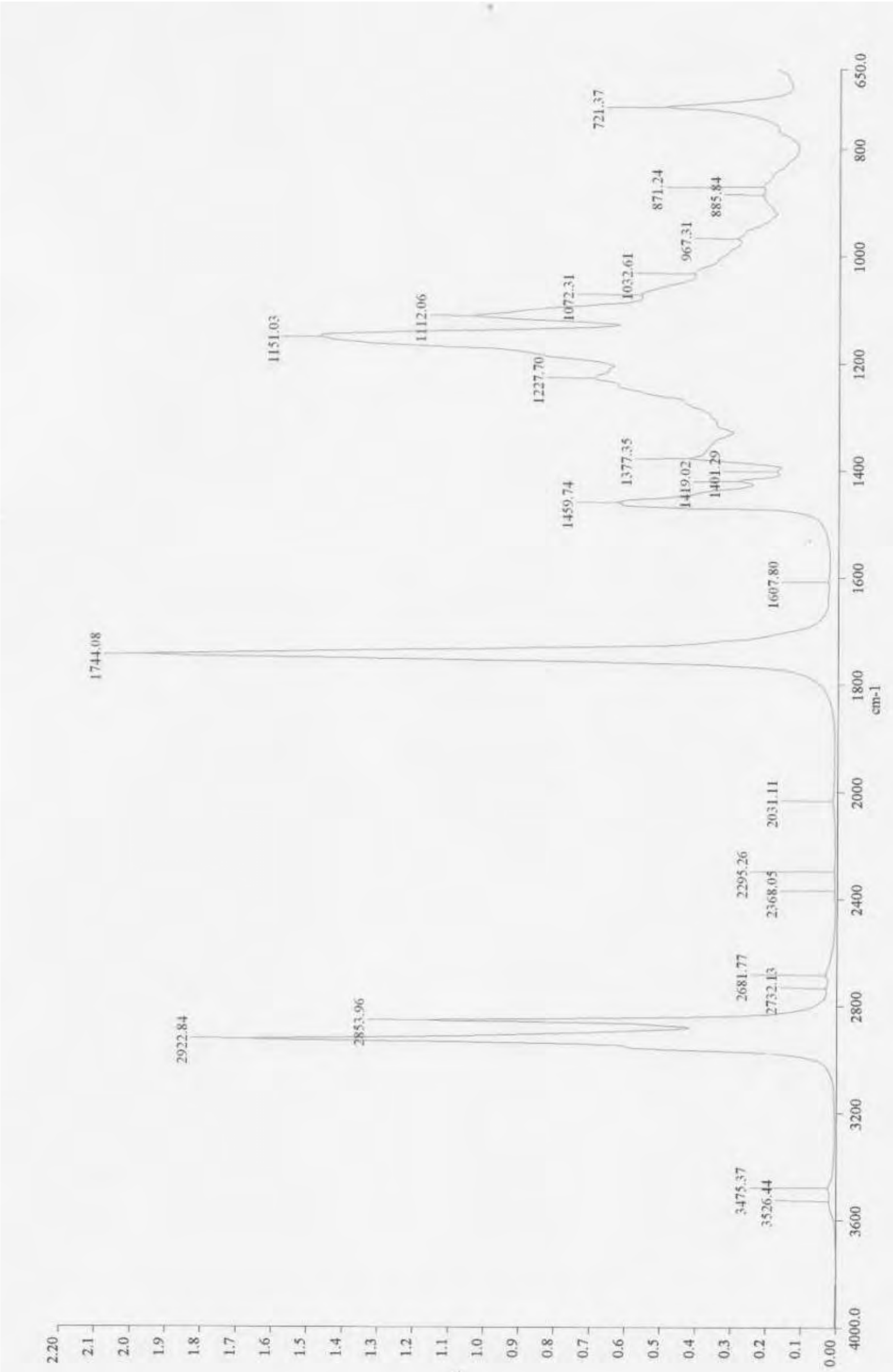
FTIR SPECTRUMS OF NON-DAIRY CREAMERS



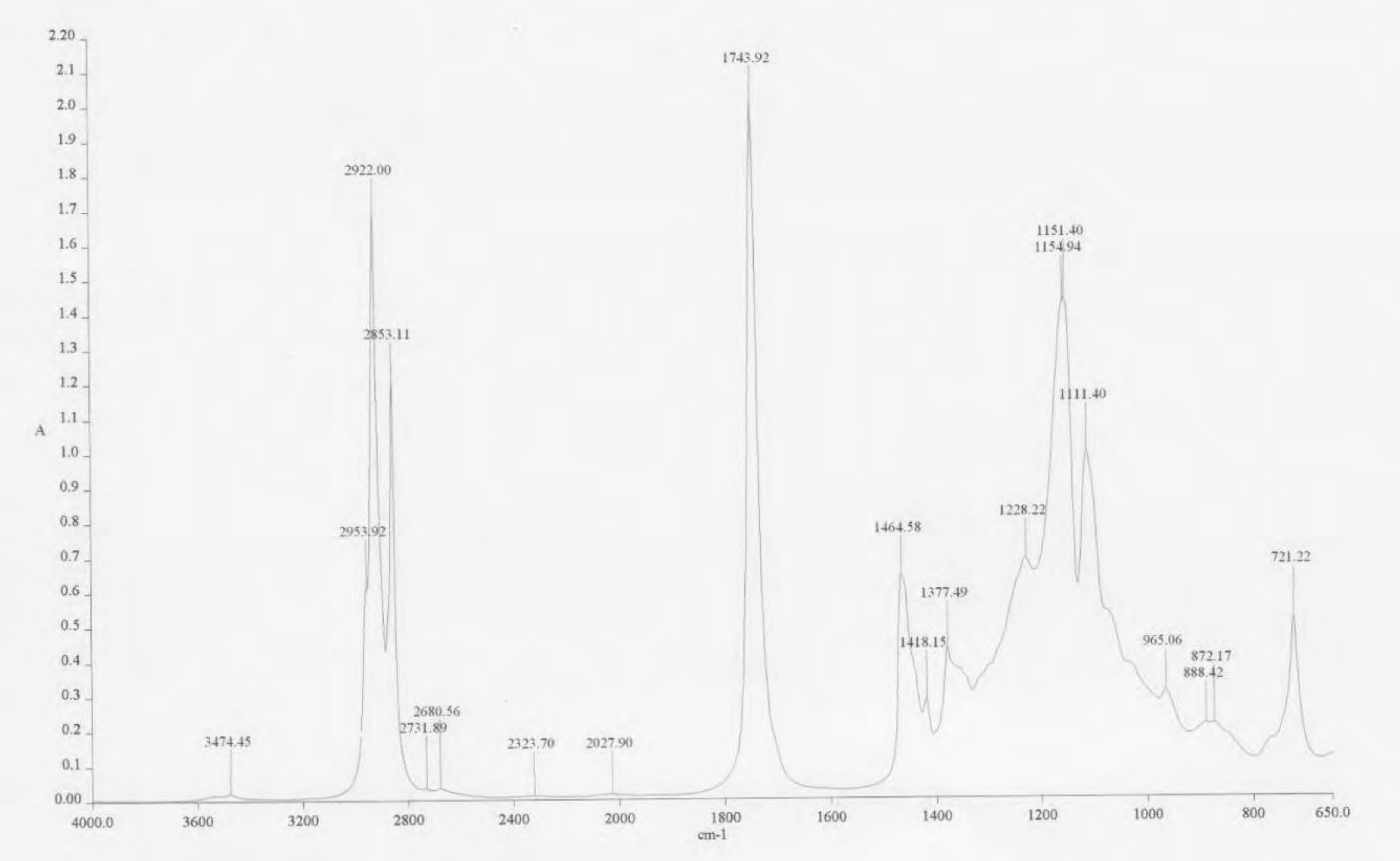
Non-dairy creamer brand A



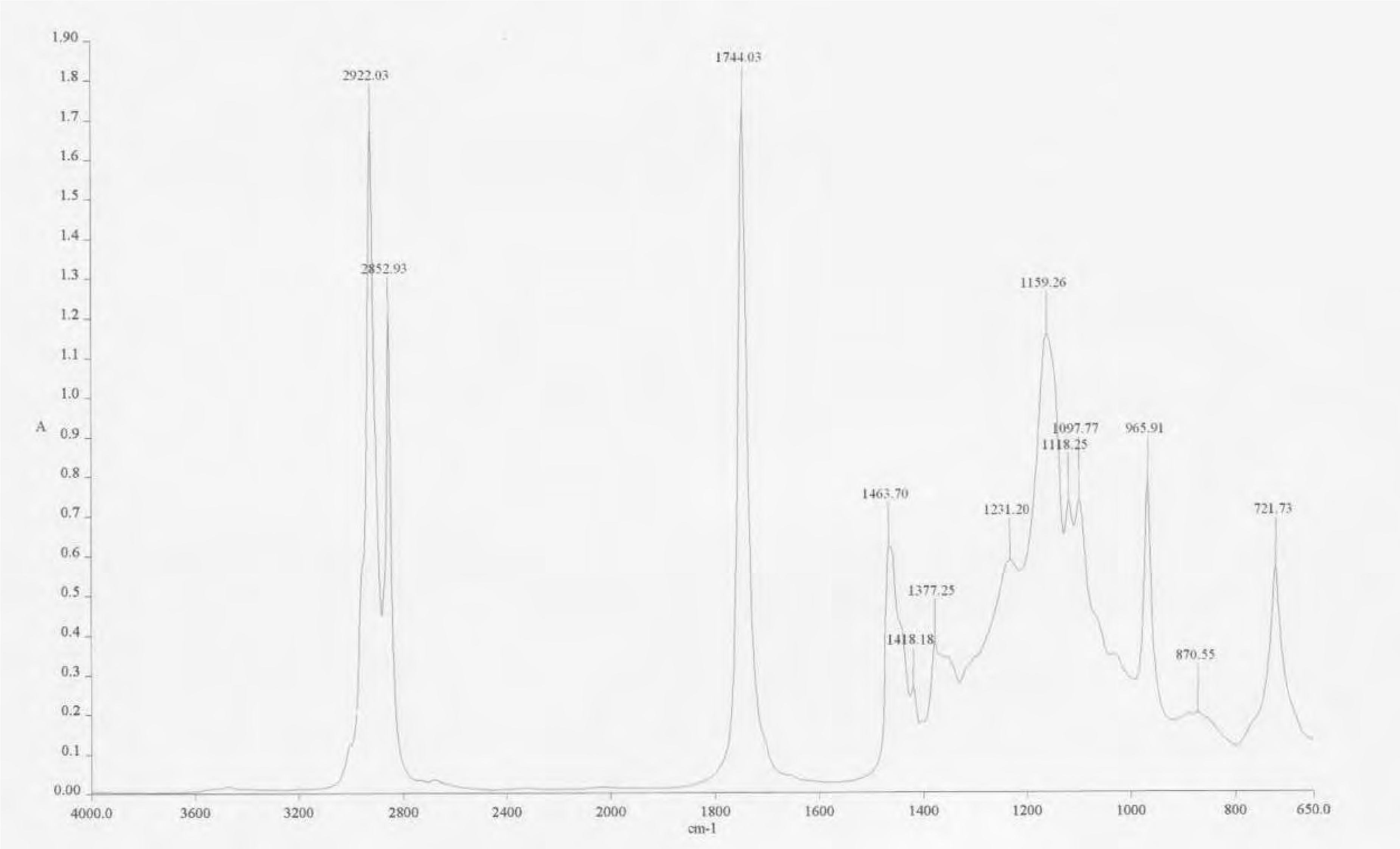
Non-dairy creamer brand B



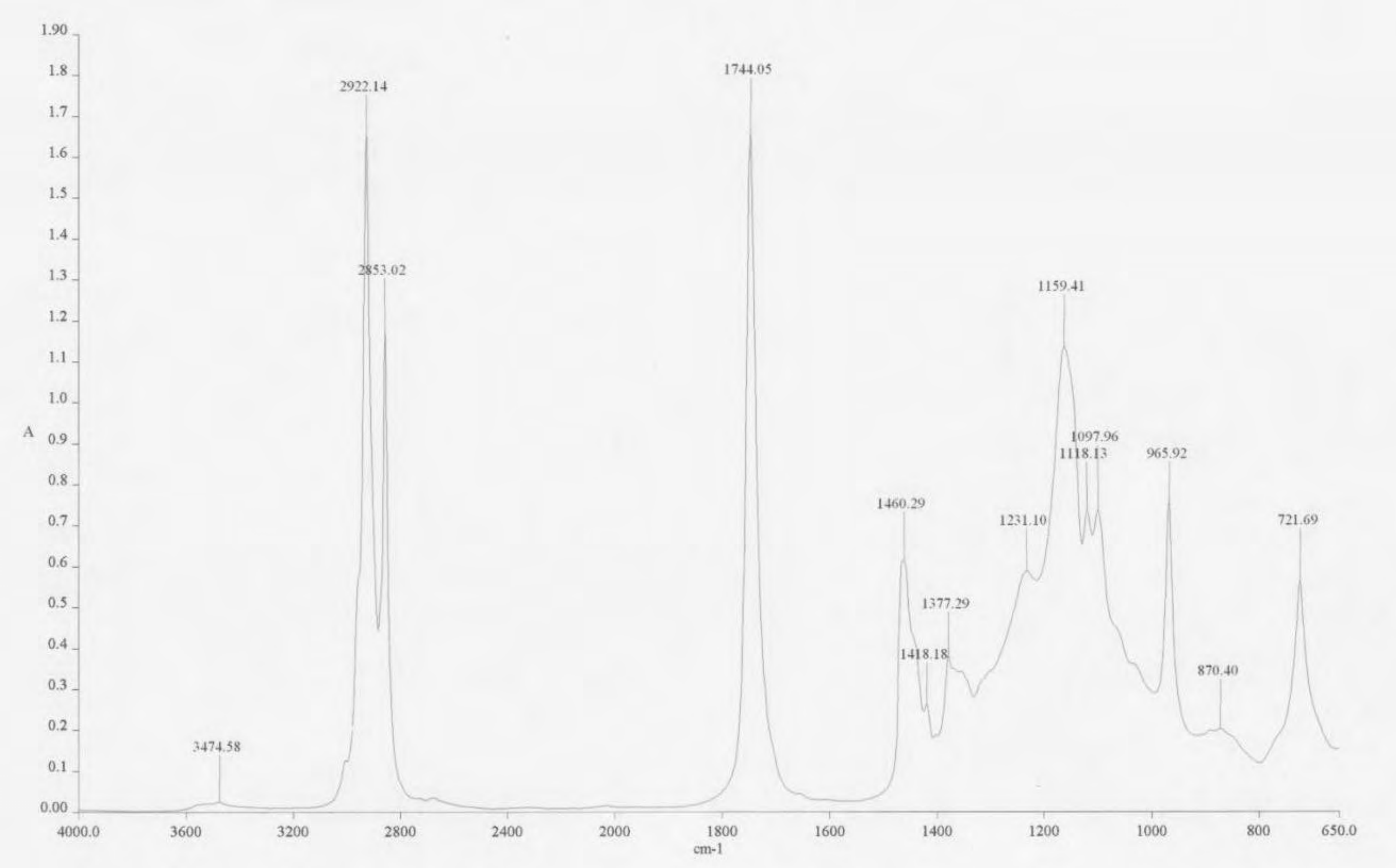
Non-dairy creamer brand C



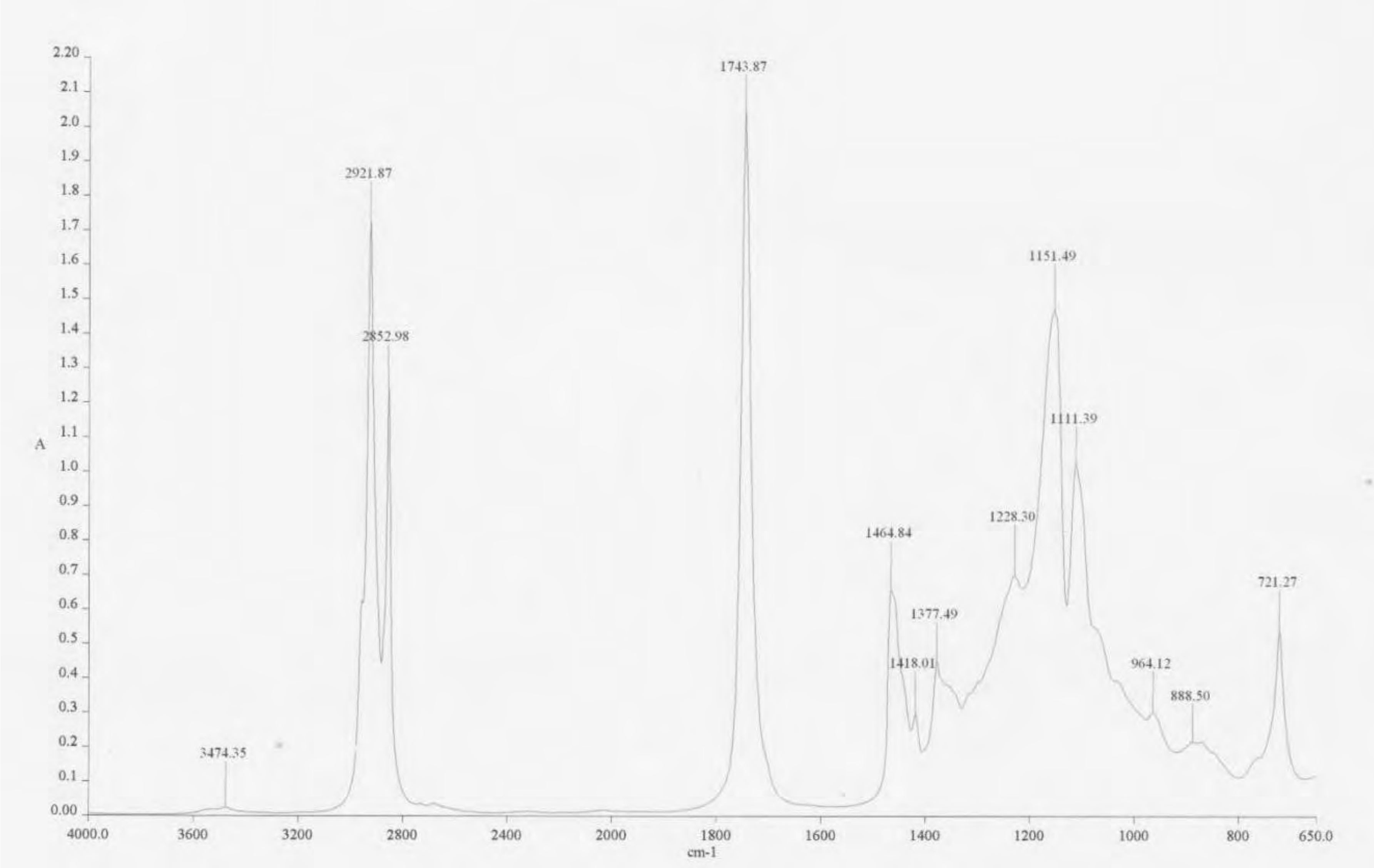
Non-dairy creamer brand D



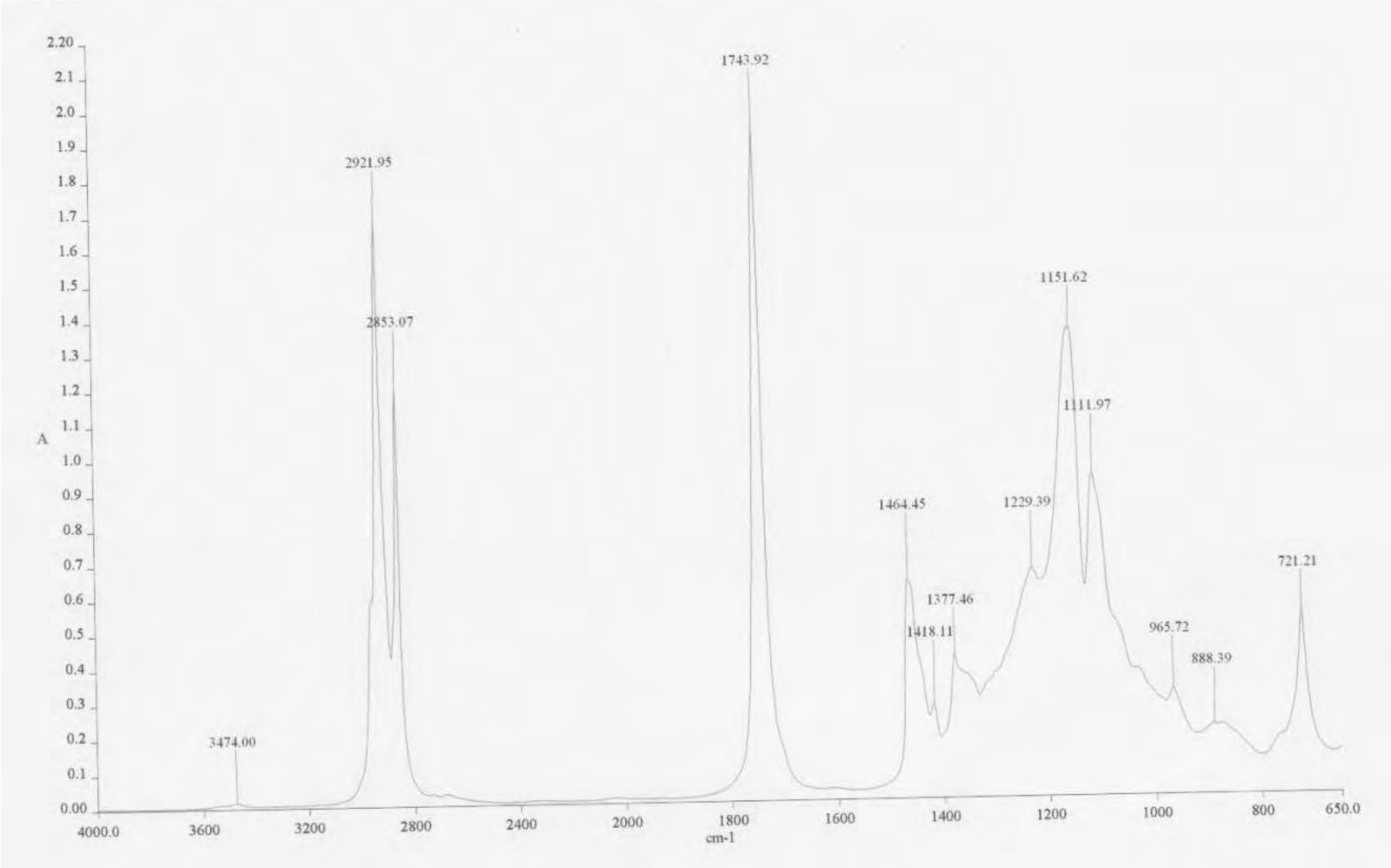
Non-dairy creamer brand E



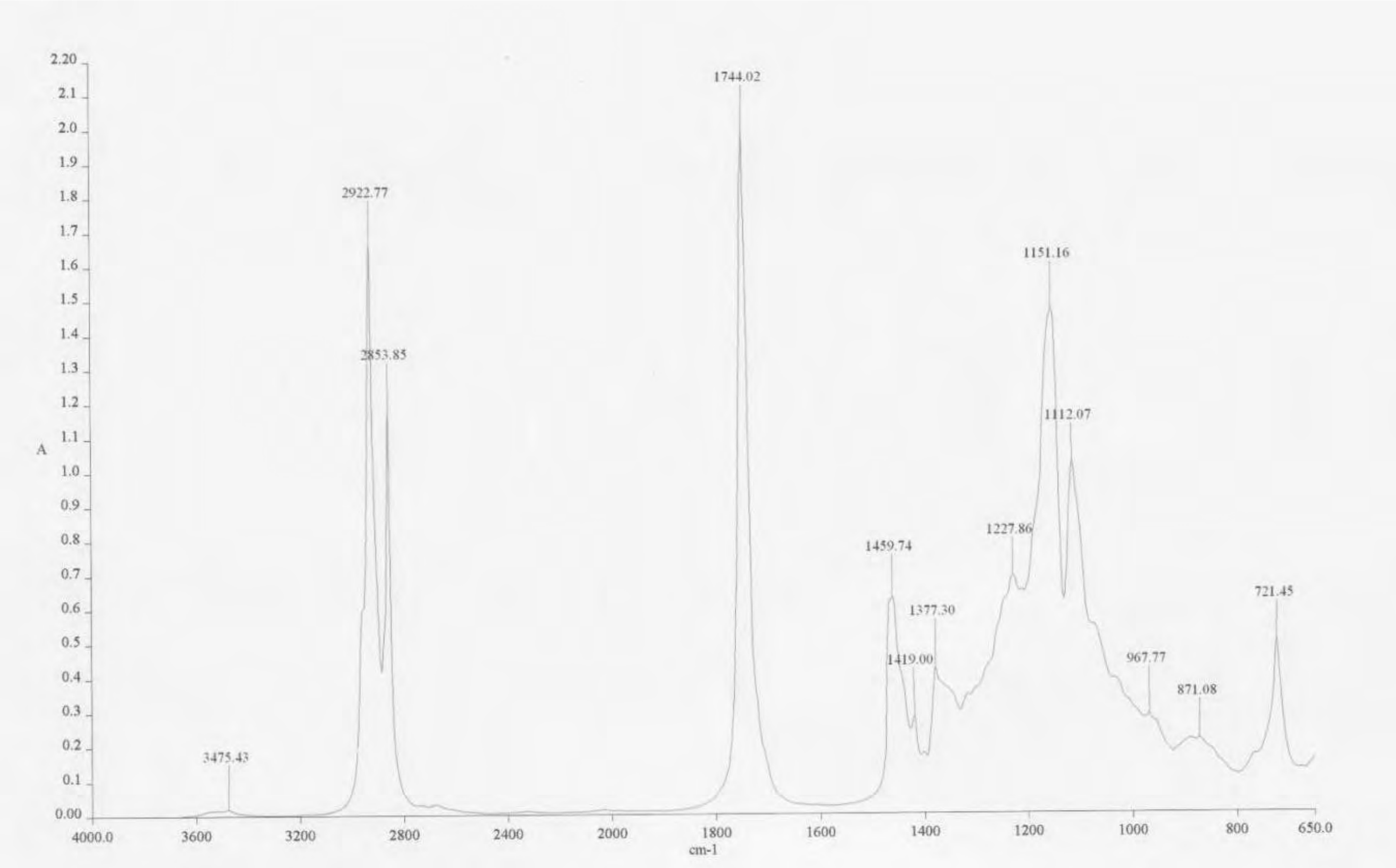
Non-dairy creamer brand F



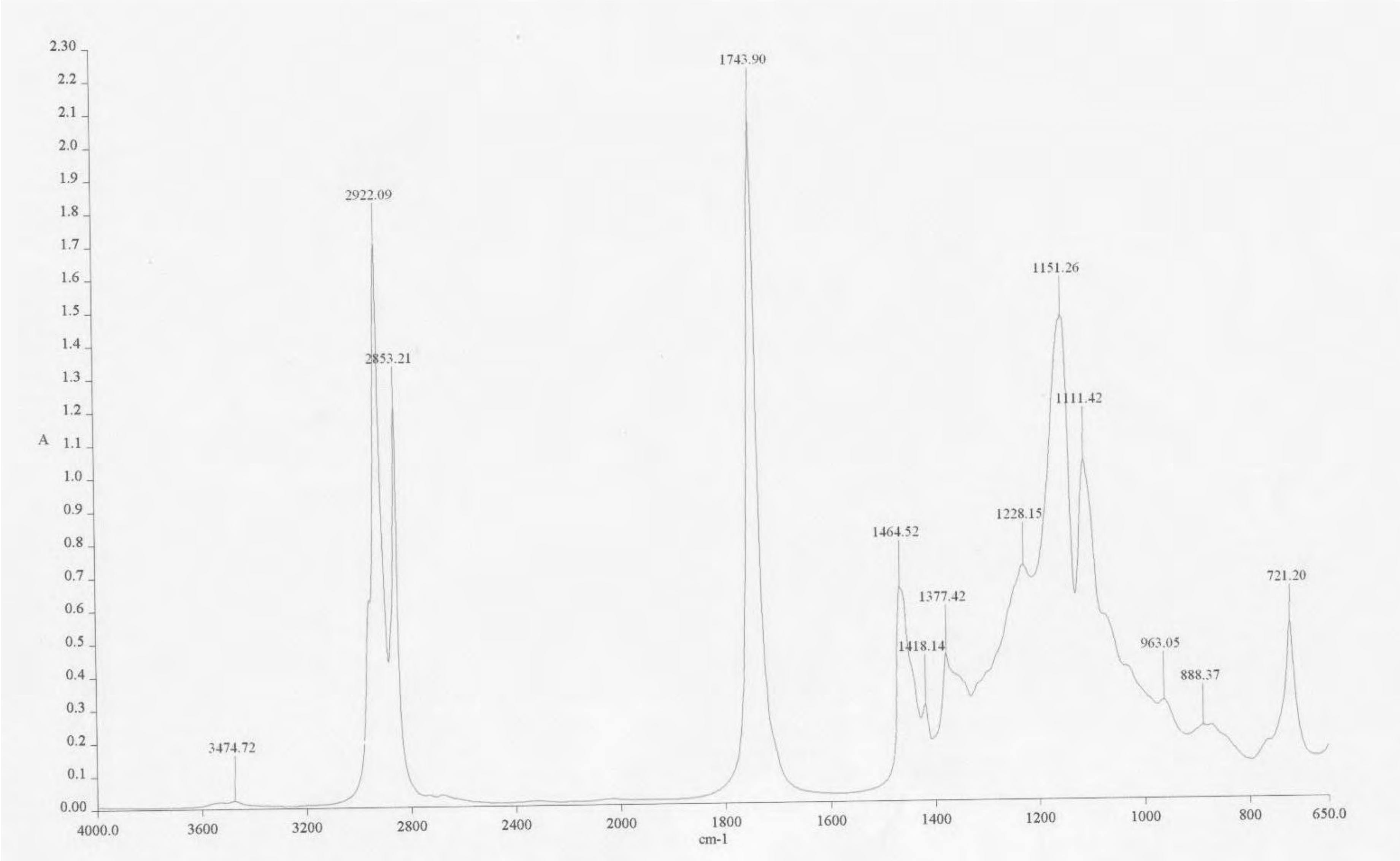
Non-dairy creamer brand G



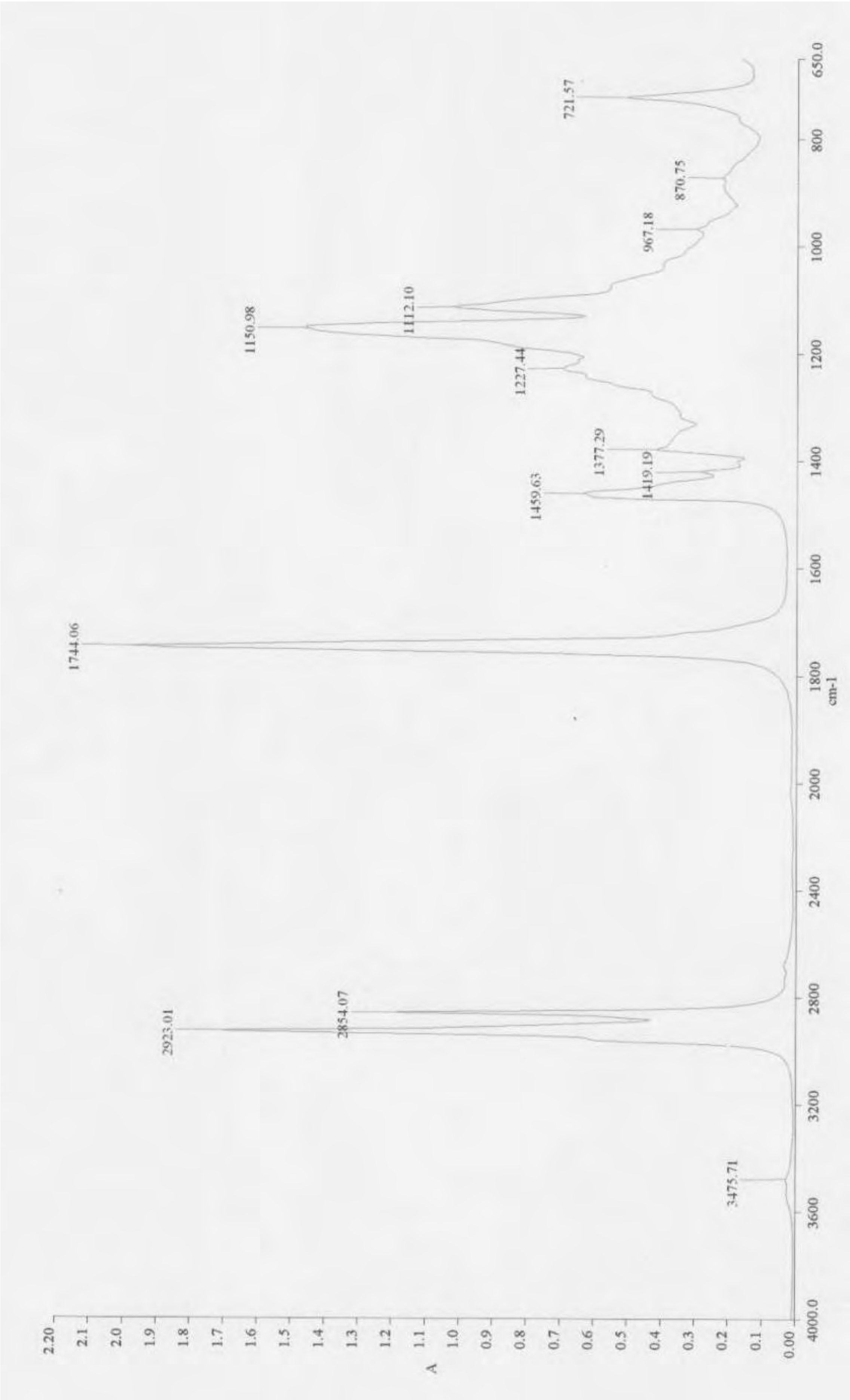
Non-dairy creamer brand H



