

DETERMINATION OF TRANS FATTY ACIDS BY MID INFRARED FOURIER TRANSFORM SPECTROSCOPY WITH OR WITHOUT NANOMATERIALS

by

MAYELI PERALTA CONTRERAS

(Under the Direction of Rakesh K. Singh)

ABSTRACT

The main concern in the Food Industry is the strict regulations on presence of *trans* fats and their accurate quantification. This reason has been the driving force for development of alternative methods to find accurate measurements. A partial least square (PLS) model was developed and used to verify the concentration of *trans* fats in margarine, butter and spread and pure vegetable oils, by Fourier-Transform Mid Infrared Spectroscopy (FT-IR) in the wavenumber region 990-940 cm^{-1} . The measurements of the *trans* fat content have shown an accuracy with a correlation coefficient of 0.98 obtained by the PLS model and no presence of outlier between the range of the *trans* fats concentrations studied (0-30%). In separate experiments, the samples were treated with multiwall carbon nanotubes and nano zinc oxide particles to determine the effect of nanomaterials in enhancement of the FT-IR spectra. Margarine, spread and butter showed the increase of the shoulder band at 1075 cm^{-1} and an additional peak at 1050 cm^{-1} as a result of nano carbon and zinc oxide nanopowder treatment, though there was no significant difference in the peak corresponding to *trans* fats. The peak at 1050 cm^{-1}

appears in the zone of the C-O band in the infrared spectra as result of the interaction of the nanoparticles with the food samples.

INDEX WORDS: trans fat; FTIR; nanoparticles; PLS model

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DEDICATION

To my parents.

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Natural oils and fats are liquids or semisolids consisting primarily of fatty acids / triacylglycerols (TAG). These triacylglycerols are found in both plants and animals, and compose one of the major food groups of our diet. The distinction between fats and oils is seen by their physical state at ambient temperature; the fats are solid and the oils are liquid. Greater than 90% of commercial oils and fats used are for human consumption and are plant-derived vegetable oils. Unrefined natural oils and fats, after extraction from the source, the main element is TAG with less than 5% of minor components such as phospholipids, free sterols and sterol esters, tocopherols (tocopherols and tocotrienols), triterpene alcohols, hydrocarbons and fat soluble vitamins.

Fatty acids are ubiquitous biological molecules that are used as metabolic fuels, as covalent regulators of signaling molecules, and as essential components of cellular membranes. It is thus logical that FA levels should be closely regulated. Indeed, some of the most common medical disorders in industrialized societies (cardiovascular disease, hyperlipidemia, obesity, and insulin resistance) are characterized by altered levels of FAs or their metabolites (Kodali & List, 2005). Fats and oils are the raw material for liquid oils, shortenings, margarines, and other specialty or tailored products that are the functional ingredients in food products prepared by food processors, restaurants and in home. Humans have used fats and oils for food and a variety of other applications since historic times, as they were easily isolated from their source. Fats and oils are the

highest energy source of the three basic foods (carbohydrate, protein, and fats) (O'Brine 2004). Hundreds of seed and fruits contain oil, all animals produce fat, and marine sources also provide oils; however, only a few of these sources are of economic importance. Trans fatty acids (TFA) are formed mainly either during the bio-hydrogenation of polyunsaturated fatty acids by ruminant animals or during the partial hydrogenation of vegetable oils. Bio-hydrogenation as well as industrial hydrogenation results in a multiplicity of geometrical and positional TFA isomers (Weggemans et al., 2004). The predominant products are *trans* octadecanoic acid (C18:1t), whereby the *trans* double bond is localized between carbon C6 and C-16 similar to their *cis*-analogues.

The majority of harmful trans fatty acids are contained in processed food items, such as cakes, buns, pastries, spreads, fried foods, crisp, and other savory snacks, which contain partially hydrogenated vegetable oils as a fat source. The hydrogenation of oils such as soybean oil, which is used to produce a more solid fat source with an extended shelf life, can increase the trans fatty acid content of the product by 10-20 fold. The major vegetable oils present in the market are soybean, cottonseed, canola, sunflower, corn, peanut, palm, palm kernel and coconut oils. Other vegetable oils like olive, rice bran, safflower, sesame and other specialty oils are not extensively used due to their availability and cost. There are approximately 21 different fatty acids found in appreciable amounts of foods, which can be classified as saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids (Table 1.1).

1.2. *Trans* Fatty acids

Edible oils such as refined or unrefined walnut, olive, sunflower, safflower, rapeseed, soybean, avocado, peanut oil or coconut oil have only negligible amounts of TFA. The TFA amounts in refined oils are influenced by duration and temperature of refining. Regular margarines contain varying contents of partially hydrogenated vegetable oils, and therefore TFA. The main TFAs in margarines are the 18:1 isomers, which have a double bond position from Δ^6 - Δ^{16} . Approximately 85% of total TFA content in vegetable margarines, sunflower margarines, and fat reduced margarines are 18:1 (Steinhart & Fritsche, 1998). Although the role of dietary lipids in causing vascular disease is now established, an influence of fat intake on cardiac arrhythmias is less well-appreciated (Siscovick et al., 2000). The potential importance of this relationship has been highlighted by researchers (Kopelman, 2000) that suggest dietary *trans*-fatty acids cause sudden cardiac death. *Trans*-fatty acids differ from the natural *cis*-isomers in the conformation around the double bond; in the former, the fatty acyl chains are on opposite (*trans*) sides of the molecule, whereas they are on the same (*cis*) side in the latter. Most dietary *trans* fatty acids are formed when unsaturated fats are “hardened” by hydrogenation and when vegetable oils become hydrogenated during frying; they occur in hardened margarines, fast foods, and some commercially baked goods and salad dressings (Katz, 2002).

The arrhythmogenic potency of these lipids is determined not only by the amounts that reach the heart, but also by their structure; for example, the ability of different FFA to lower ventricular fibrillation threshold depends on the length and saturation of the fatty

acyl chain. A role for FFA intake has been shown in experimental animals, where diets rich in polyunsaturated fats and fish oil were found to have antiarrhythmic effects.

The two primary reasons for hydrogenation are to improve the oxidative stability and to increase the solid fat content (SFC), which are the reasons why the food industry has widely used this process as its main manufacturing technique for the production of vegetable oils. The improved oxidative stability also improves the flavor and the shelf life of the products. The increase of the SFC increases the melting temperature and provides improved food texture and functionality (Kodali & List, 2005). In the case of the autoxidation of oils this is an important deteriorative reaction that has significant commercial implication in the terms of the product value (Man, 1999). It is generally accepted that autoxidation of unsaturated fatty acids proceeds as a free-radical mechanism.

In the hydrogenation process, hydrogen atoms are added to the unsaturated (double) bonds thereby converting them into saturated C-C (single) bonds (Fig 1.1). The hydrogenation is done in the presence of a metal catalyst and pressurized hydrogen gas at high temperature. The level of unsaturation of oil is measured by the adsorption of iodine and expressed as iodine value (IV). Industrial hydrogenation reactions are more commonly carried out in batch reactors with nickel catalyst. Various reactions involved in the hydrogenation of dienes to saturated fatty acids follow the Horiuti-polani mechanism (Horiuti, 1934), because this mechanism proposed a half-hydrogenated intermediate. Even though the addition of hydrogen to the double bonds is the primary reaction during the hydrogenation, other side reactions occur, such as isomerization of double bonds from *cis* to more stable *trans* configuration and migration of the double

bonds along the chain (shifting the position of the double bonds in the chain). Isomerization conditions should lead to an equilibrium between the *cis* and *trans* isomers and this implies an enthalpy difference of 4.1 kJ/ mol (Dijkstra, 2006) as function of temperature. With the current hydrogenation technology, the *trans* formation can be reduced but not eliminated. *Trans* formation increases with hydrogenation temperature but decreases with an increase of pressure, catalyst concentration and agitation. TFAs are also formed in varying amounts during the industrial hydrogenation of vegetable or fish oils. The hydrogenation improves oxidative and thermal stability, in particular for the polyunsaturated oils, which contain linoleic acid. The extreme case is a fully hydrogenated oil that contains zero percent TFA. In practice, the TFA content is limited by the thermodynamics of the *cis-trans* equilibrium to approximately 75% of the total number of double bonds.