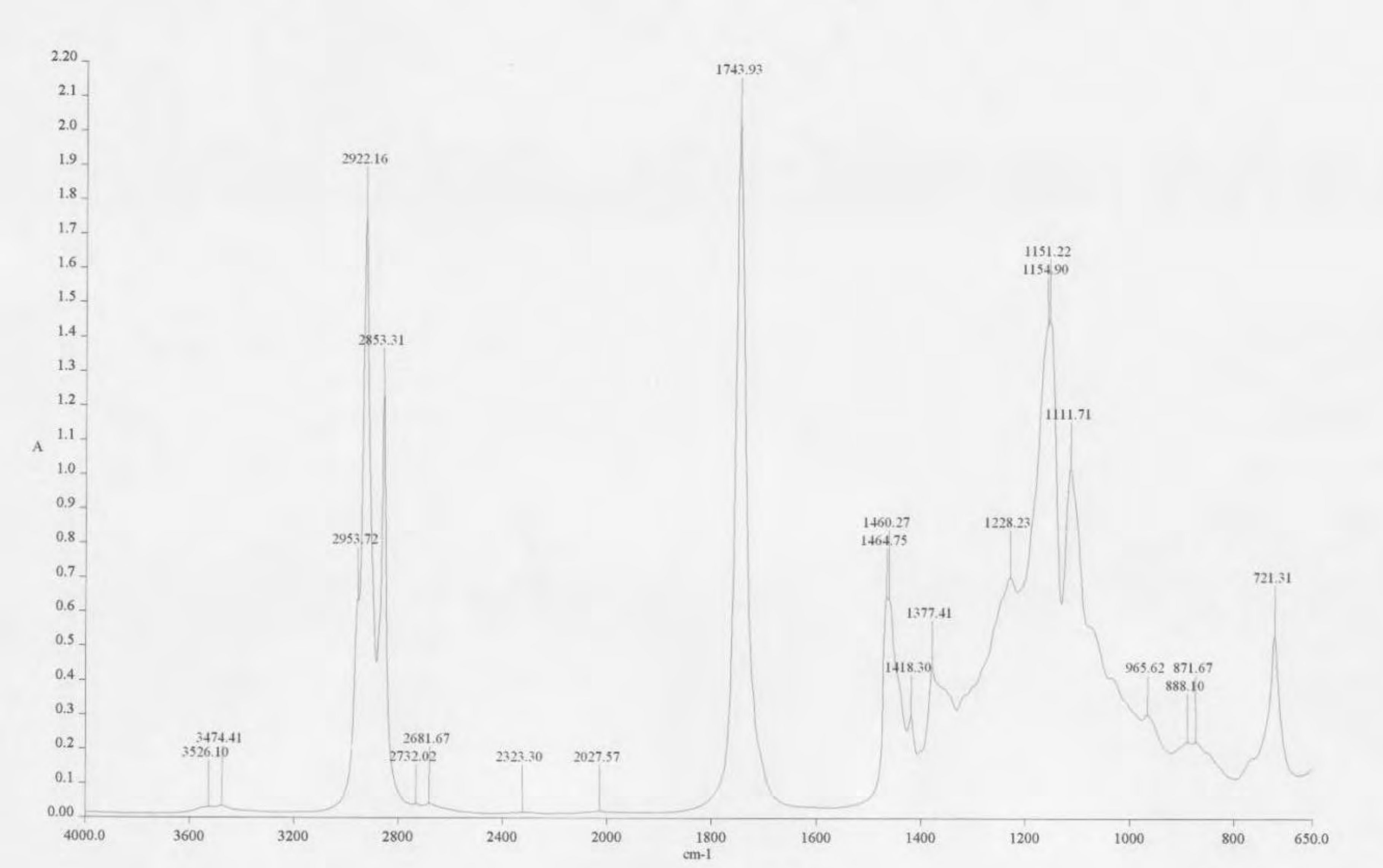
Non-dairy creamer brand I



Non-dairy creamer brand J



Non-dairy creamer brand K



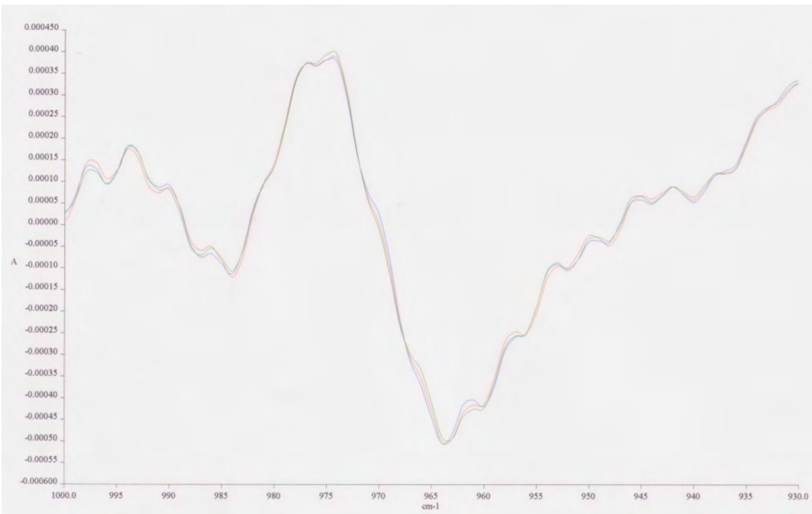
Non-dairy creamer brand L

APPENDIX E

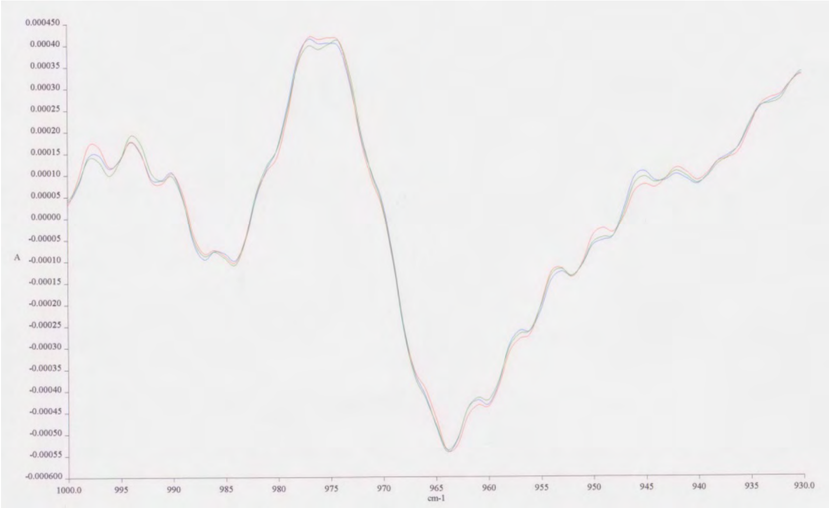
THE NEGATIVE SECOND DERIVATIVE OF SNACKS

Cracker brand A (beaker 1)

Cracker brand A (beaker 2)

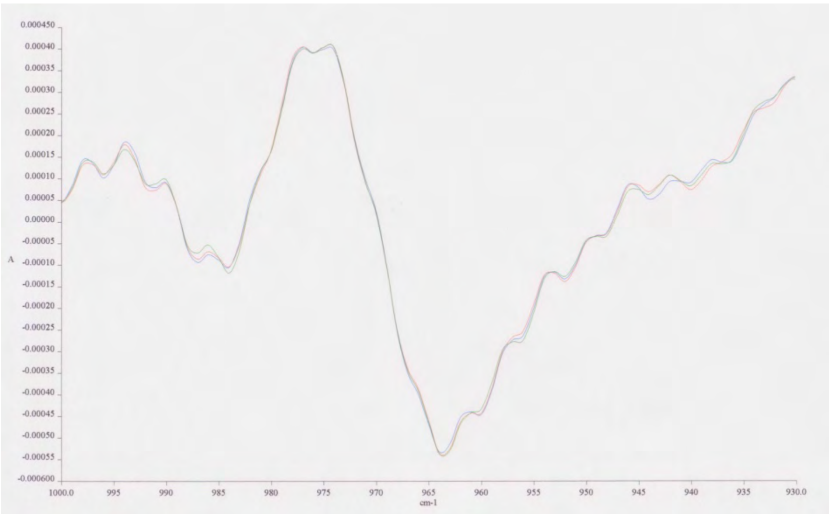


Cracker brand A (beaker 3)

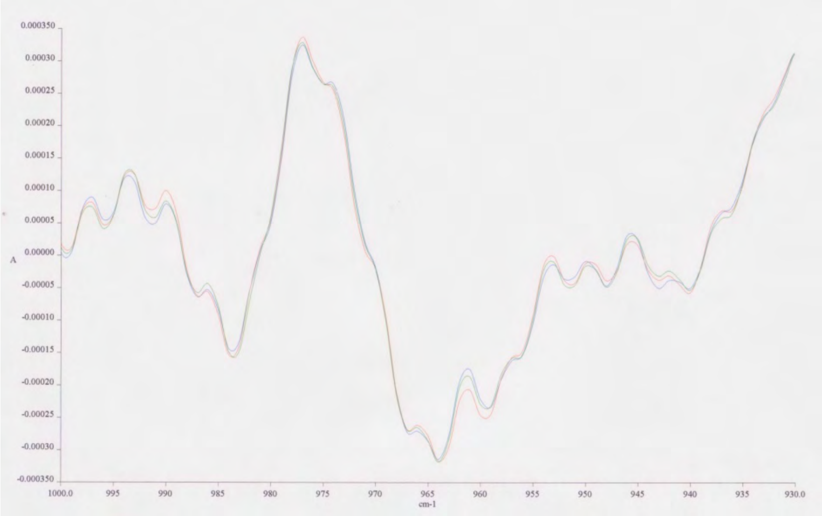


Cracker brand B (beaker 1)

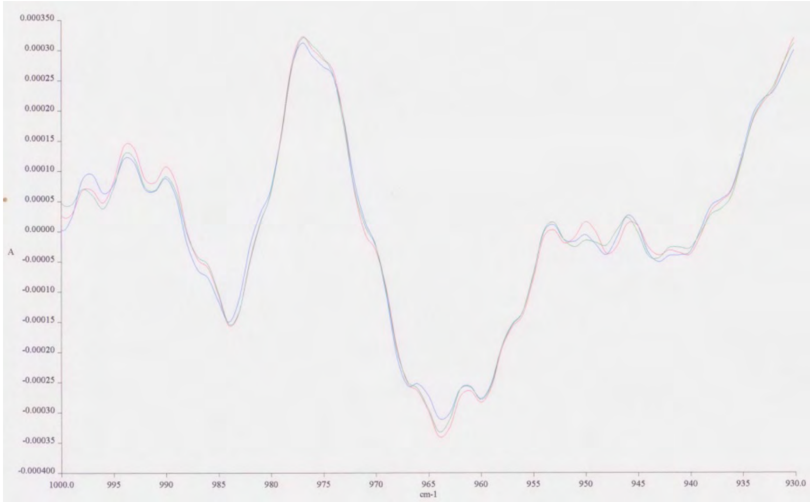
Cracker brand B (beaker 2)



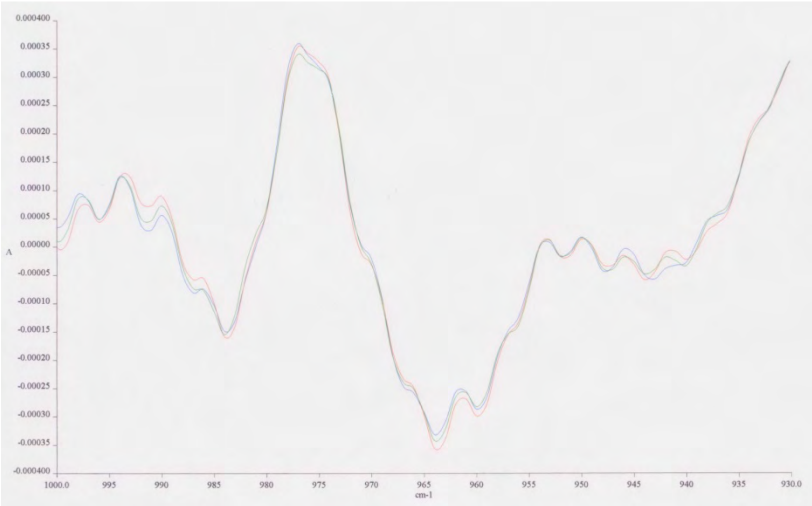
Cracker brand B (beaker 3)