1.3. Regulations for Trans Fat

In 1999, the U.S. Food and Drug Administration (FDA) published proposed rules for labeling the *trans* fatty acid content of food products (Kodali & List, 2005). In the 1999 proposal, FDA proposed to amend its nutrition labeling regulation to require, in part that the amount of *trans* fatty acids in a food or dietary supplement be included when the product contains 0.5 or more grams (g) *trans* fatty acids per serving. Specifically, it proposed that the amount of *trans* fat be included in the amount and percent Daily Value (% DV) declared for saturated fatty acids with a footnote indicating the amount of *trans* fatty acids in a serving of the product. In the 1999 proposal, FDA concluded that dietary *trans* fatty acids have adverse effects in blood

cholesterol measures that are predictive of CHD risk. Consequently, to avoid misleading claims, the agency proposed that the amounts of trans fatty acids be limited wherever saturated fat limits are placed on nutrient content claims, health claims, or disclosure and disqualifying levels.

The relationship between dietary fats and health and disease is one of the most active areas of biochemical, nutritional, and medical research. One of the main protagonists has been the conjugated linoleic acid (CLA), especially in the cis-9, trans-11, and cis-12, trans-10 isomers, considered essential to human diet (Kulmyrzaev et al., 2007). Continuing interest in Trans fat labeling and discussions of the nutritional significance of trans fatty acids have prompted efforts to develop more efficient methods for rapidly determining the trans fat content of foods. The necessity of the food industry for procedures that assure quality control in the process as well as finished products motivated the use of techniques from a wide spectra of fields such as the analytical techniques and one of the biggest problems is the detection o the trans fatty acids (TFA) and is becoming a major concern since it impacts in the health of consumers.

1.4. Estimation methods of *Trans* fats

For several decades analytical methods, such as gas chromatography (GC) (AOCS, 1998) and infrared spectroscopy (AOAC, 2002), for determination of total trans fatty acids in partially hydrogenated vegetable oils have been widely used and repeatedly modified in order to improve accuracy. Researchers have found that capillary GC separations can be optimized by highly polar stationary phases for the accurate determination of trans fatty acids in partially hydrogenated vegetable oils and in refined

oils (deodorized or stripped) oils, it has also been found that the measurement can be improved with long capillary columns (50 to 100 m) which provides better accuracy and resolution for the separation of the complex cis/trans mixture in the hydrogenated fat (Ulberth 2001). However the main disadvantage for the capillarity column method is that some cis/trans octadecenoates co-elute (Ratnayake & Beare-Rogers, 1990). In such cases it is necessary to optimize the method where the GC separation is preceded by silver-ion chromatography plates impregnated with AgNO₃ or silver ion (Ulberth & Haider, 2001).

The GC Official Method AOCS Ce 1c-89 for partially hydrogenated vegetable oils underestimates some trans C18 monoene (C18:1) positional isomer in favor of cis 18:1 isomers, with which they overlap. Several researchers have often compared the determination of total trans fatty acids by GC and IR techniques (Mossoba et al., 1999), and it was found that for the GC technique the amount reported as *trans* fat was lower than the reported by the IR spectroscopy technique. It has been found that for the IR Official Methods, such as AOAC 994.14 and AOCS Cd 14-95, are not fully satisfactory because the problem was that the method report relatively higher trans levels, particularly below 5%. One of the fundamental problems is that at 966 cm⁻¹ the overlap of the trans absorption by other bands in the spectra produces strongly sloping background that converts the trans band into a shoulder at levels below 2% and reduces the accuracy of the determination (Steinhart & Fritsche, 1998).

Therefore, many procedures have proposed changes to overcome these problems. This optimization of the method include options such as applying arithmetical compensation to eliminate biases, using double beam differential spectrophotometers to eliminate background interferences (Huang & Firestone, 1971), applying the internal

absorption band rationing procedure (Belton et al., 1988), eliminating the volatile toxic carbon disulphide solvent by using attenuated total reflection (Dutton, 1974; Mossoba et al., 2003) or 0.1 mm transmission cells, modifying the calibration procedure for two component or multiple component instead of calculating the absorptivity of a standard (Firestone, 1965; Huang & Firestone, 1971), and applying partial least-squares chemometric procedures (Ulbert & Buchgraber, 1992) or post-measurement spectral subtraction manipulations (Toschi et al., 1993).

To eliminate the strong overlapping background of the 966 cm^{-1} trans band, the single beam spectrum of the trans fats is “ratioed” against that of an unhydrogenated oil or a reference cell containing only *cis* double bonds. Thus, a symmetric absorption band on a horizontal background is obtained (Fig 1.2).

The area under the *trans* band can be accurately integrated between the same limits, 990 and 940 cm^{-1} , for all the trans levels. In order to speed up the analysis it is used, into which oils, melted fats or their methyl esters are poured without weighing or by quantitative dilution with carbon disulfide.

In general the main advantage for IR spectroscopy techniques is that it is a non-destructive technique and thus another nutritional constituent can be measured in foods since it is a complex system. One of the methods that have been widely used in food applications is FT-IR, which has been successful for the quantification of conjugated linoleic acid (CLA). Another application is the quantification of fat in milk and *trans* fatty acids with the application of chemometrics. The main advantage with this analytical method is automated, robust and rapid (<2 min/sample) measurement compared to the AOCS method (Kulmyrzaev et al., 2007).

1.5. Nanotechnology approach to food science applications

Recent studies have reflected different perspectives about Nanotechnology (Moraru et al., 2003; Hirose, 2005; Berne, 2006). Some researchers consider the study of microstructures of materials using electron microscopy and the growth and characterization of thin films to fall under the category of nanotechnology. Other researchers consider a bottom-up approach in materials synthesis and fabrication, such self assembly to form specific nanosensors (Kopelman et al., 1998; Kisaalita et al., 2006), or the ability to work at the atomic, molecular and supramolecular levels (on a scale of 1–100 nm) in order to understand, create and use material structures, devices and systems with fundamentally new properties and functions resulting from their small structure (Roco, 2003). The U.S. National Nanotechnology Initiative has defined Nanotechnology as the science, engineering, and technology related to the understanding and control of the matter at approximately 1-100 nm (NSTC, 2005).

All biological and man-made systems have the first level of organization at the nanoscale (such as a nanocrystals, nanotubes or nanobiomotors) where their fundamental properties and functions are defined. The goal of nanotechnology might be described by the ability to assemble molecules into objects, hierarchically along several length scales, and to disassemble objects into molecules.

Molecular self assembly is a powerful approach for building nanostructures through “bottom up” procedures (Wang, 2006). Nanofibers, bionanotubes, nanowires, protein scaffolds, microribbons and nanocages are possible to manufacture and assembly with different biomolecules such as amphiphilic peptides, lipids and proteins. The links

between molecules are possible to “weak” molecular interactions such as hydrogen bonds, hydrophobic interactions, ionic bonds, and van der Waals interactions.

This kind of material can be prepared by nebulized spray pyrolysis and the alignment process using acetylene and ammonia mixtures. It can be also prepared by the pyrolysis of mixtures of organometallic precursors and hydrocarbons. One would expect that transition metal particles produced in situ, not only nucleate formation but also align themselves. Therefore, the diameter of the rods can be controlled by changing the thermal transfer and diffusion (Rao, 2005). One of the advantages for this material is the specific functionalization, in the specific case a single carbon nanotube, which is a cylindrical aromatic macromolecule, and is chemically inert without functional groups attached. In their geometry the curvature-induced pyramidization of the pi orbital of the carbon atoms induces local strains and the nanotubes are expected to be more reactive than a flat graphite sheet.