Cracker brand C (beaker 1)



Cracker brand C (beaker 2)



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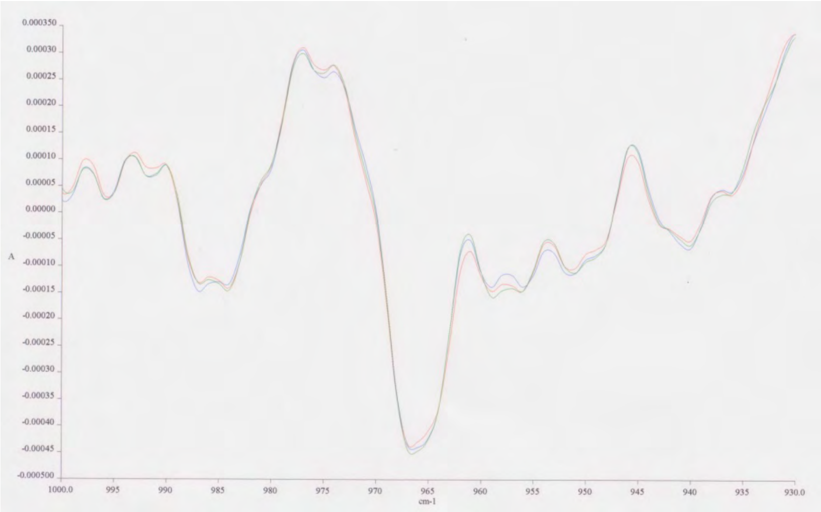
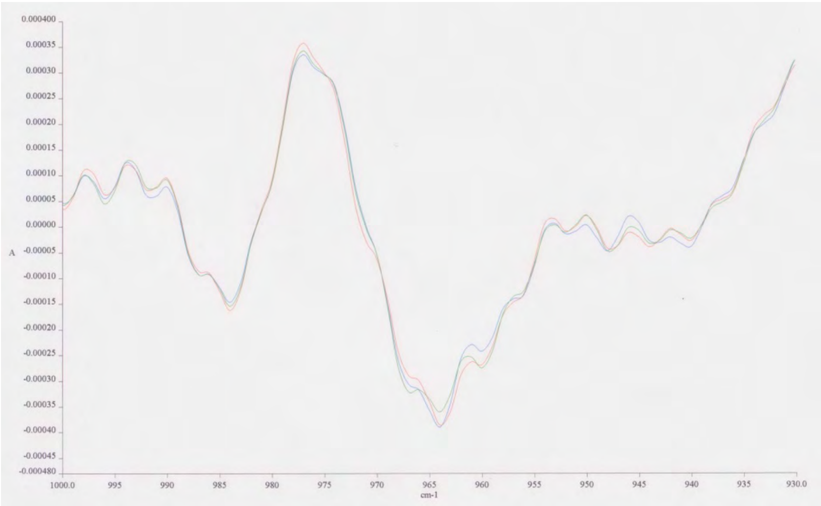
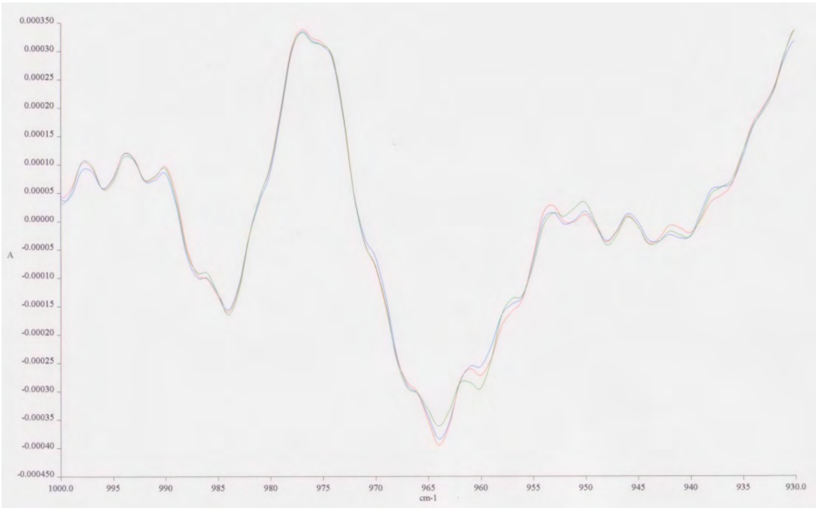


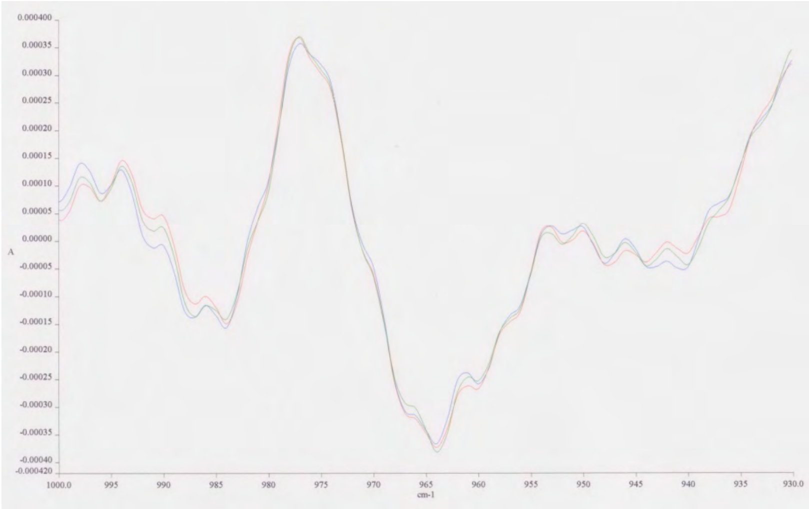
Stick biscuit brand A (beaker 1)



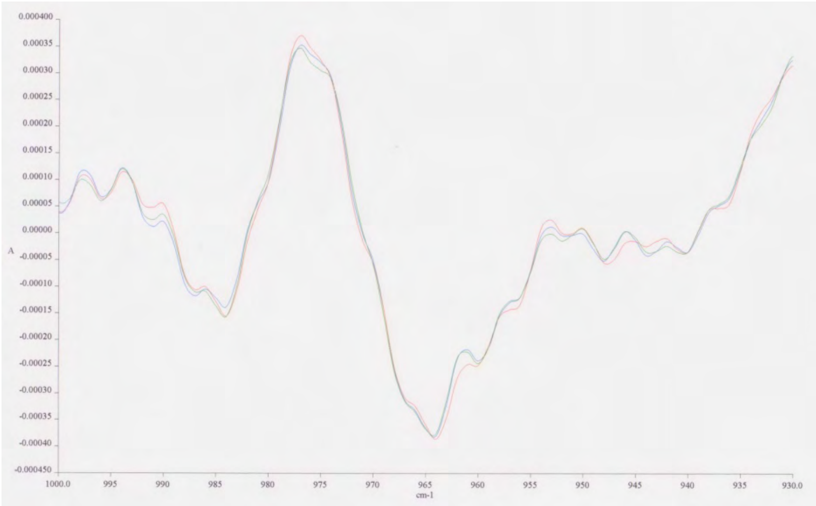
Stick biscuit brand A (beaker 2)



Stick biscuit brand A (beaker 3)



Stick biscuit brand B (beaker 1)



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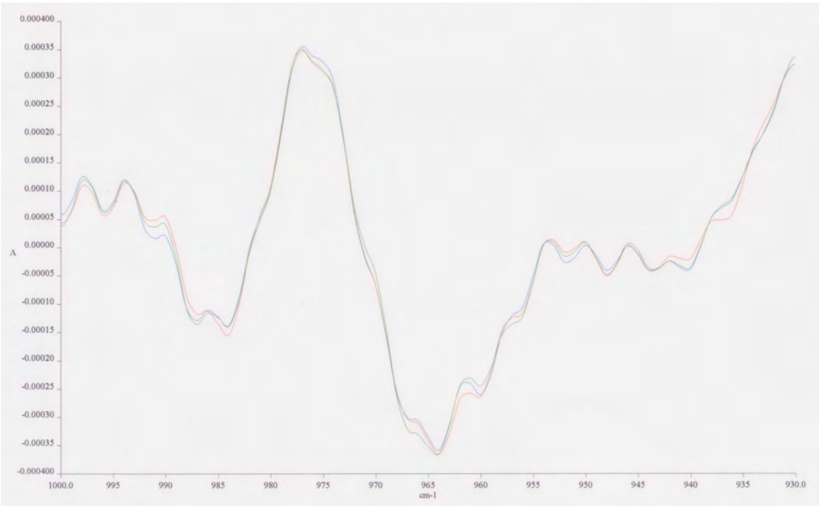
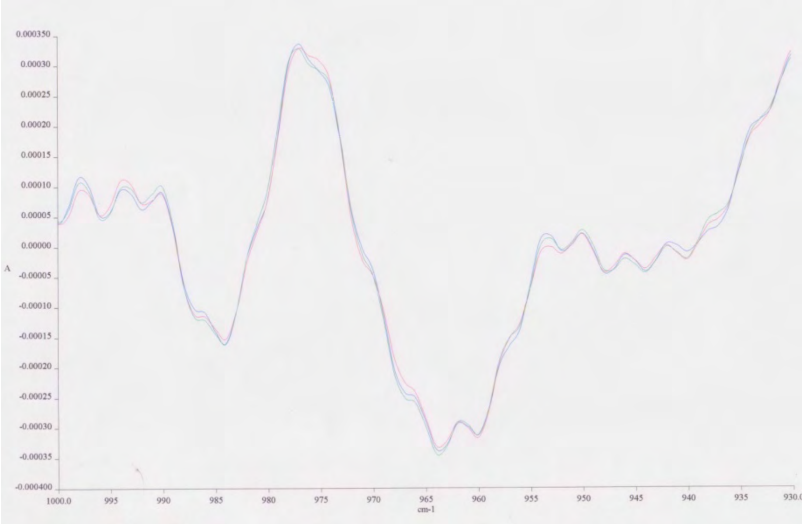
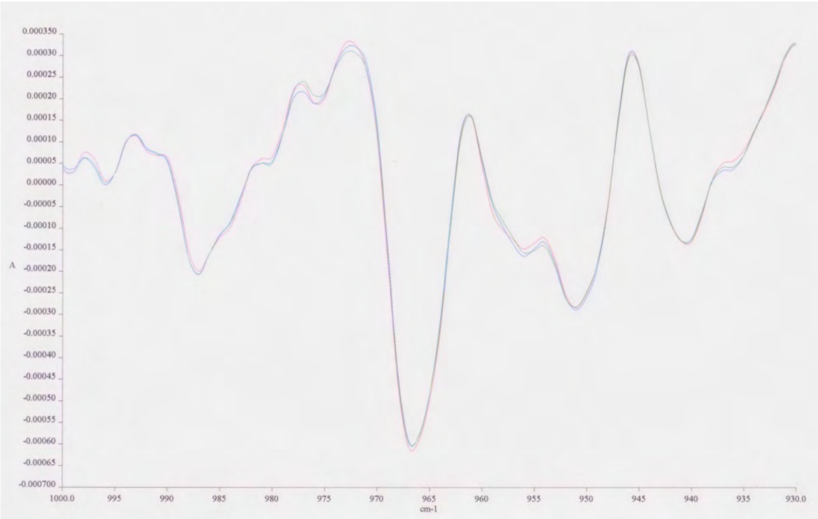