The applications of these new materials can be related to different disciplines such as medicine, agriculture, biotechnology, drug delivery and bioprocessing (in industry) provide creative solutions and a great potential. Although nanotechnological applications have been less common in Food Science, many investigators are recently interested in the properties of the nanoparticles (Fellman, 2001; Pompeo & Resasco, 2002) and nanosensors for the development of different areas such as: food safety (Hartley & Baeumner, 2003), flavor technology (Moraru et al., 2003), and food analysis (Baeumner, 2003). In the specific case of the carbon nanotubes, it have been reported that the carbon nanotubes can detect beta alkaloids in foods and beverages with a electrochemical

modification (Sedeno et al., 2007), and sulfamides in food of animal origin with a online solid extraction (Wang et al., 2006).

The better understanding of the properties of macromolecules makes it possible for the manipulation of molecular conformation to deliver active compounds improving more efficient relationships with food systems (Berne 2006) as well as the interaction with the nutrients. The enzymatic interesterification of fat reactions has increased interest in both academia and industries (Xu, 2000; Xu et al., 2005). The nanoapplications of existing food analysis techniques would bring a new useful tool for monitoring and detecting different element in the compositions as well as the different alterations that can be derived during the food processing (Li, 1999).

The field of the spectroscopic research is currently experiencing dramatic technological development, and the use of spectroscopy in novel applications is growing rapidly. Some of the applications have been focused to the molecular study of surfaces in biomolecules such as phospholipids' membranes (Granick & Xie, 2002), proteins, and other biological systems. Many researchers have been involved in biotechnology (Lowe, 2000; Sarikaya et al., 2003) at the nanoscale level, therefore, the resulting biological applications holds the promise of revolutionary changes into several aspects of biology ranging from fundamentals questions of receptor function to drug discovery and personal medicine (Rosenthal & Wright, 2005). Since agriculture and food science are mostly involved in biological systems, it is a fact that in the near future, the influence of this novel technology in the food science field will be come more reliable and common (Fig 1.3).

1.5.1 Food safety

Several factors make nanotechnology promising for expanding the possibilities in Food Science. First, because nanotechnology focuses on smaller physical/biological structures, it is ideal for analyzing specific pathogenic organisms, for example *Bacillus anthracis* spores with an amplification reaction (Hartley & Baeumner, 2003). The principle that makes biosensor technology works includes developing a technique using pathogens such as *bacillus anthracis*, Dengue virus and generic *E. coli* (Baeumner, 2004) as model analytes, then fabricating a bioanalytical microsystem with the help of nanotechnology, containing a microfluidic biosensor with all the desired characteristics for the pathogen detector. The nanosensors open the possibility of more precise tools for detection in specific areas like biosafety, biosecurity and clinical diagnostics, therefore most of the success for these areas are based on the development of sensors specificity for rapid detection of biological agents in food or water. Fellman (2001) improved a method to produce nanoparticles with a triangular prismatic shape that can be used in detecting biological threats such as anthrax, smallpox, and tuberculosis.

1.5.2 Food analysis

The better understanding of nanoscale materials' properties of macromolecules makes the manipulation of molecular conformation possible to deliver active compounds improving more-efficient relationships with food systems (Berne, 2006: Honda & Fereidoon, 2004). The importance to incorporate nanoapplications with existing food analysis techniques, it would bring a new useful tool for monitoring and detecting

different elements in the compositions as well as the different alterations on composition that can be derived for the processing such as trans fatty acids (Li, 1999).

The field of the spectroscopic research is currently experiencing dramatic technological advancement, and the use of spectroscopy in novel applications is growing rapidly. These remarkable developments have created a need for an up-date and more precision in the measurements. Among the different spectroscopic methods in food analysis are: Nuclear Magnetic Resonance (NMR) for food additive and contaminants (Stefanaki & Tsatsou-Dritsa, 2003), Gas chromatography for fatty acids, Super critical fluid chromatography for detection of non volatile compounds, Raman spectroscopy and Fourier transform infrared spectroscopy (FT-IR) which can be used for identification, confirmation, and quantification of analytes in complex food (Mossoba et al, 1999).

Successful applications of Fourier transform infrared spectroscopy (FT-IR) in the field of edible oils and fats analysis (Xu et al., 2005) imply potentially utilities for trans fatty acids detection. However, the central issue for this technique is the quantification part due to the quality of the signal at the *trans* bond region (Li, 1999; Knothe, 2000; Xu, 2000), that is in a 966 cm^{-1} wavelength. Thus by using nanoparticles the signal to noise ratio can be enhanced further to identify the *trans* fatty acids at a lower concentration in food system.

Thus the big challenge in the present context is the fast detection methods for food applications, delivering and maintaining quality as well as safety for the consumers. Nanotechnology brings powerful tools to Food Science research and opens up the possibility of applying this new method not only for research but also for practical purposes in order to guarantee healthy and safe food.

One of the most important characteristics in a food product is the flavor release because it is one of the key elements that determine the quality of food more pleasurable or palatable (Reineccius, 2006). For that reason, there is great interest in the improvement of flavor release techniques among the food science community. The application of nanoparticles can be also related with flavor encapsulation, due to the exceptional small size of the particles that can be generated by liquefying a high melting fat as an emulsion droplet by cooling from lipid phase to solid lipid (Porzio, 2004). In addition, microencapsulation could confer better stability to specific molecules such as the conjugated linoleic acid by preventing contact with oxygen and light. It also produces a free-flowing powder which can be readily incorporated into foods. The functional properties of the microencapsulated material is influenced by the composition of the wall, having good emulsion-stabilization properties, low viscosity at high concentrations and effective redispersion from the core material on rehydration. It has been reported that the implementation of an accurate spray freezing for production of microparticles resulted in improving the ability to produce large surface area with minimal aggregation in the case of the linoleic acid (Jimenez et al., 2004), and it is also possible to produce stable protein nanostructured particles, with spray freezing technique into liquid nitrogen in order to encapsulate uniformly into microspheres to reduce a release over the first 24 h with a large surface area particles due to ultra-rapid freezing and the absence of a liquid-air interface (Leach et al., 2004). Thus, it may be an advantage in many drug delivery applications including depot delivery from biodegradable microspheres.

1.6. Objectives:

1. Determination of *trans* fats by Fourier Transform Infrared spectroscopy and to quantify and validate the *trans* fats by developing a partial least square (PLS) model.
2. To study the effect of pretreatment of fat samples with nanomaterials on enhancement of FT-IR spectral peaks.

Table 1.1 The principal fatty acids of the diet

Fatty acid	Structural title
Saturated	
Lauric	C12:0
Myristic	C14:0
Palmitic	C16:0
Stearic	C18:0
Monounsaturated	
Oleic	C18:1 <i>cis</i> (n-9)
Elaidic	C18:1 <i>trans</i> (n-9)
Vaccenic	C18:1 <i>trans</i> (n-11)
Polyunsaturated	
Linoleic	C18:2 <i>cis</i> (n-6)
Alpha-Linoleic	C18:3 <i>cis</i> (n-3)
Arachidonic	C20:4 <i>cis</i> (n-6)
Eicosapentaenoic acid	C20:5 <i>cis</i> (n-3)
Docosapentaenoic acid	C22:5 <i>cis</i> (n-3)
Docosahexaenoic acid	C22:6 <i>cis</i> (n-3)

(Minihane Anne M., 2007)