Stick biscuit brand B (beaker 3)

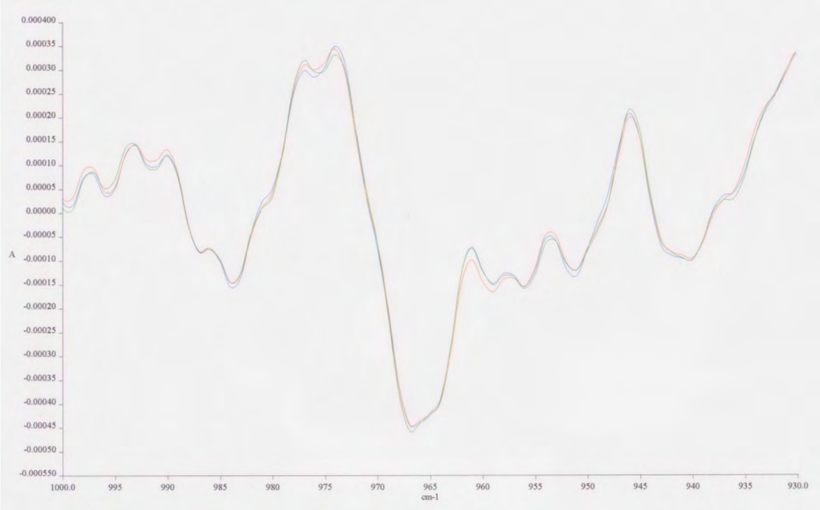


Stick biscuit brand C (beaker 1)

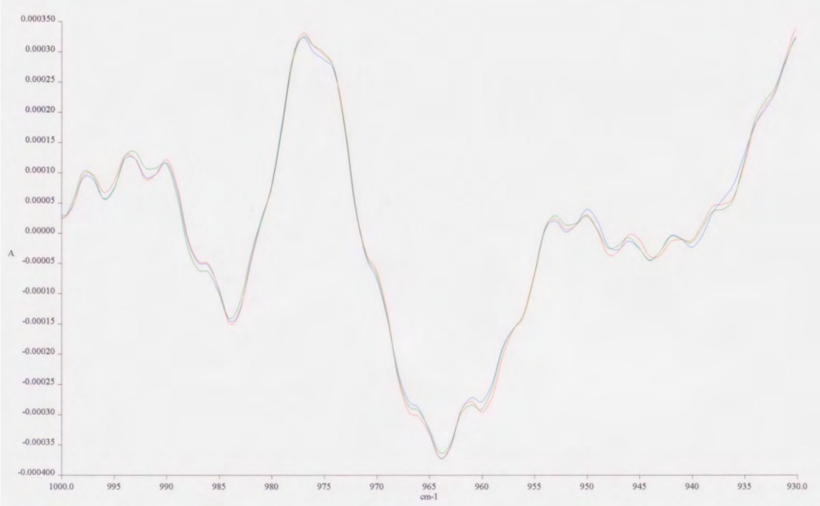
Stick biscuit brand C (beaker 2)



Stick biscuit brand C (beaker 3)



Potatochip brand A (beaker 1)



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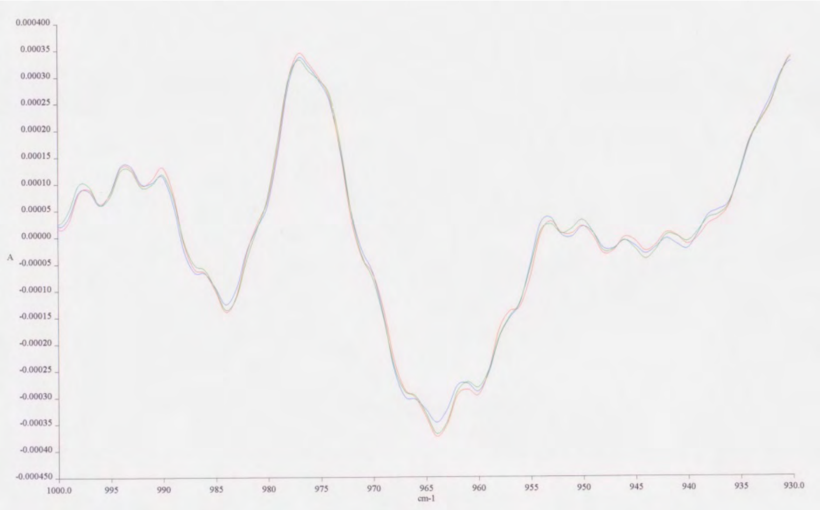

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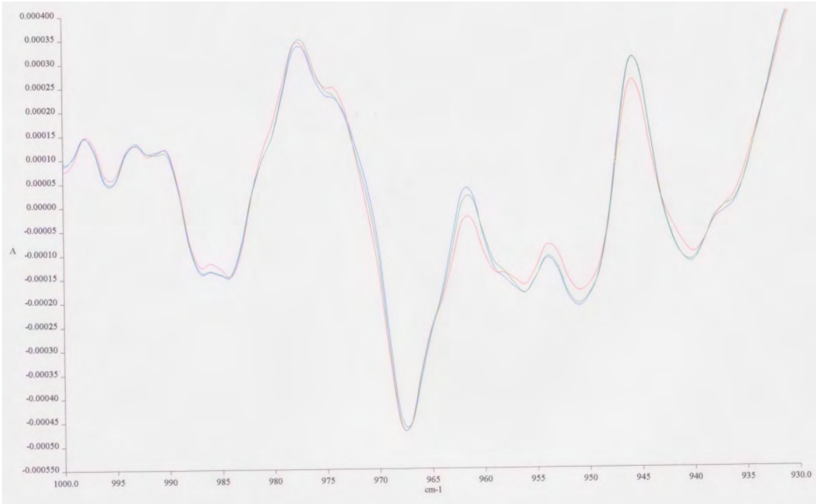

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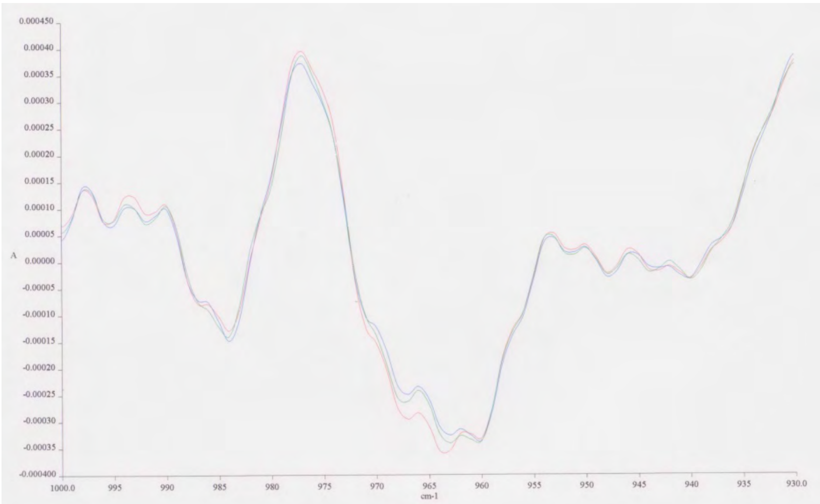

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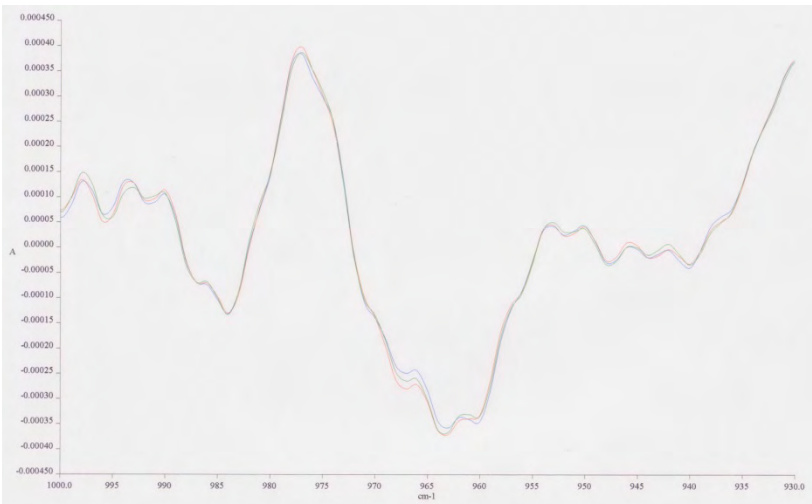
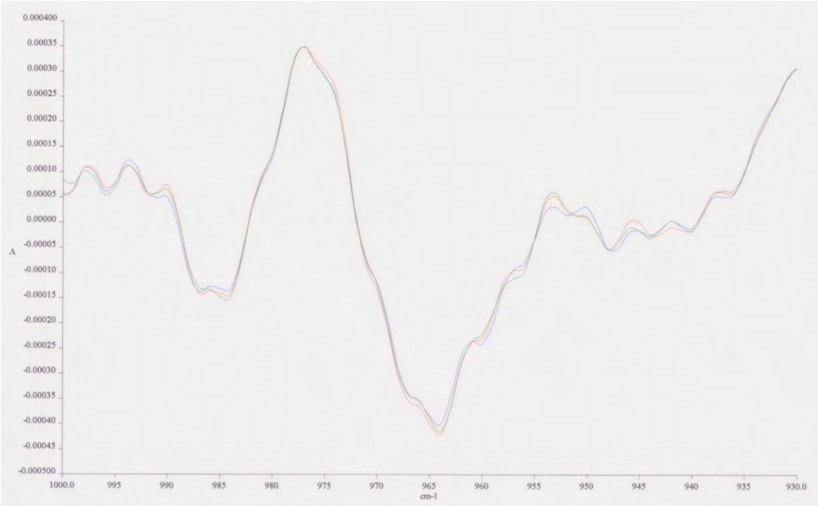
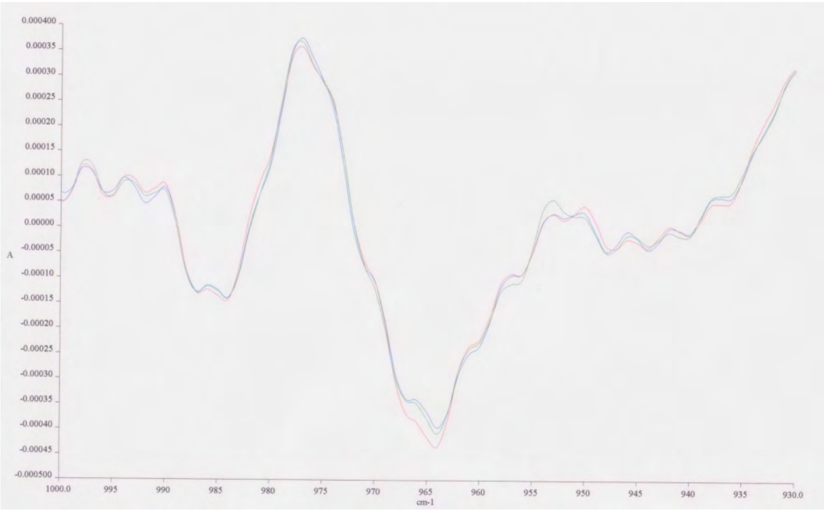


Potatochip brand B (beaker 3)



Potatochip brand C (beaker 1)



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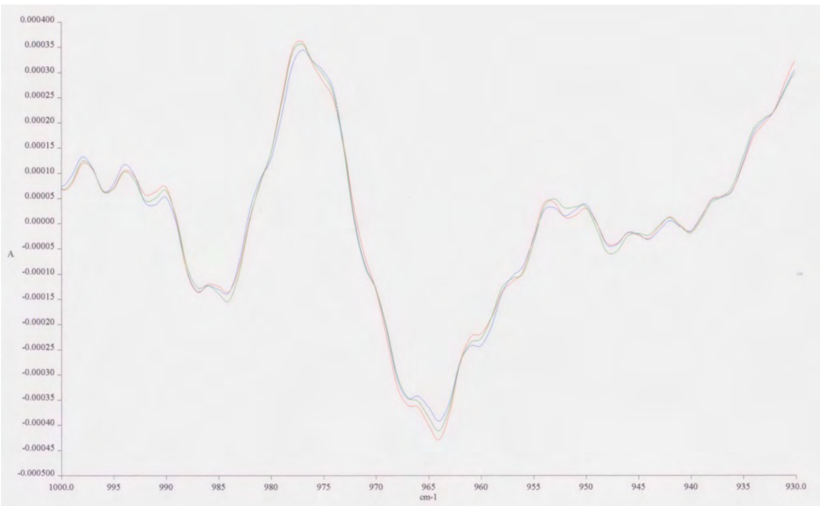
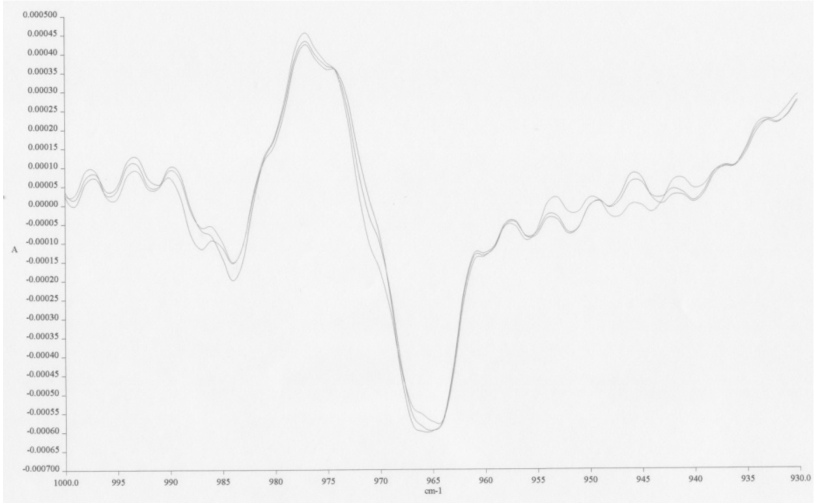
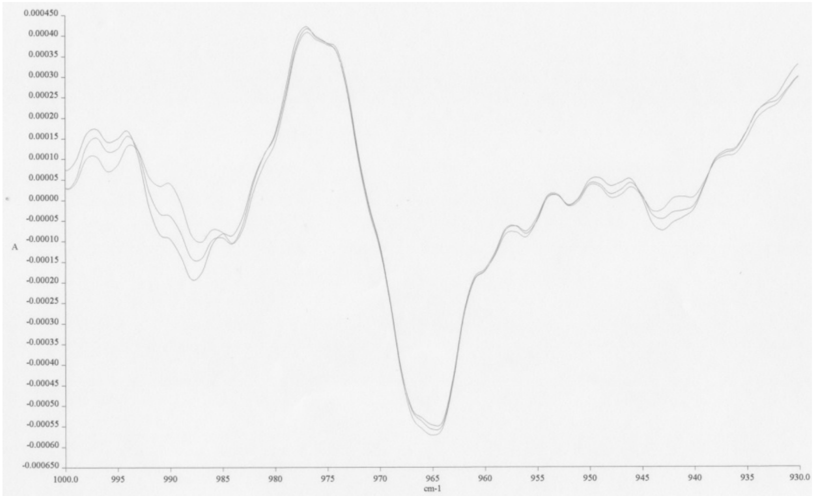


Potatochip brand C (beaker 3)



Biscuit brand A (beaker 1)



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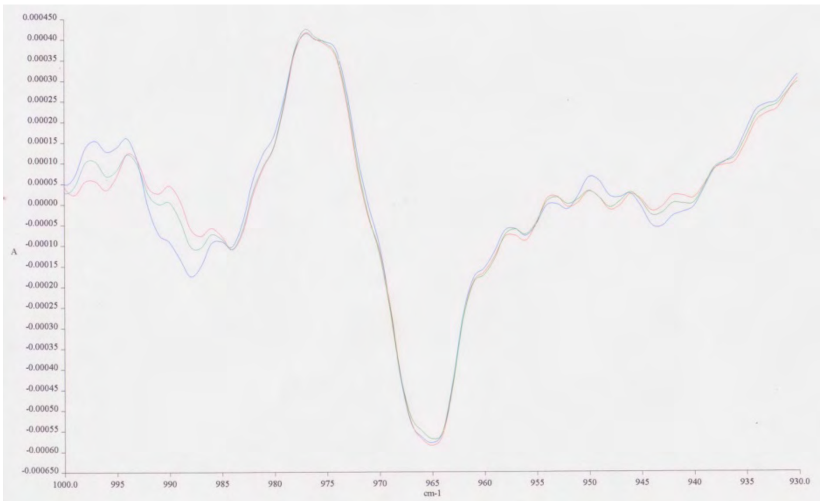

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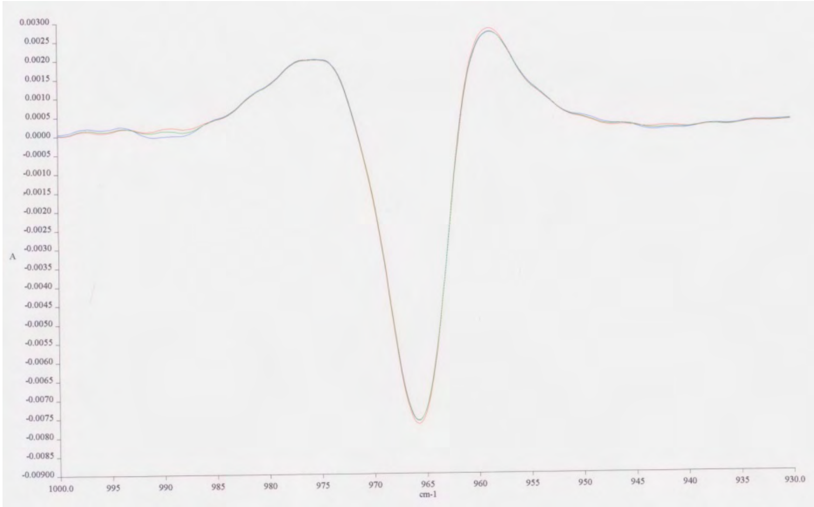

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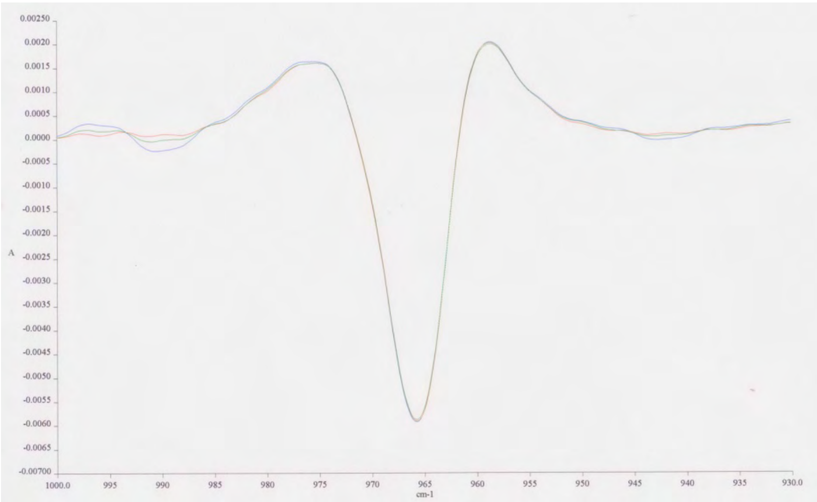
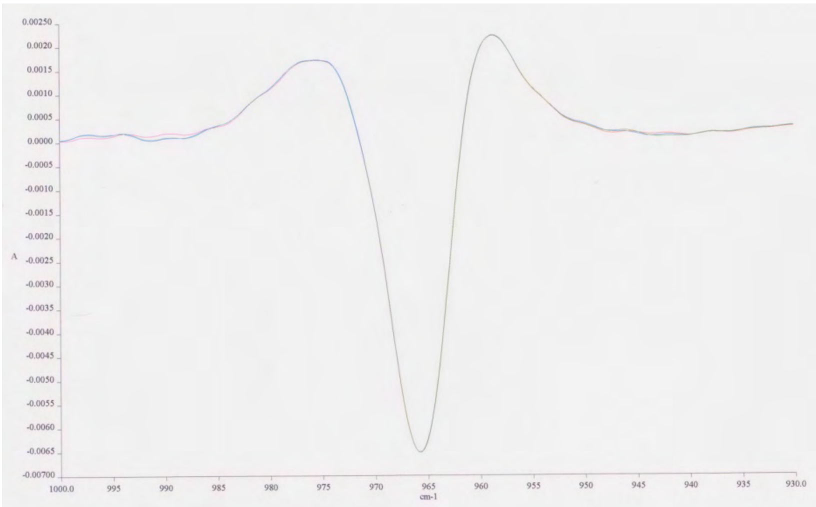


Biscuit brand B (beaker 2)



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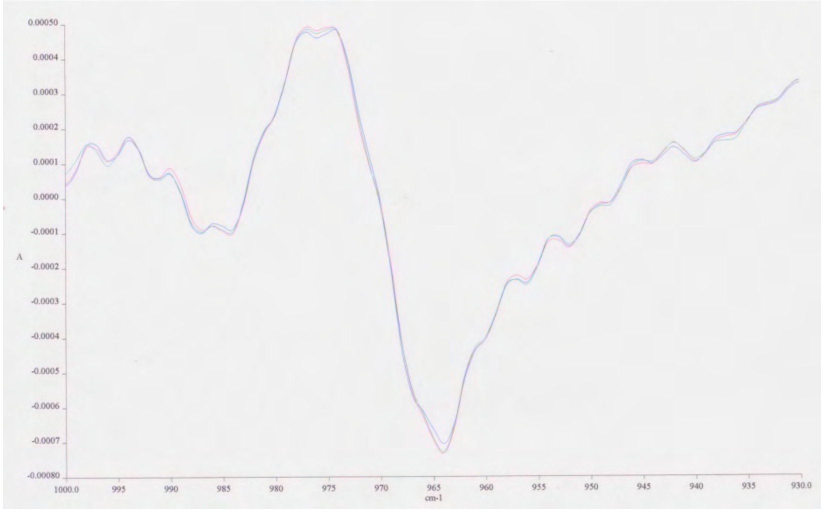

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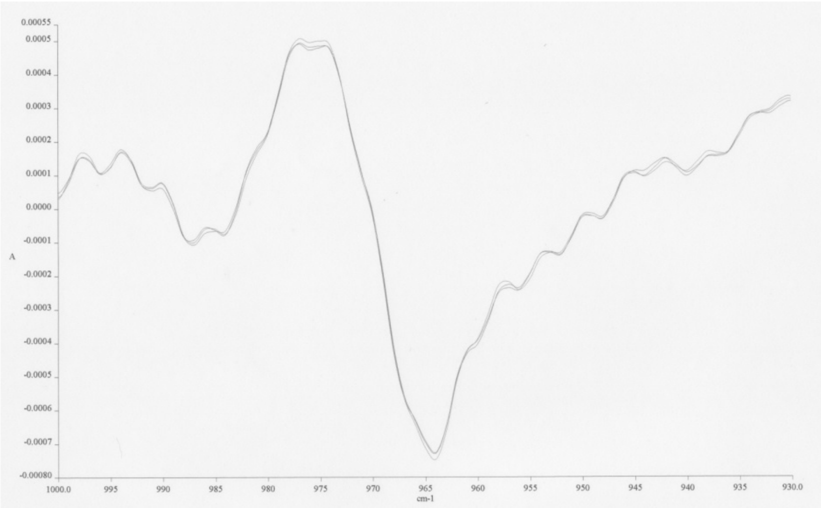

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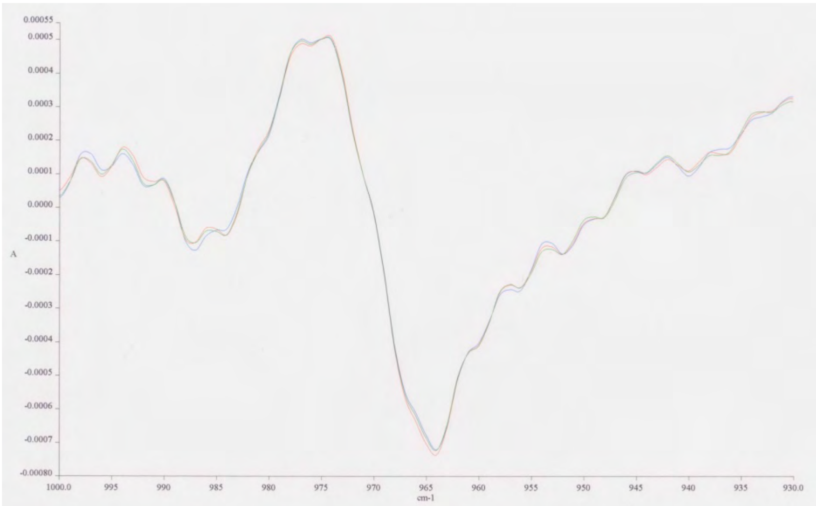

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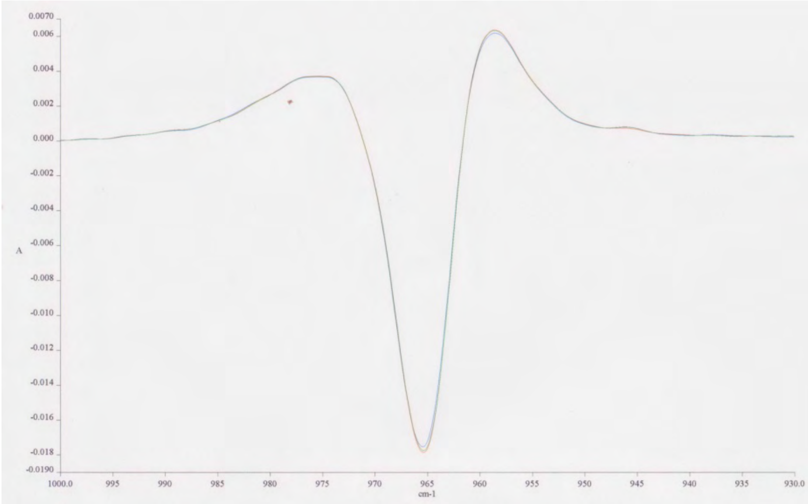

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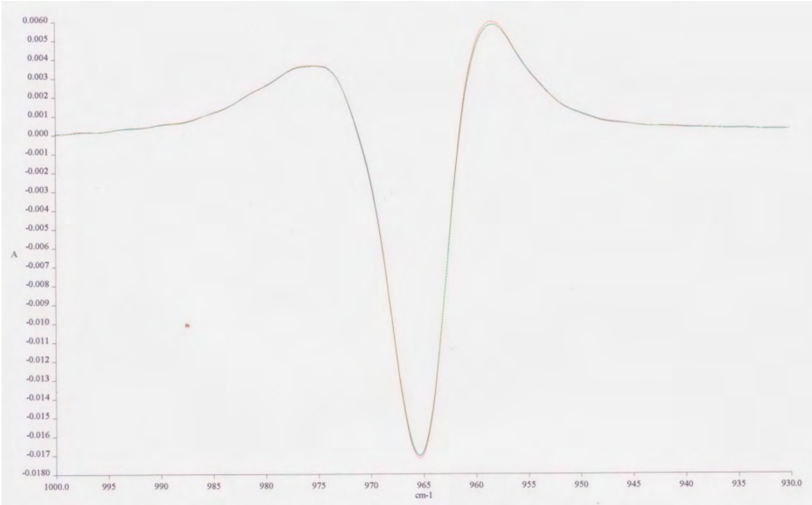

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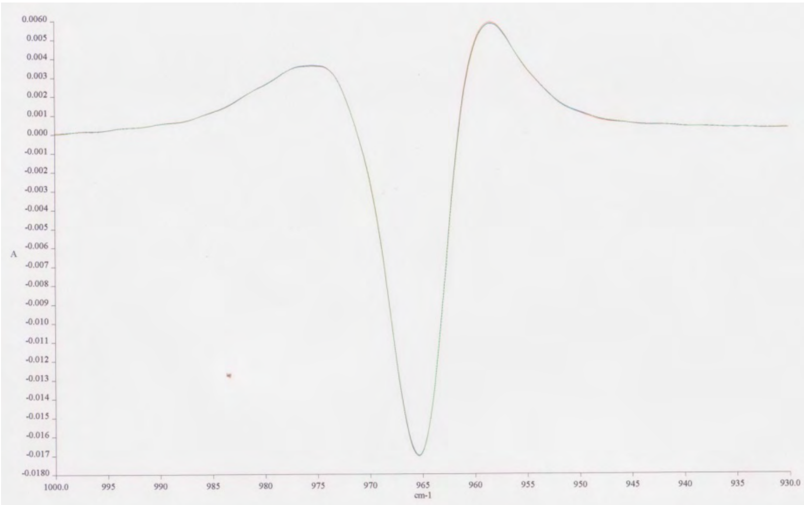

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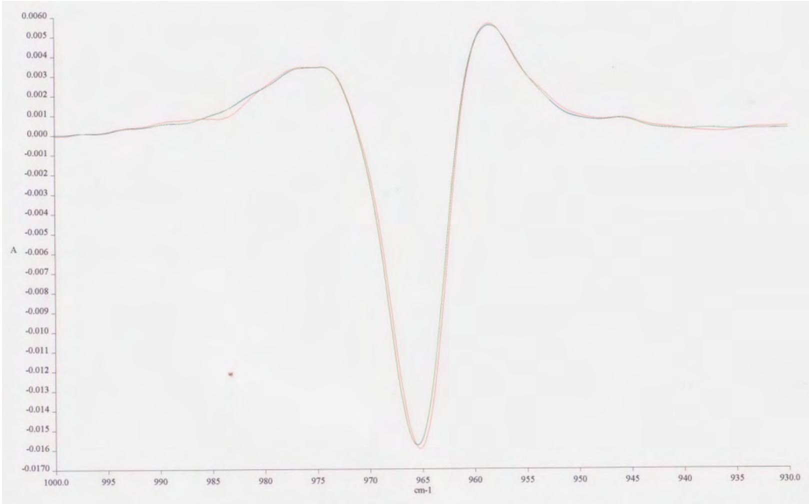

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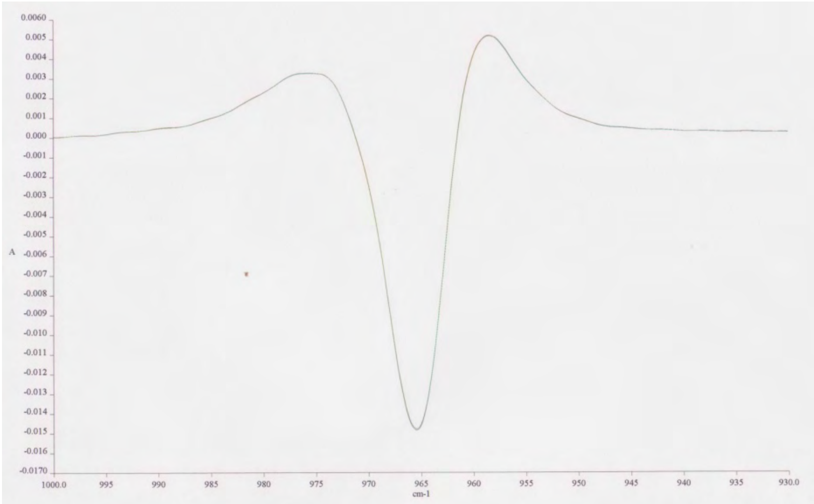

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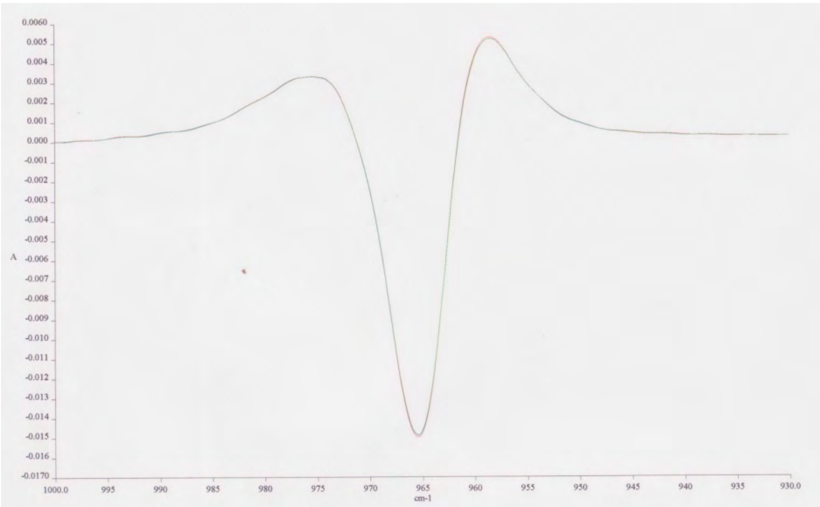

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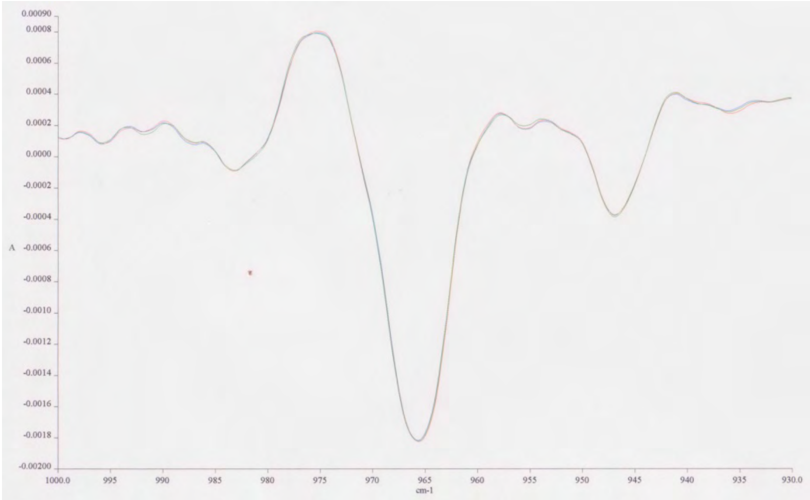

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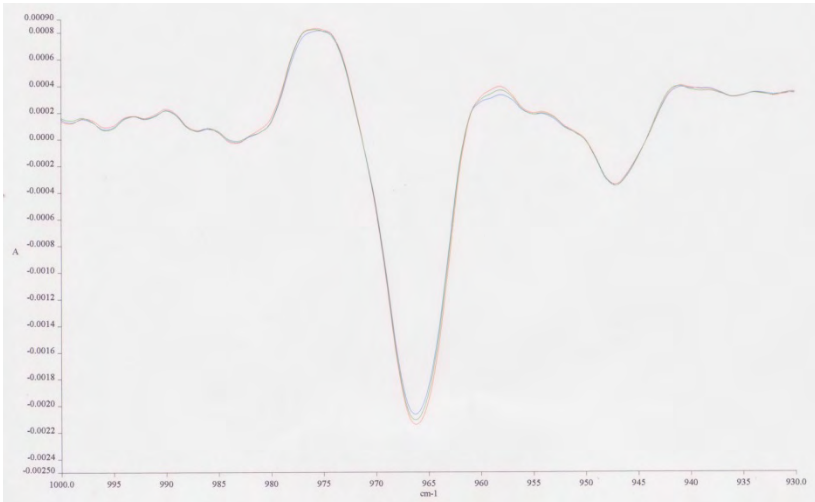

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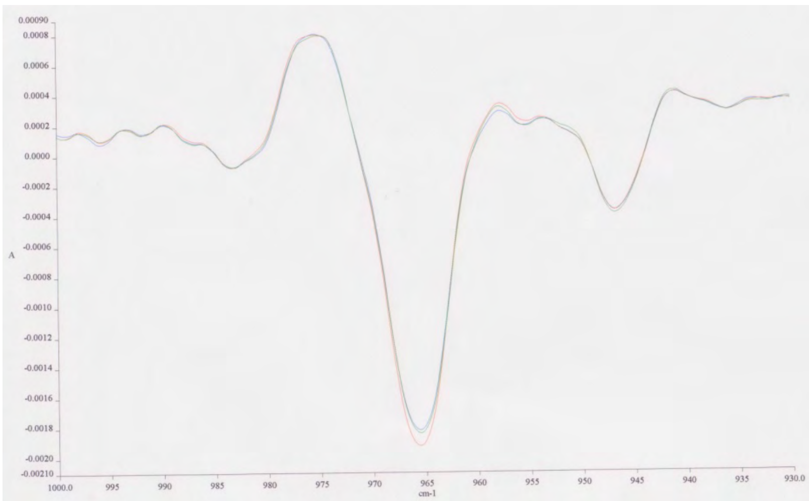