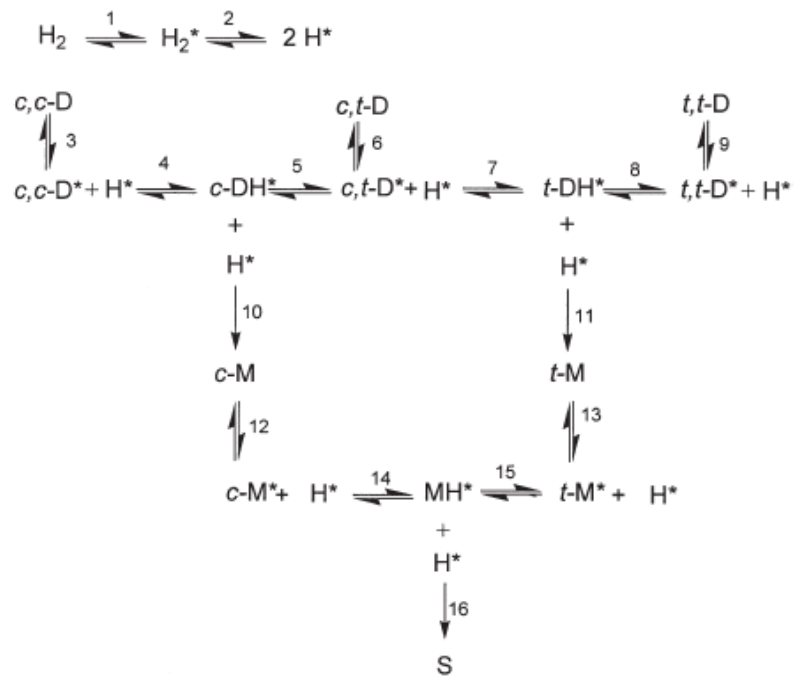


Fig. 1.1 Hydrogenation of unsaturated fatty acids. (Horiuti, 1934)

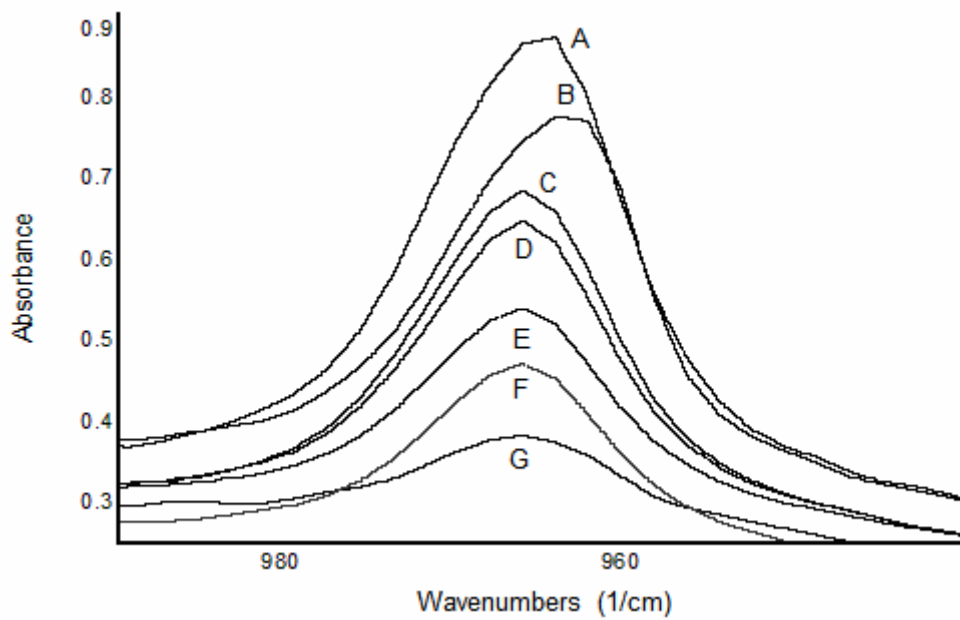


Fig. 1.2 Spectra of fatty acid (Trielaedin) at different concentrations. A: 50 %, B:40%,C:25%,D: 20%, E :10%, F: 7%, G:3%.

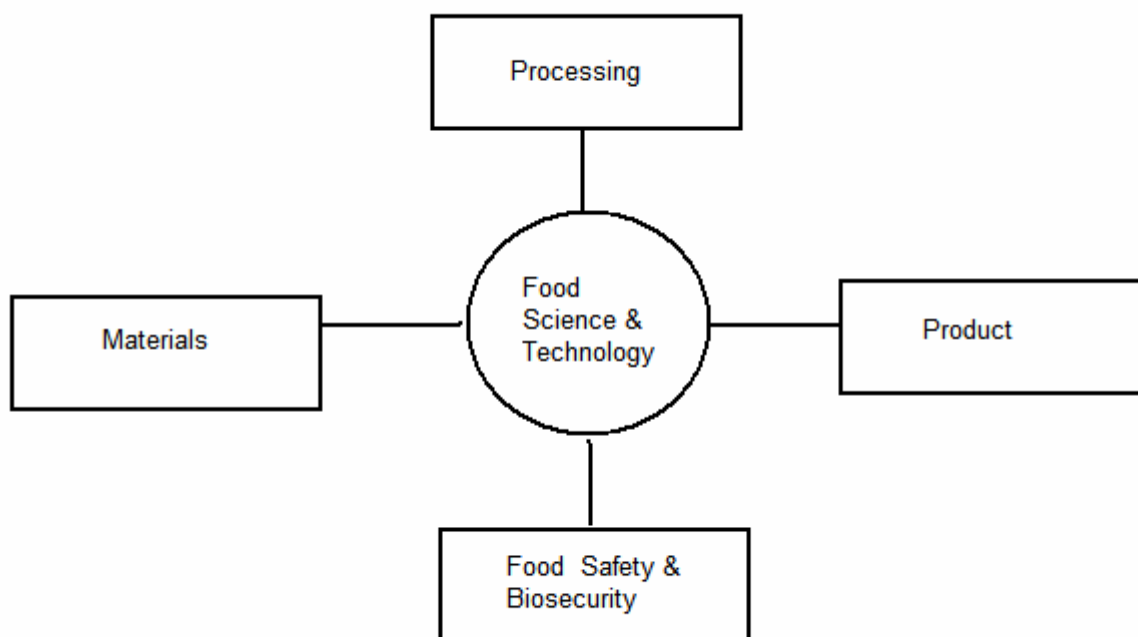


Fig. 1.3. Application matrix of nanotechnology in food science and technology

CHAPTER 2. MATERIALS AND METHODS

2.1 Materials

Margarine, spread, butter, olive oil and sunflower oil were purchased from local stores and stored under refrigeration until the experiment was performed. Multi walled carbon nanotubes (length 60 nm; dia 10 nm) were procured from Nanocyl S.A., Belgium. Nanoparticles of Zinc Oxide (particle size 50-70 nm) were purchased from Sigma, USA.

2.2 Attenuated total reflection (ATR)- FTIR analysis of trans fats

2.2.1 FTIR measurements.

Infrared analyses were performed with a Fourier Transform Infrared Spectrometer (Thermo Nicolet 6700 FTIR, USA) equipped with deuterated triglycine sulfate (DTGS) detector. FT-IR operating parameters used were as follows: 4000-350 cm^{-1} wavelength range with 32 scans collected and averaged; resolution of 4 cm^{-1} , and a Happ-Genzel apodization function. Oils / melted fats were deposited using disposable pipettes onto the ZnSe Smart ARK ATR cell until the sample completely covered the surface of the cell. The single beam sample absorbance spectrum was collected. A background was taken before spectrum of each sample was collected. The crystal was cleaned between runs by rinsing with 70% ethanol and wiping with soft lint-free tissue paper. This procedure was followed for the calibration and validation standards.

2.3 Treatment with nanomaterials

The oil samples were treated with nanoparticles (carbon nanotubes and nano ZnO) at different levels. The nanoparticles were mixed with Triton-X (1 mg/ml), and 4 ml of

ethanol (70%) , and the final mixture was placed in a sonicator for 5 minutes. One ml of this solution was added to 2 ml of the oil sample in a test tube, mixed thoroughly, vortexed for 10 s or until the mixture became homogeneous, followed by storage for 4 h under refrigeration. Then, the refrigerated samples were placed in a water bath at 50 °C for 20 min. Three hundred µl of this sample solution was drawn with a micropipette to determine the spectra by FT-IR.

2.4 Preparation of calibration curve

Standard solutions of *trans* fats were prepared by mixing Trielaidin (TE) and Triolein (TO) to get a range of 1-50 % *trans* fat (Table 2.1) as percent of total fat (AOAC 2002). The glycerides standards (Trielaidin and trioleins) were melted before the preparation of the calibration curve.

2.5 Partial Least Square Model

The analysis model employed was Partial Least Square Regression (PLS) to quantify the percentage of *trans* fats (Fig. 2.2). The model was developed with TA Analyst v6 (Thermo-Nicolet) software package. Calibration model between chemical data and FTIR spectra were developed using PLS regression with cross validation. The optimum number of terms in the PLS calibration model was determined by the PRESS (Predicted Error Sum of Squares) function in order to avoid over fitting of the models (Cozzolino et al, 2005). This process was repeated until all the calibration and validation samples had been recorded in the model once (Fig. 2.3). The spectral region (990-940 cm^{-1}) where the

variations were expected was chosen for developing the PLS model analysis with four factors.

2.6 Validation of samples

Known quantity (0.1 g) of trielaidin (TE) was added to olive oil in different ratios in order to get series of dilutions with different levels of % *Trans* fat. Samples were validated with the data obtained on integrated area under the absorption band at 966 cm^{-1} when each combination of TE and olive oil was subjected to FT-IR analysis.

Table 2.1 Trans fats calibration

TO	TE	% Trans fats
0.297	0.003	1
0.285	0.015	5
0.27	0.03	10
0.24	0.06	20
0.21	0.09	30
0.18	0.12	40
0.15	0.15	50

TO: Triolein. TE: Trielaidin

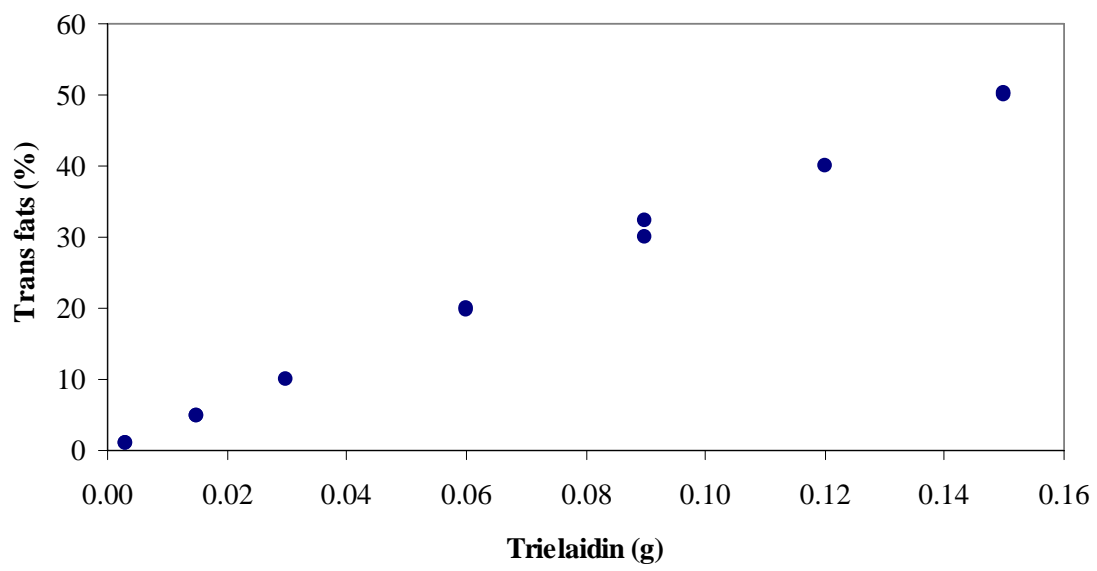


Fig 2.1 Trans fats levels as a function of Trielaidin amount (grams). The values of the trielaidin are based on the trans fat calibration table.

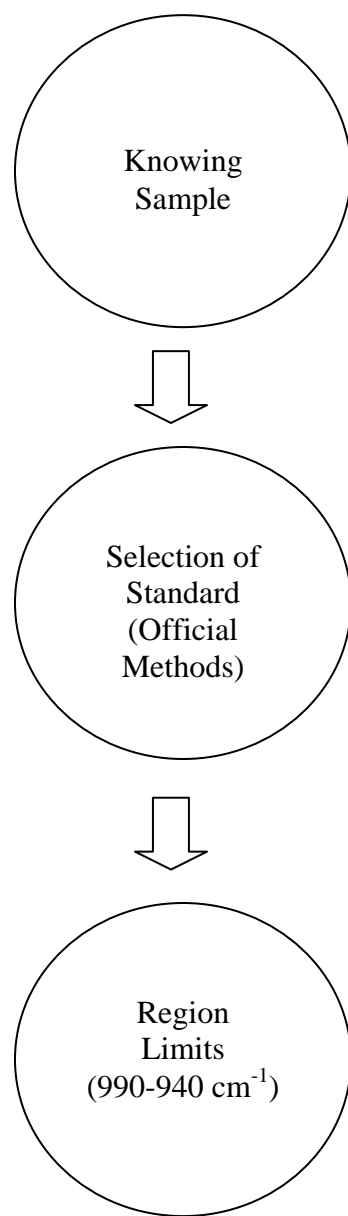


Fig. 2.2. Information for the PLS method. For the quantification of trans fats is necessary to know enough information about the samples, the component that is necessary to measure based on the Official Methods of Analysis for Trans Fats (2002).

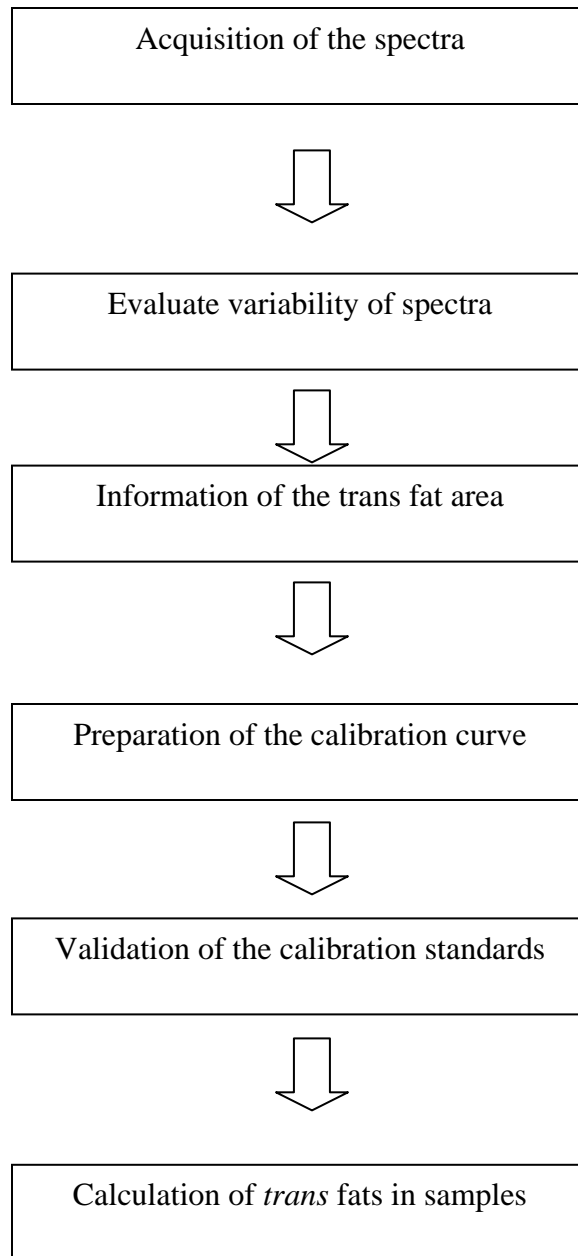


Fig. 2.3. Steps for the PLS algorithm.