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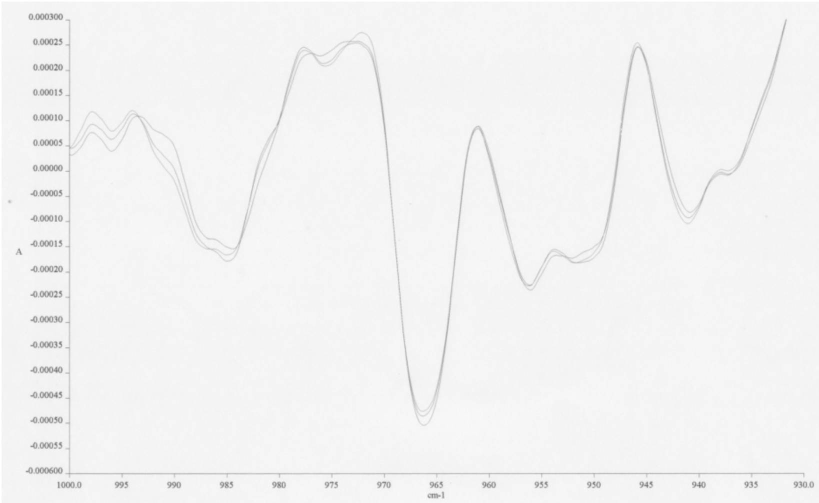

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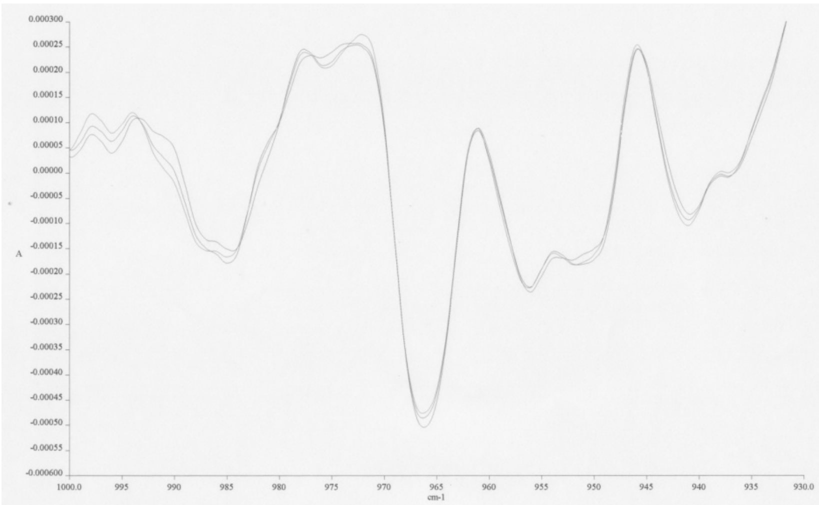

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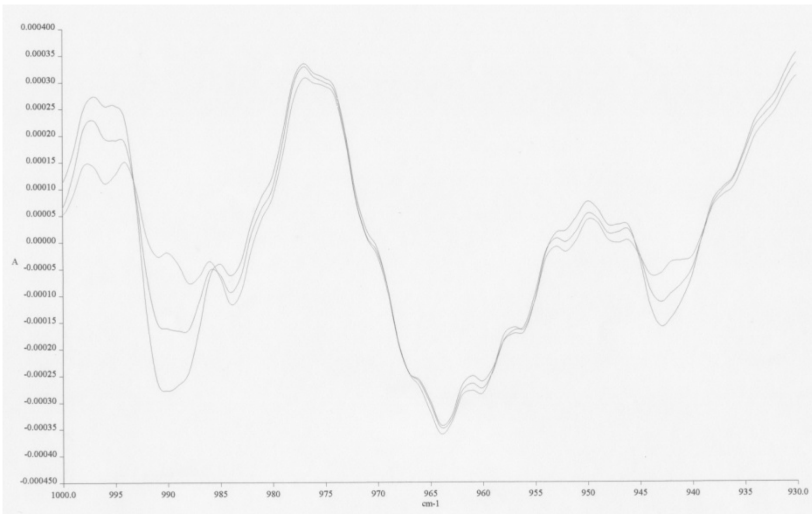

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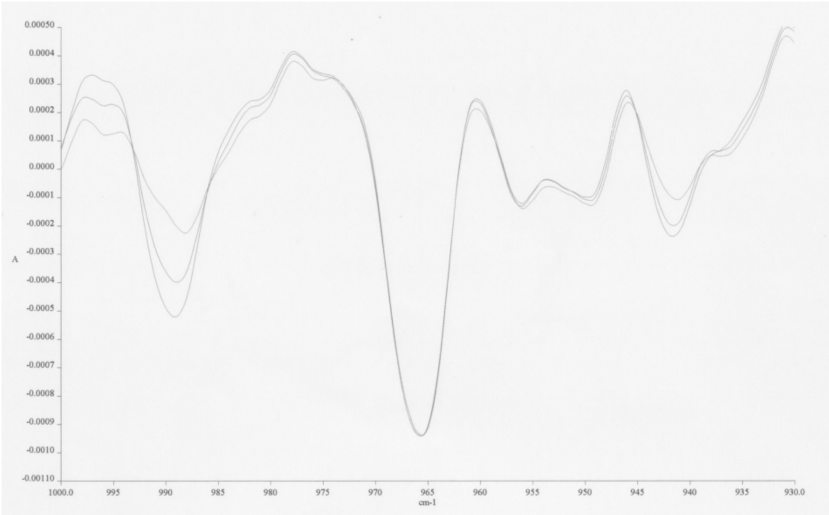

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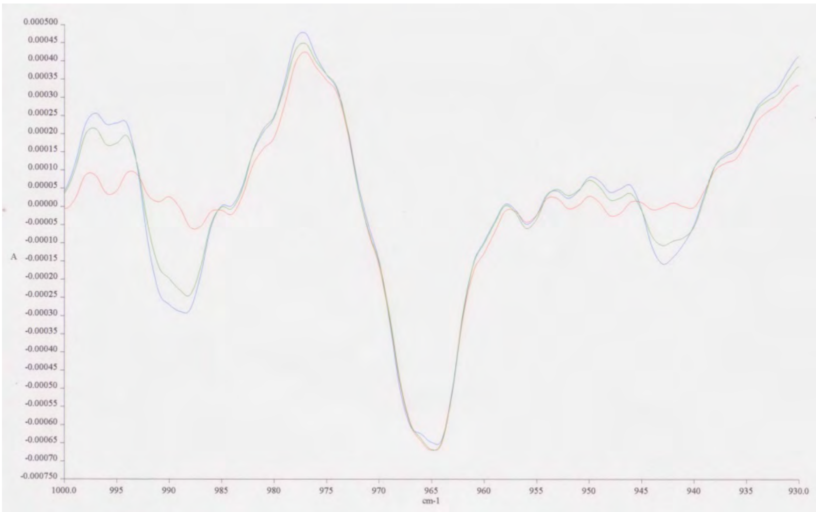

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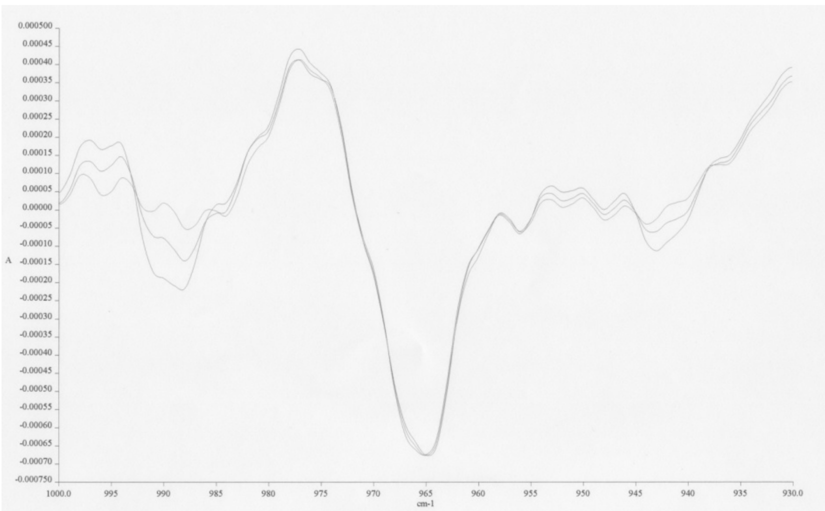

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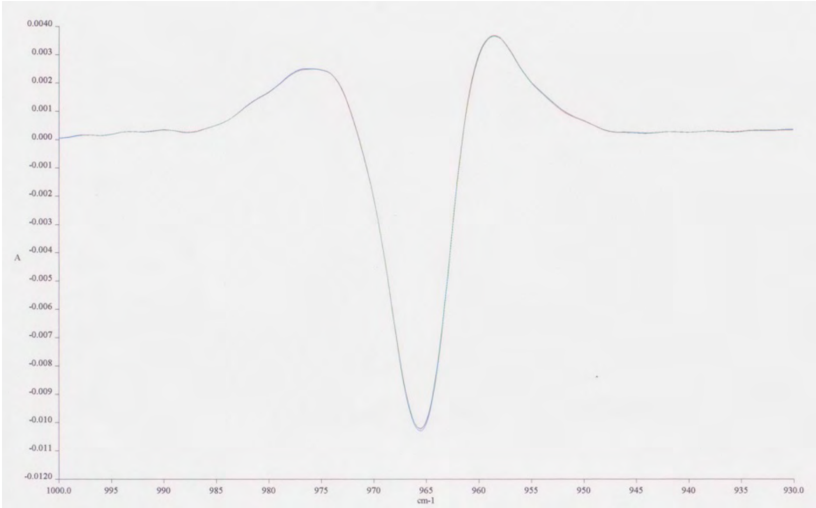

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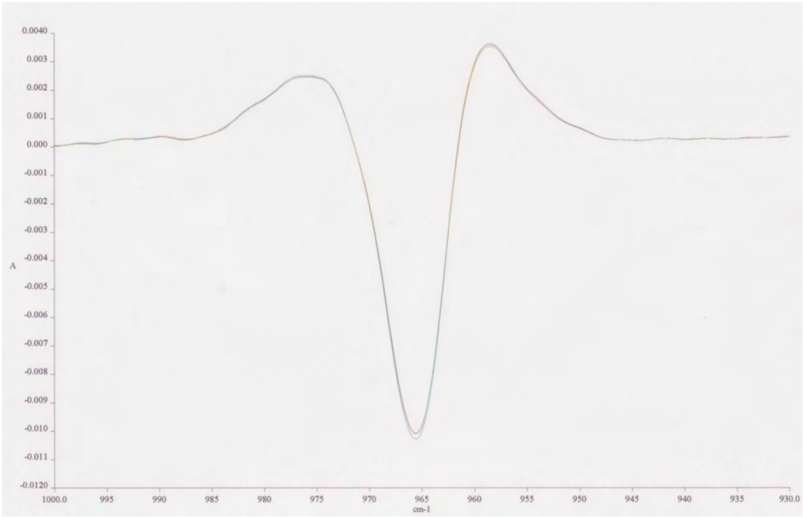

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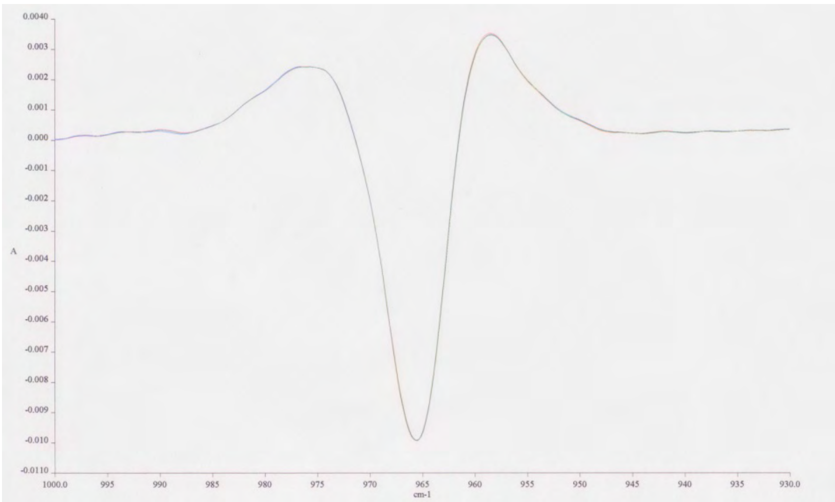
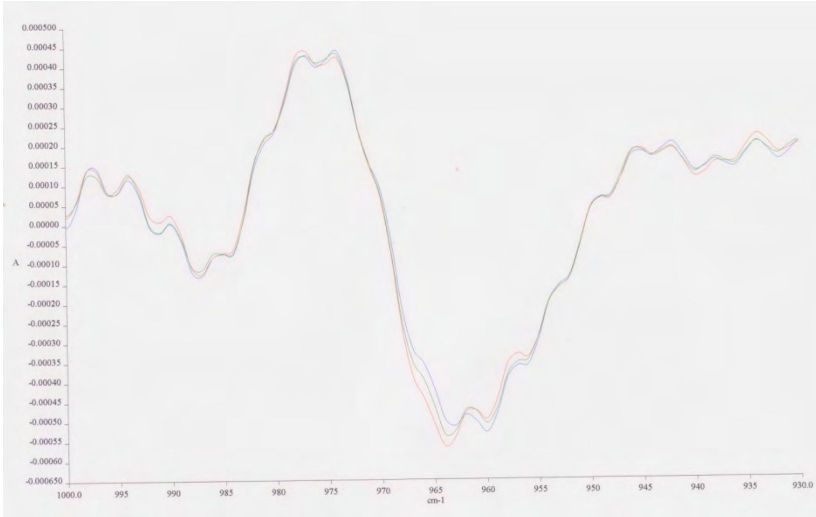


Wafer brand C (beaker 3)

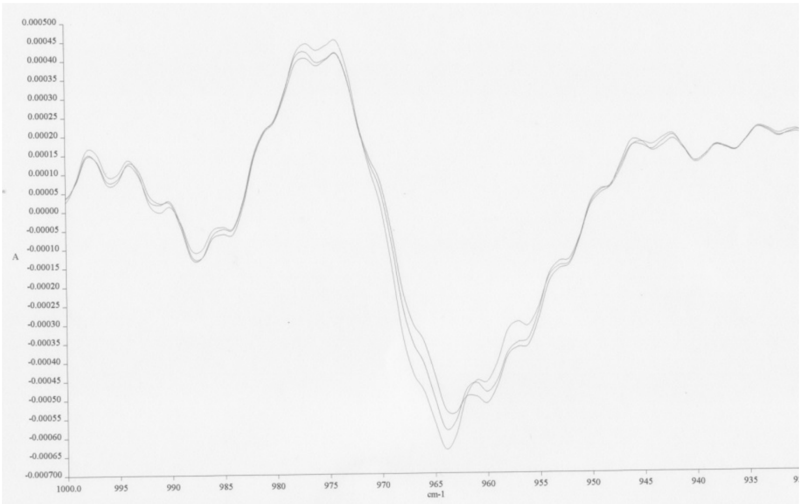
APPENDIX F

THE NEGATIVE SECOND DERIVATIVE

OF NON-DAIRY CREAMERS



Non dairy creamer brand A (beaker 1)



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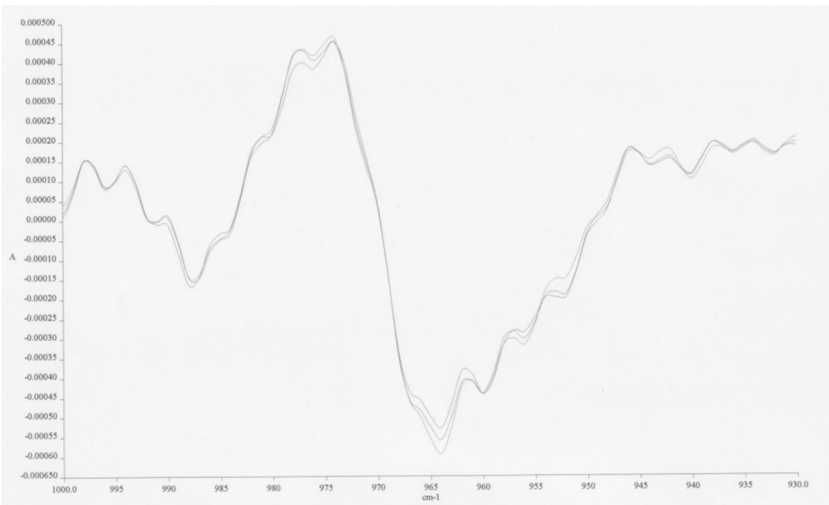

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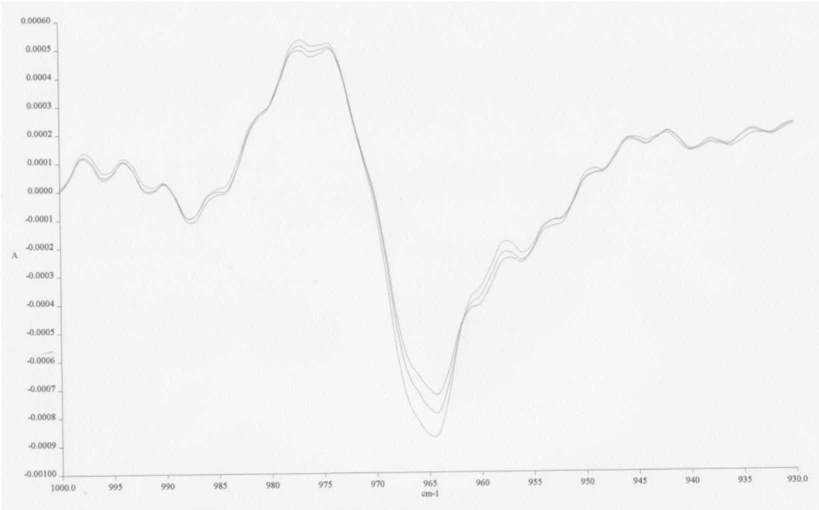