CHAPTER 3. RESULTS AND DISCUSSION

3.1 Results

In the typical spectra of the vegetable oils and shortenings, the main functional groups were studied in the mid infrared region with special focus on spectral peak related to *trans* fatty acids (the spectra of saturated oil is shown as additional spectra in Appendix D). Researchers have made assignment bands for functional groups in oils and fats in the mid infrared spectra (Guillen & Cabo, 1997a; Safar et al, 1994).

The spectra of the trielaidin and triolein glycerides are shown in figure 3.1 and 3.2 with the characteristic peak of the *trans* double bond at 966 cm^{-1} wavenumber. The typical spectra of shortenings and the vegetable oils analyzed in the present study are shown in Figs 3.3-3.7. The prominent peaks due to the C-H bending and C-O-C stretching can be found in the region of $900\text{-}1400\text{ cm}^{-1}$. The C=O stretching is seen in the region of $2800\text{-}3100\text{ cm}^{-1}$. The small absorption band observed at 1400 cm^{-1} is related to O-H stretch. The wave number for the *trans* fats peak (966 cm^{-1}) was based on the C-H out of the plane that is a unique characteristics of isolated double bonds with *trans* configuration (Tay *et al*, 2002). Peaks corresponding to *trans* bond (966 cm^{-1}) were conspicuous in margarine, spread, and butter whereas the spectra of olive oil sunflower oil did not show any peak at 966 cm^{-1} , indicating their absence in oils.

The analyzed samples have shown the typical functional groups that can be in edible oil spectra. Guillen and Cabo (1997b) reported bending vibrations of the methylene group which are produced between 1350 and 1150 cm^{-1} , and also stretching bands for

vegetable oils for -C-O bond of esters are around 1118 and 1097 cm^{-1} with a medium intensity. The C-C(=O)-O band of saturated esters usually appear between 1240 and 1163 cm^{-1} and the unsaturated esters the vibration is produced at lower frequencies. In this context, the presence of the band at 914 cm^{-1} , which appears in some oil samples, has been related to the bending vibration of *cis*-disubstituted olefinic groups (Van de Voort et al, 1995).

Trans fats of shortenings and oil samples can be quantified for a correlation of the area under wavenumbers of $990\text{-}940\text{ cm}^{-1}$ considered as a baseline (Table 3.2) but the variation can be decreased through PLS method. The results of the measurements of the trans fat content of the food samples are given in Table 3.1. The hydrogenation process of vegetable oils in spread and margarine and the trans fats present in animal fat (butter) are responsible for exhibiting higher levels of trans fats in these products.

3.2 Analysis of data by PLS method

For the current experiment, the spectrum from the samples was analyzed by a calibration model that was built with the PLS method with a “cross validation” sample set and defined by the PRESS function (Predicted Error Sum of Squares) in order to choose the better number of representative factors, appropriate for the food matrix of analysis (Fig 3.8).

For building the method, the factors to represent the variability of set samples was determined by PRESS algorithm. This method considers three principal components from the spectra of the samples, the principal component for this study was considered by the method as the distance in determined group of values in the 966 cm^{-1} wavelength. Due to the multivariable nature of this type of models, the direct relationship between the

spectral response and the constituent concentration (univariate) is not the main concern. These models do not examine the absolute relationship between the values; instead they calculate the relative change in the spectra and attempt to correlate that to a corresponding change in the constituent concentrations. This is why the model can be considered as robust and is able to calibrate for the constituent of interest in the present study (*trans* fat region with peak at 966 cm^{-1}) even in the presence of many other interferences or functional groups. Thus, the representative variation of study was for the *trans* bond in the infrared spectra. This was the main distinction considered for the calibration of standards and with this principle was analyzed the variations between the concentration of the standards. Fig 3.11 is a plot of the chemical reference, the actual concentration of the Trielaidin standard against the values calculated in the independent validation set.

The plots illustrate the relationship between the chemical data and the PLS calibration as well as the presence of the outlier samples (Fig 3.9), which represent a typical sample included in the population.

The high R^2 value (Correlation Coefficient: 0.98) found in the calibration indicated that the model proposed by the PLS method was able to predict the 98 % of viability of oil samples in this experiment on *trans* fats of lipids. After completion of the model building, the model was validated and the variation between the readings of the actual concentrations of the samples against the values estimated for the PLS model had a relative error of 0.02% (Fig 3.10). The validation samples in the model have a range of *trans* fats concentration between 10.2 -31.8 %.

3.3 FT-IR analysis of nanomaterial treated samples

3.3.1 Treatment with carbon nanotubes

Margarine, spread and butter treated with multiwall carbon nanotubes have shown peak at 966 cm^{-1} , corresponding to *trans* fats and the peak intensity of all the samples reduced as a result of treatment (Figs 3.12-3.16). However, an additional sharp peak at $1050\text{-}1045\text{ cm}^{-1}$ was formed in the treated samples, which was not present in the native samples. This could be the characteristic absorption peak of aliphatic functional groups that appeared at the wave number between $1160\text{-}1060\text{ cm}^{-1}$ (Skoog et al 2004). Olive oil and sunflower oil samples which did not contain *trans* fats also showed the additional peak at 1050 cm^{-1} , as a result of nano carbon treatment. Further, the peaks at 1075 cm^{-1} observed in the native samples were found to be sharpened with nano carbon treatment in all the samples studied. Researchers have reported for edible oils that the O-C-C band of esters derived from primary alcohols appear in the zone between 1064 and 1031 cm^{-1} , while for those derived from secondary alcohols, the band appears approximately at 1100 cm^{-1} . Both kind of esters are present in triglyceride molecules (Guillen & Cabo, 1997b). Ahmed (2005) had reported close to the 1100 cm^{-1} wavenumber the presence of gamma tocopherol peaks in the infrared spectra of soybean. Shift in wave number of the 1075 cm^{-1} peak was also conspicuous as a result of treatment. It can be related to some interaction with functional group of the fatty acids.

The studies thus showed that nanocarbon treatment did not enhance the area/intensity of the peak corresponding to *trans* fats. Further studies to investigate this phenomenon will help in understanding the mechanism aided by nanomaterial and also to

introduce new materials of appropriate source to enhance the spectral peak corresponding to *trans* fats.

The interactions of oil with nanomaterials samples were monitored by the zeta potential at room temperature. The zeta-potential is the term describing the electrokinetic properties at determined position of the solid–liquid interface which is accessible for interactions (Stana-Kleinschek & Ribitsch, 1998).

When the voltage was applied to the solution in which the particles were dispersed, the particles are attracted to the electrode of the opposite polarity, accompanied by the fixed layer and part of the diffuse double layer. The zeta potential is the electrical potential of the inner area. As this electric potential approaches to zero the particles tend to aggregate. The readings given by the electrode were zero therefore the interactions weren't strong enough to detect a charge change of the surface between the lipid and the nanoparticle (Fig. 3.15-3.25).

3.4 Treatment with nano ZnO

No apparent changes were observed in the peak corresponding to *trans* fats (966 cm^{-1}) in nano zinc oxide treated margarine and spread samples, whereas the peak became inconspicuous in butter samples as a result of nano zinc oxide treatment (Fig 3.26-3.30). However, additional peak for all the above samples at different wave numbers were observed. Nano ZnO treated samples of margarine, spread and butter showed an additional peak at 1050 cm^{-1} , and the increase of the intensity of a shoulder band at 1075 cm^{-1} , respectively. The shift in wavenumber would have resulted in appearance of additional peaks which may require further studies to confirm the peaks.