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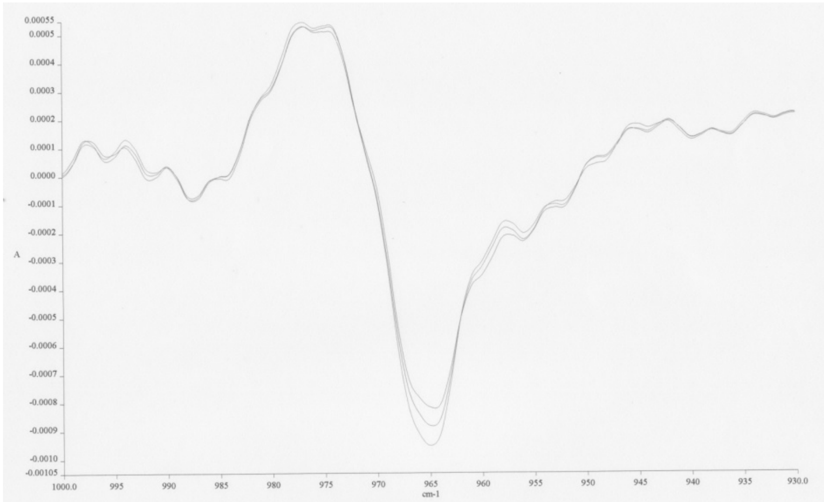

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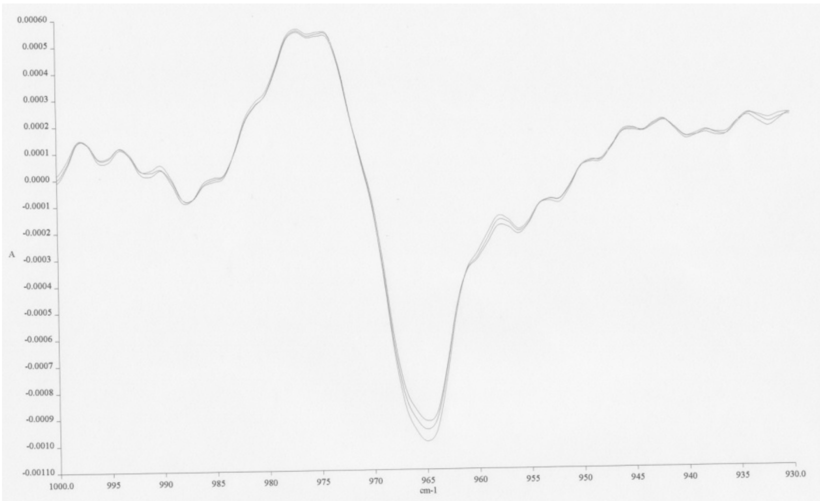

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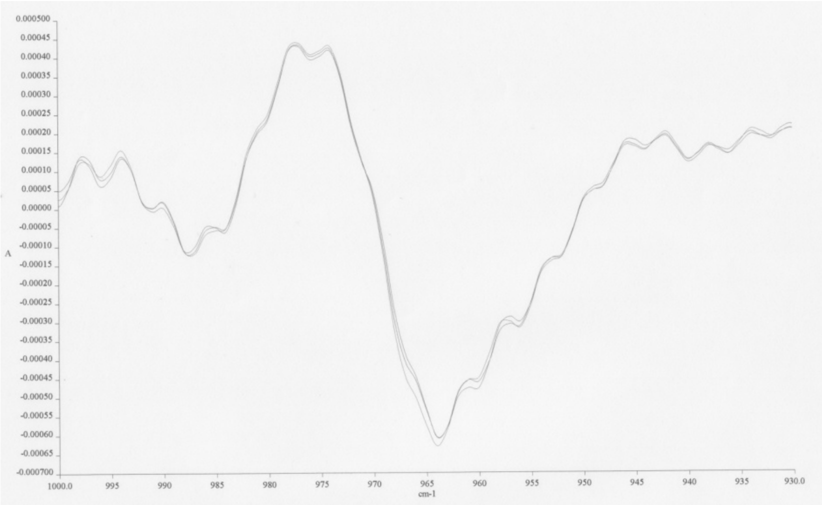

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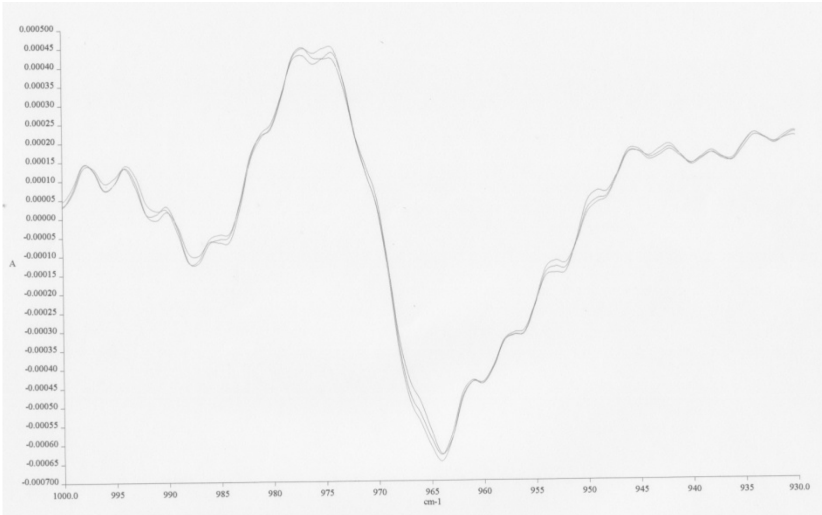

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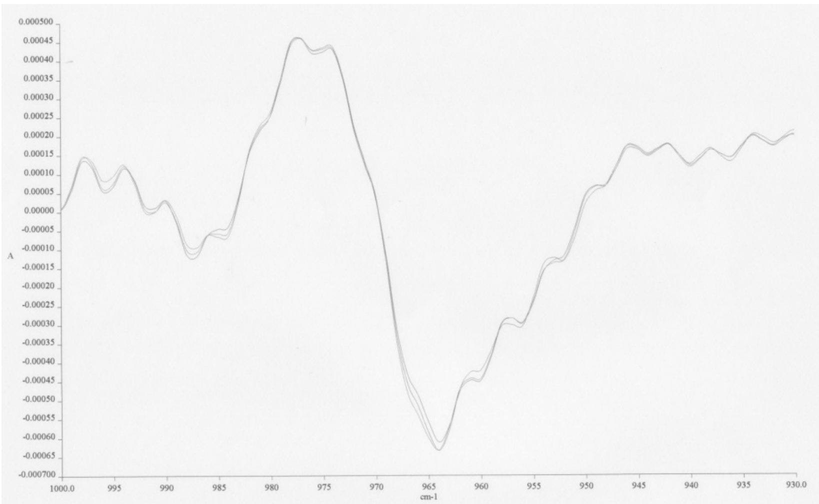

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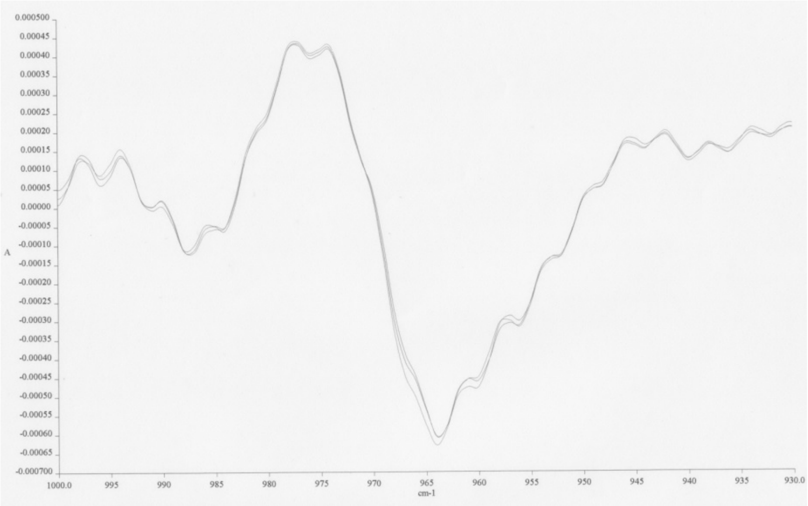
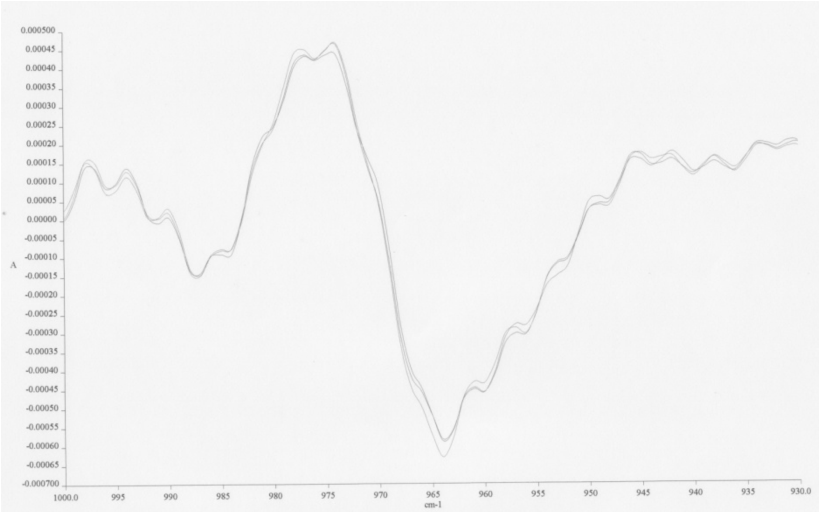


Non dairy creamer brand D (beaker 1)



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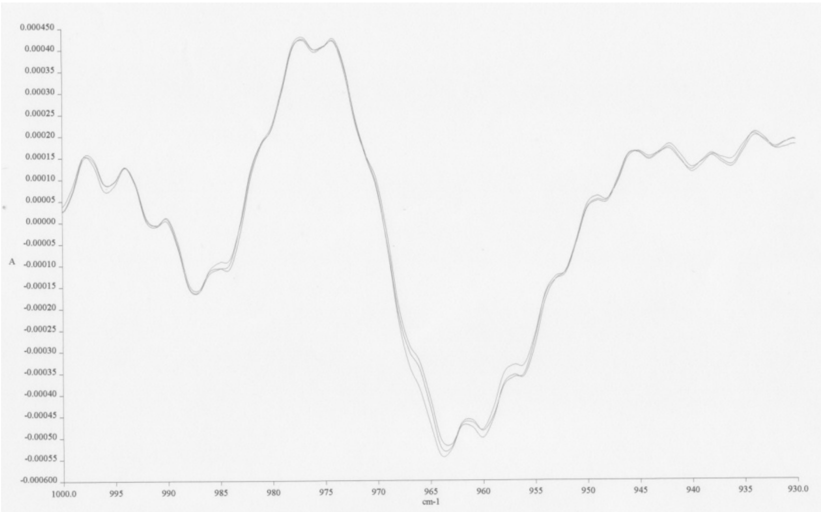

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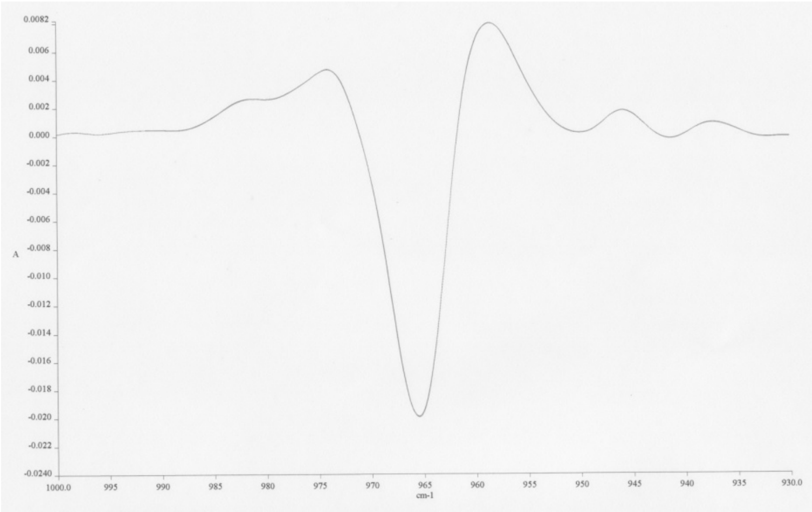

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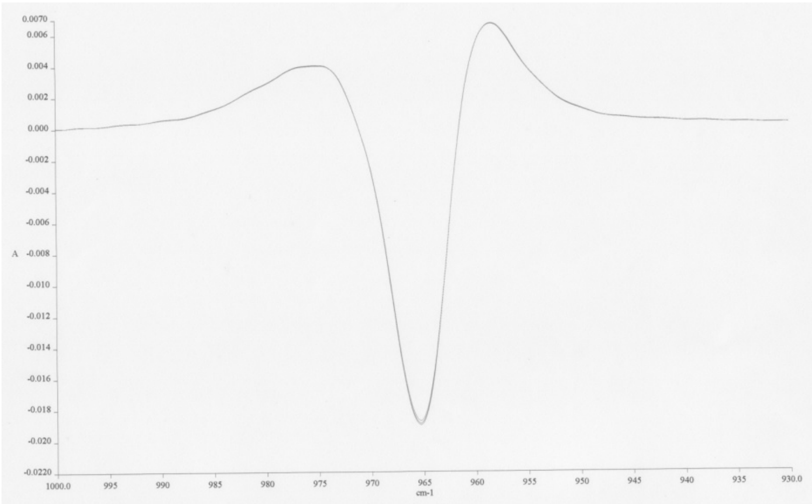

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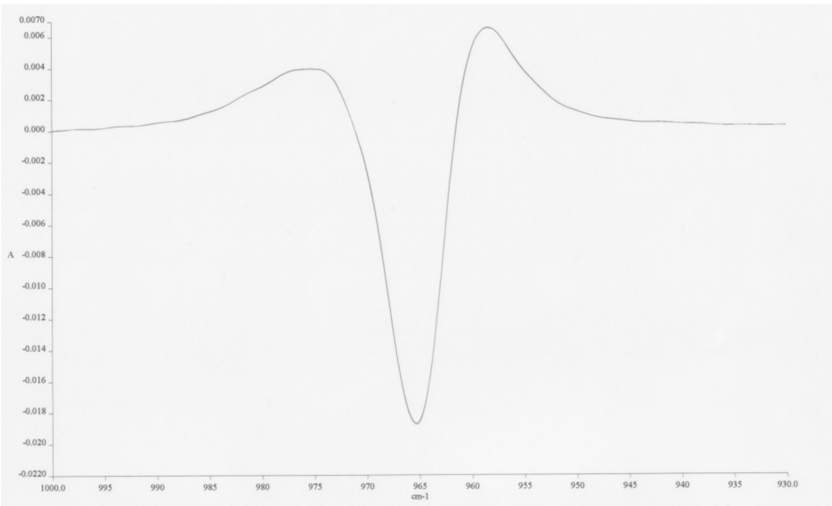

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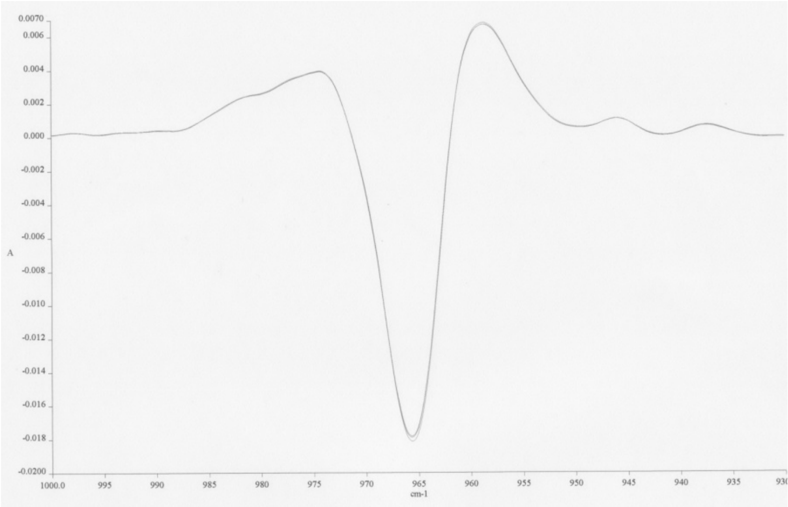

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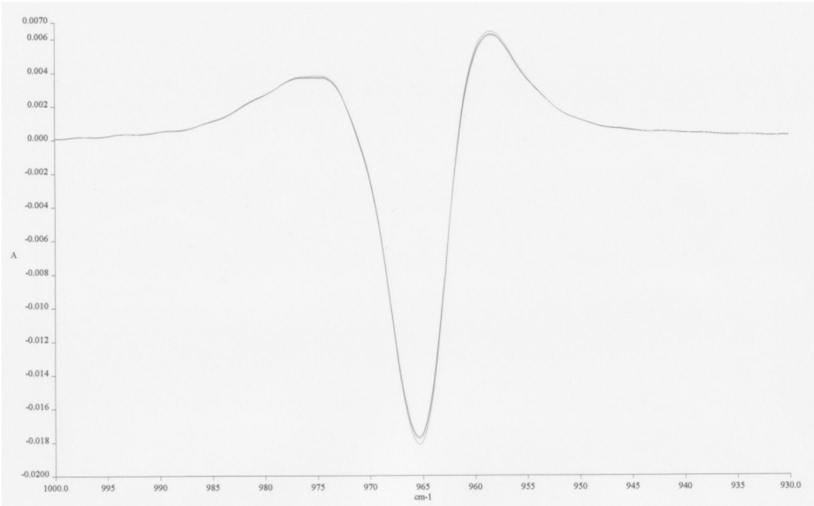

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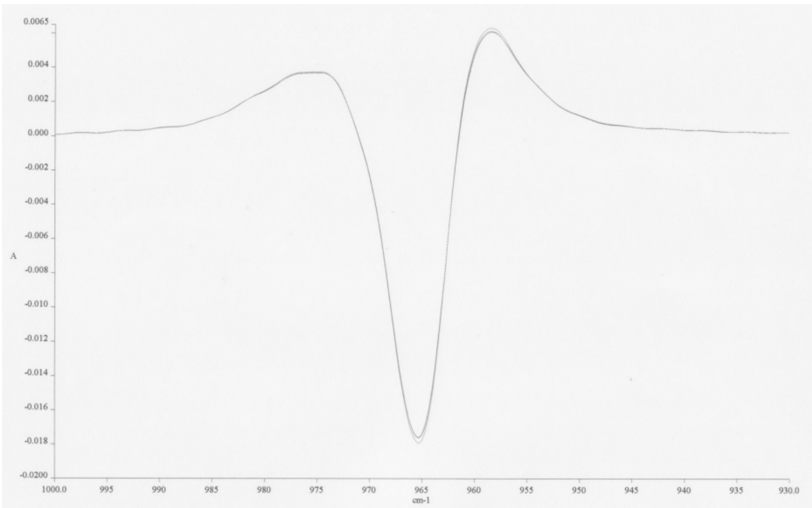
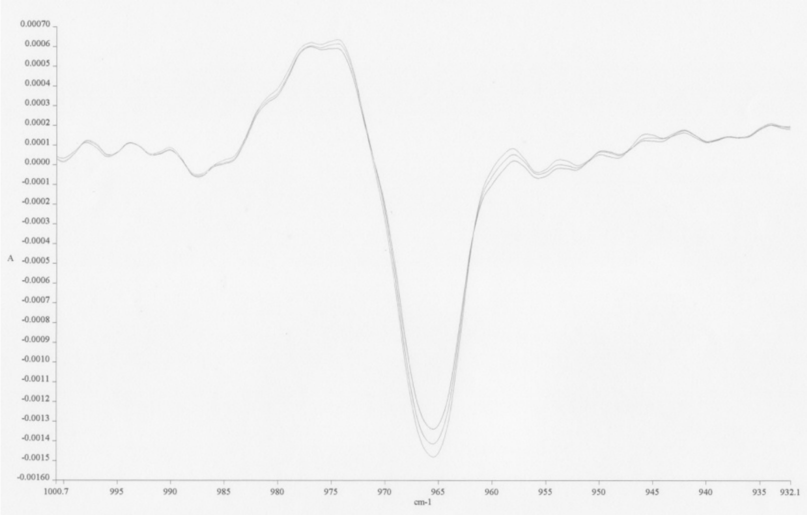


Non dairy creamer brand F (beaker 3)

Non dairy creamer brand G (beaker 1)

Non dairy creamer brand G (beaker 2)

Non dairy creamer brand G (beaker 3)



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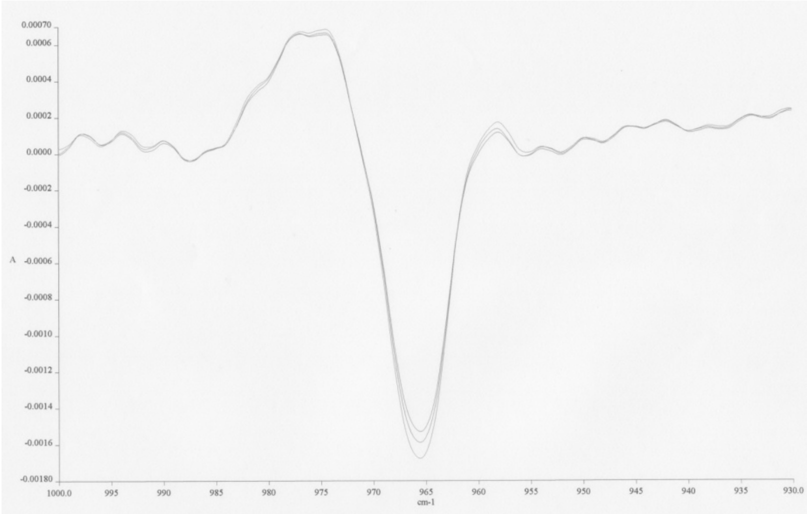

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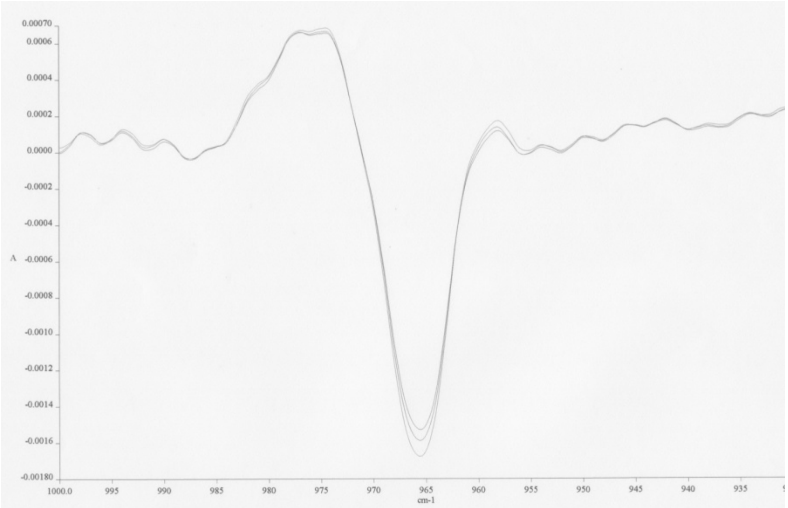

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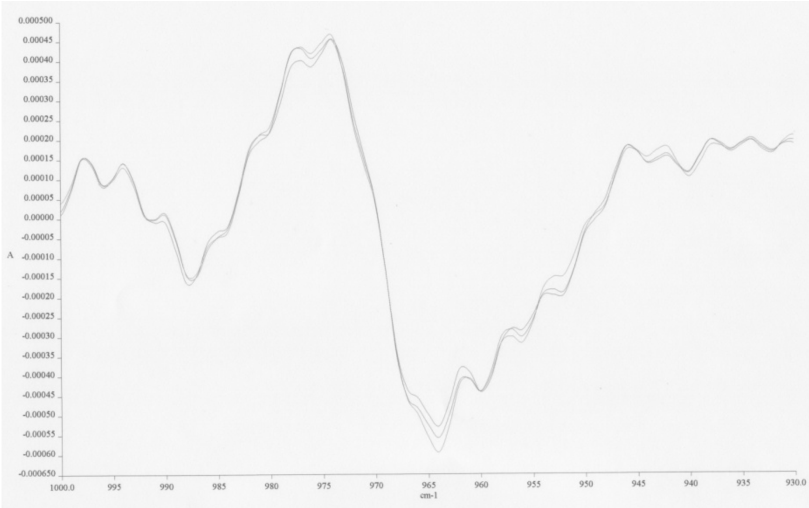
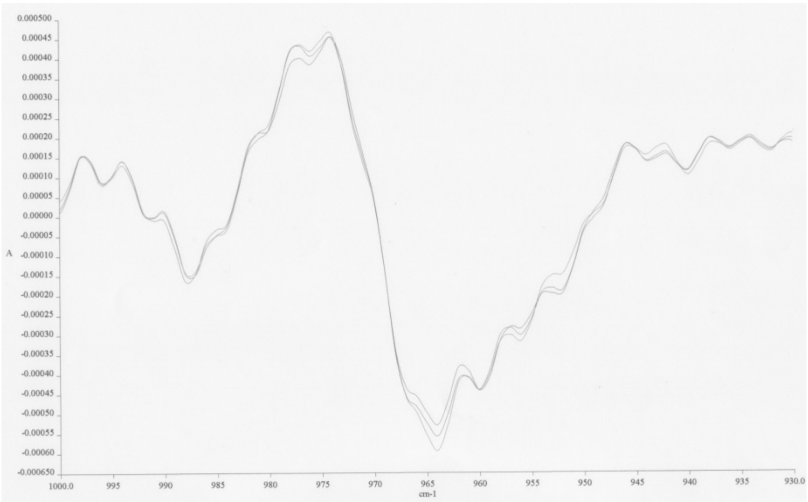


Non dairy creamer brand I (beaker 1)

Non dairy creamer brand I (beaker 2)



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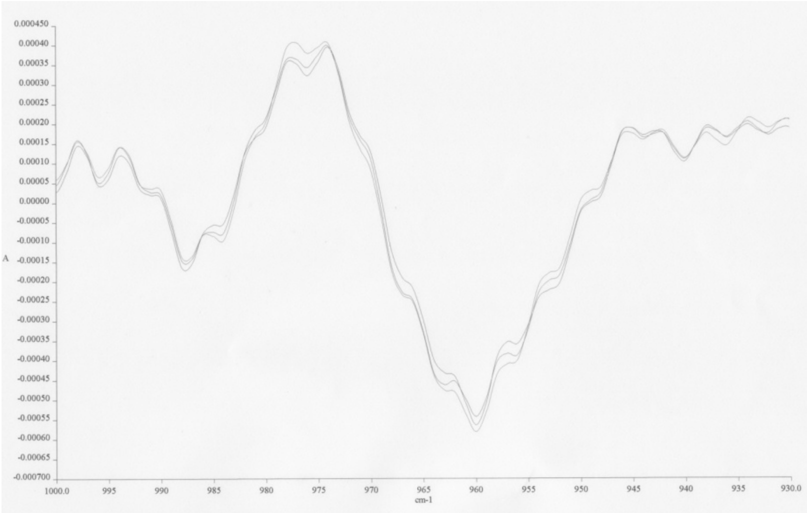

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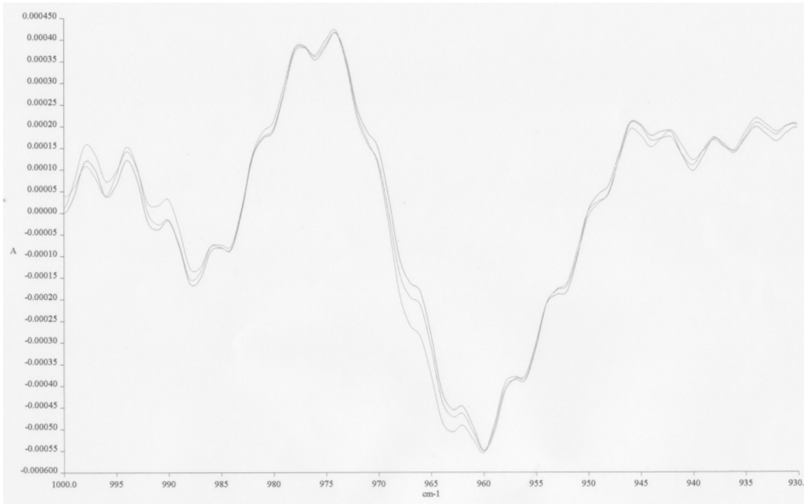

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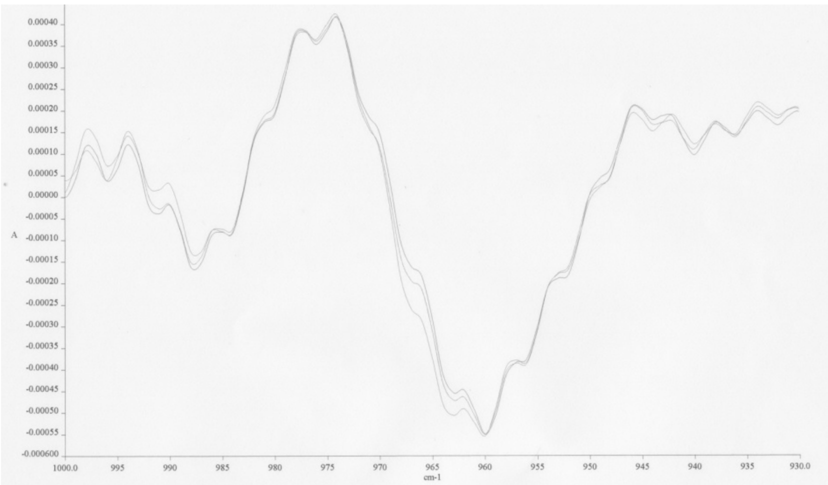

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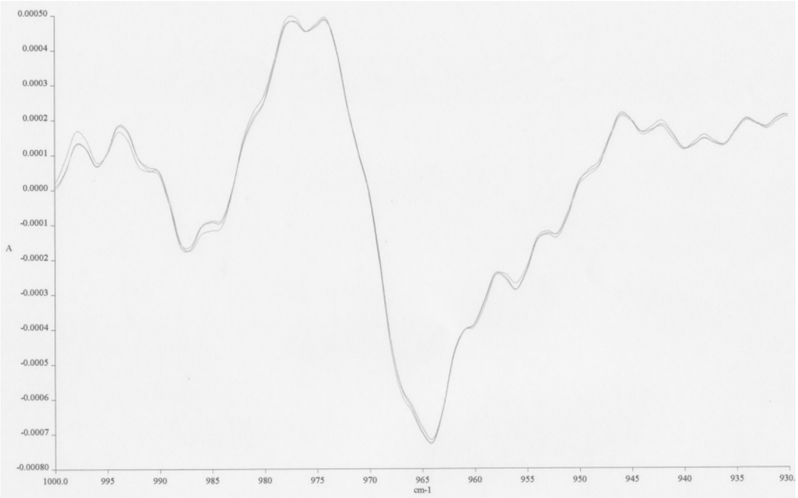

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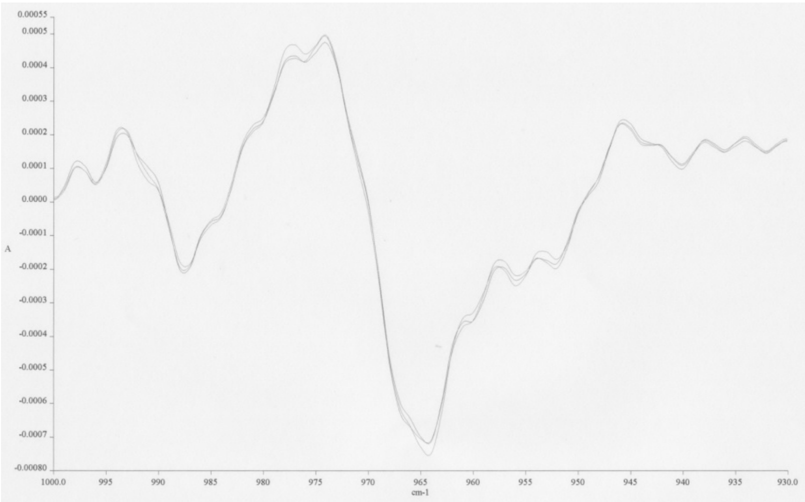

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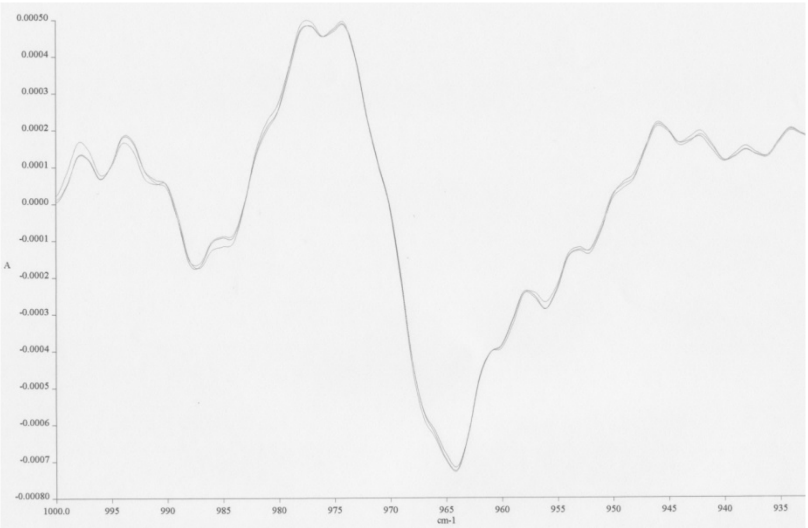
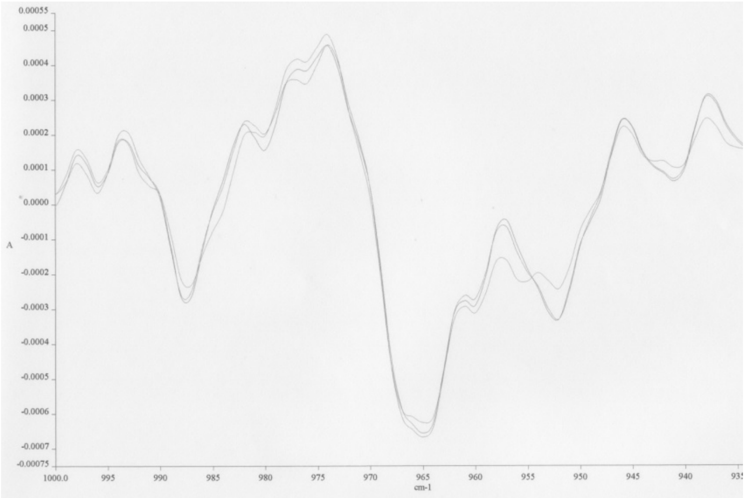


Non dairy creamer brand K (beaker 3)



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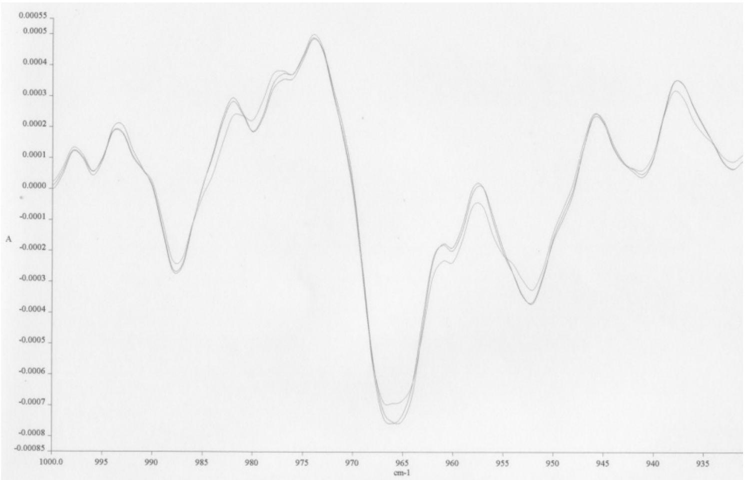

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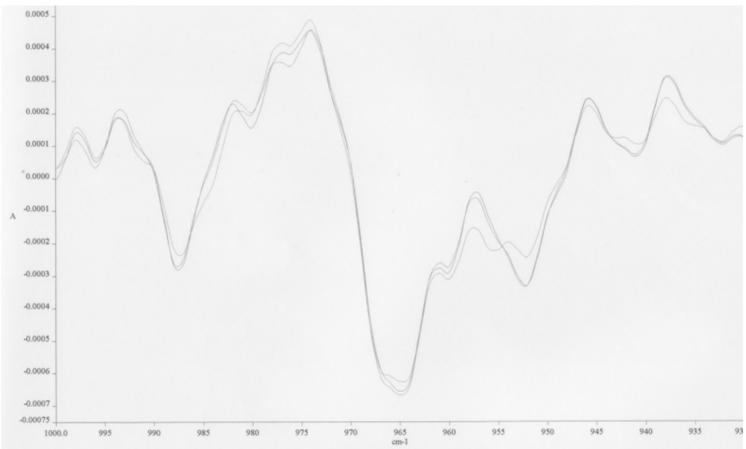

Non dairy creamer brand L (beaker 3)

BIOGRAPHY

NAME	Miss Waraporn Suwannakood
DATE OF BIRTH	27 July 1979
PLACE OF BIRTH	Ubonratchathani, Thailand
INSTITUTIONS ATTENDED	Mahidol University, 1998-2003;
	Bachelor of Science in Pharmcy
	Chulalongkorn University, 2008-2010
	Master of Science in Pharmacy
	(Food Chemistry and Medical Nutrition)
POSITION OFFICE	2003-present, Import and Export Inspection
	Division, Food and Drug Administration,
	Thailand.