Table 3.1 Trans fats values of oils and shortenings

Sample	Trans fats (%)	SD
Olive oil	-	-
Sunflower	-	-
Butter	7.23	0.4
Spread	26.07	0.12
Margarine	26.37	0.52

SD: Standard Deviation

Table 3.2 Measurements for the characteristic trans fat peak obtained by electronic integration of the spectra

Sample	Heigh*	Wavenumber (cm ⁻¹)	Area (cm ⁻¹)	Baseline
butter	0.116	966.11	2.356	989-941
	0.117	966.11	2.298	989-941
	0.116	966.11	2.294	989-941
margarine	0.387	966.17	6.318	989-941
	0.383	966.17	6.226	989-941
	0.378	966.17	6.249	989-941
spread	0.458	964.25	7.24	989-941
	0.376	966.17	6.102	989-942
	0.352	966.17	5.764	989-943

*Height of Absorbance peak (dimensionless)

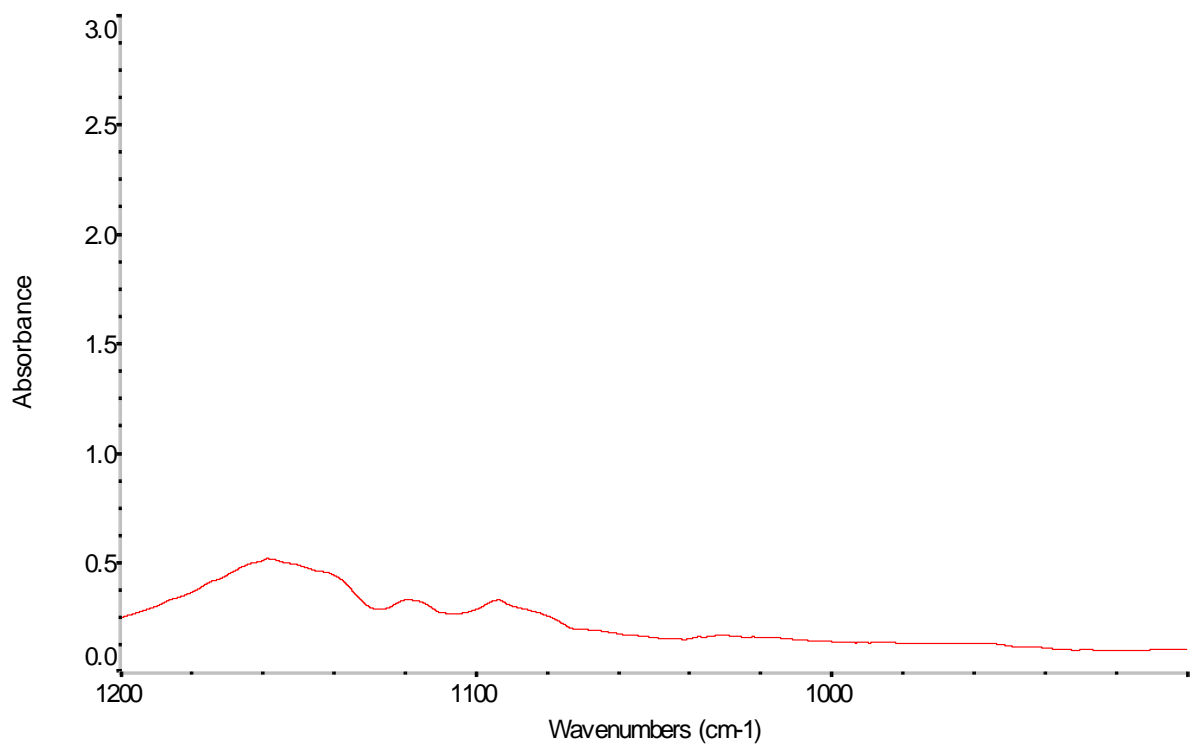


Fig. 3.1 FT-IR spectra of Triolein. This standard contains the *cis* double bond isomers.

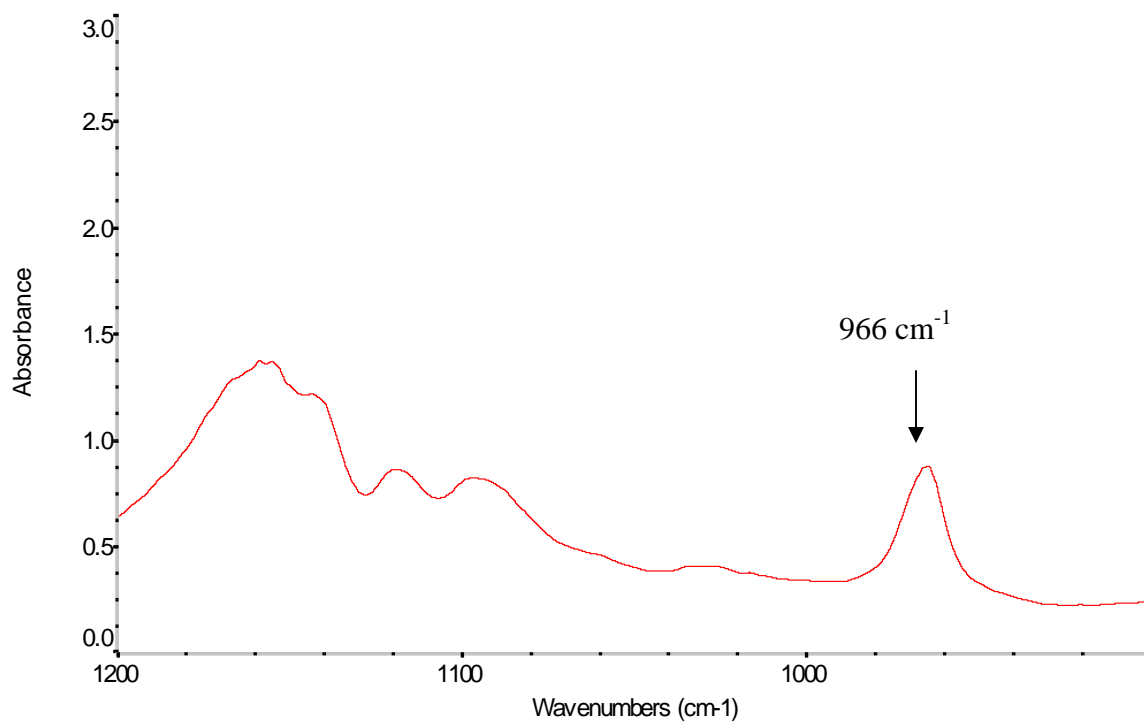


Fig 3.2. FT-IR spectra of Trielaidin. This standard contains the trans double bond isomers.

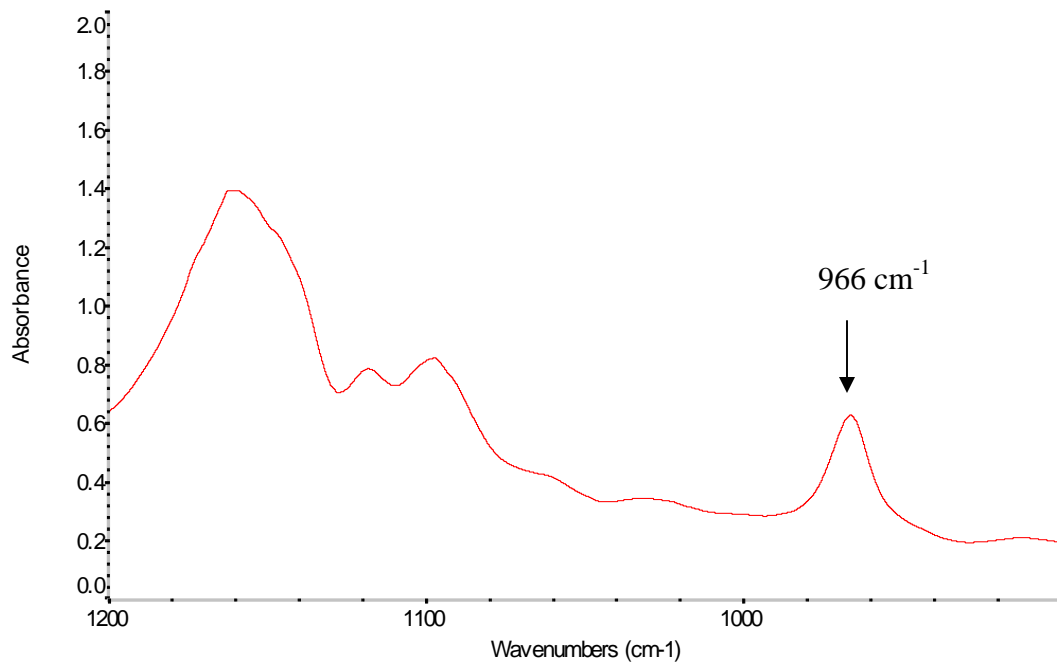


Fig 3.3 FT-IR spectra of Margarine. The margarine analyzed has as ingredients partially hydrogenated soybean oil, soy bean oil, water, salt, whey, soy lecithin, potassium sorbate, citric acid, and beta carotene.

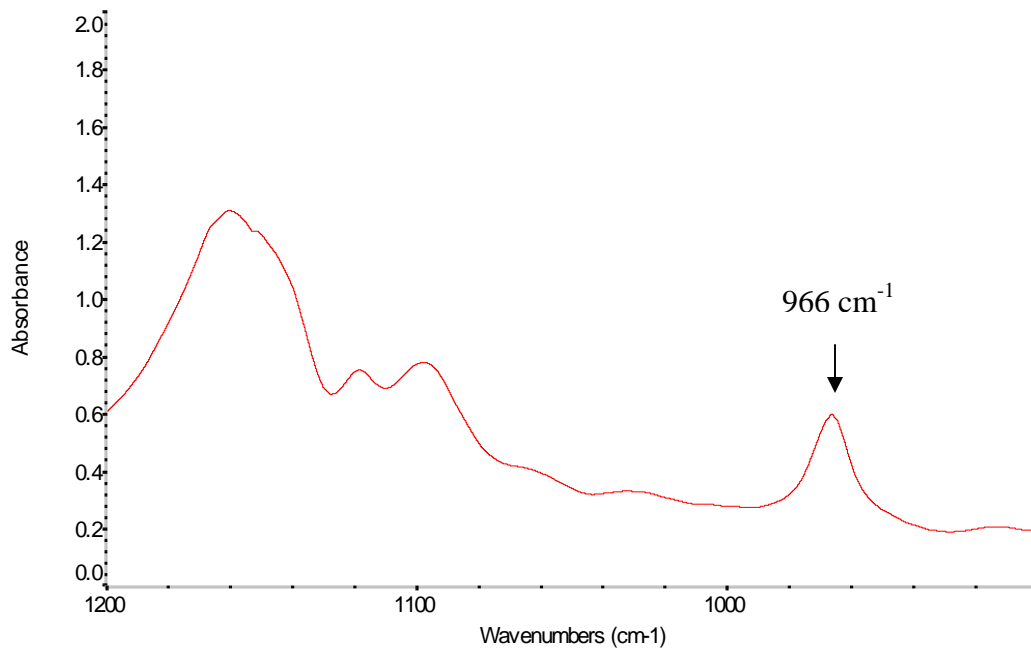


Fig 3.4 FT-IR spectra of Spread. The composition of the spread is 65 % vegetable oil.

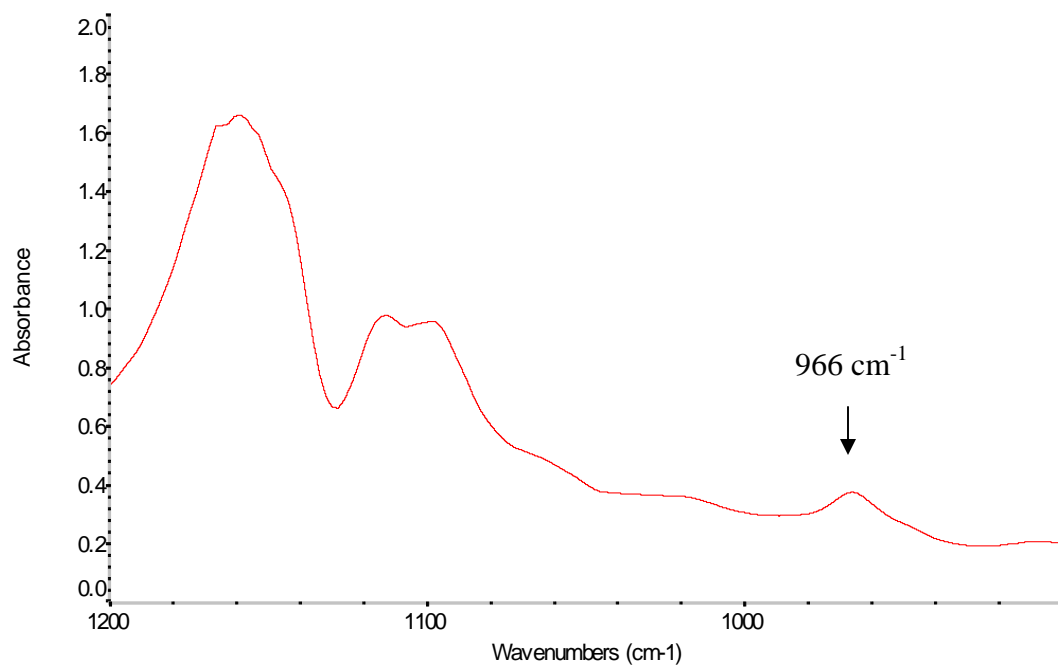


Fig 3.5 FT-IR spectra of Butter. The butter has an ingredient pasteurized sweet cream and salt. Commercial Brand: Land O Lakes.

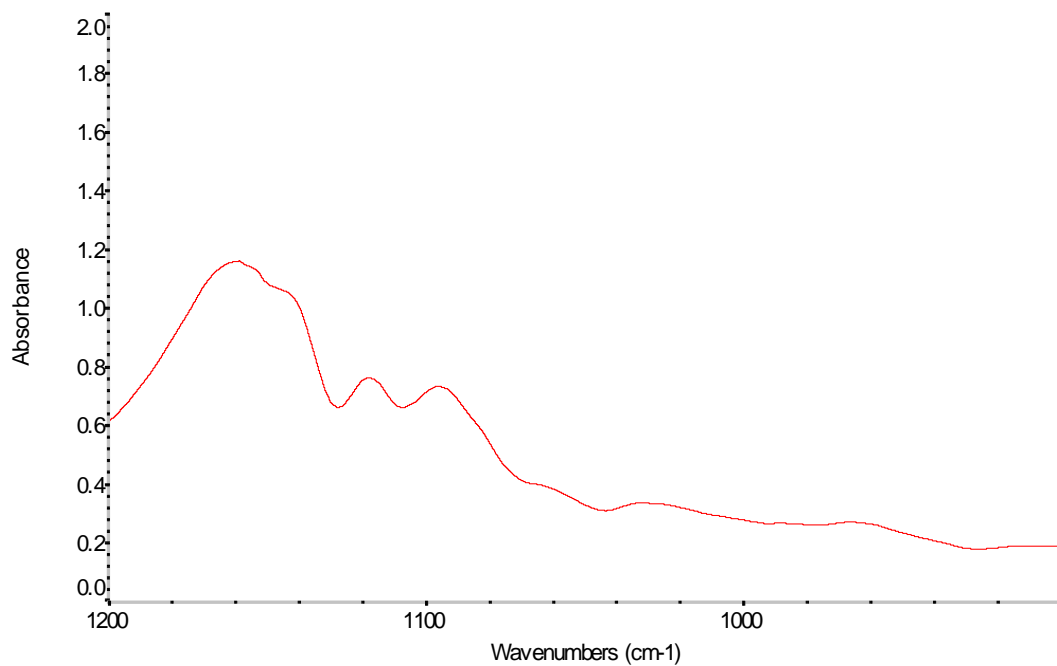


Fig 3.6 FT-IR spectra of Olive oil. This oil is characterized by a high level of oleic acid and it contains squalene at high level (150-170 mg/100 ml) than other vegetable oils (usually only 5-50mg/100ml)

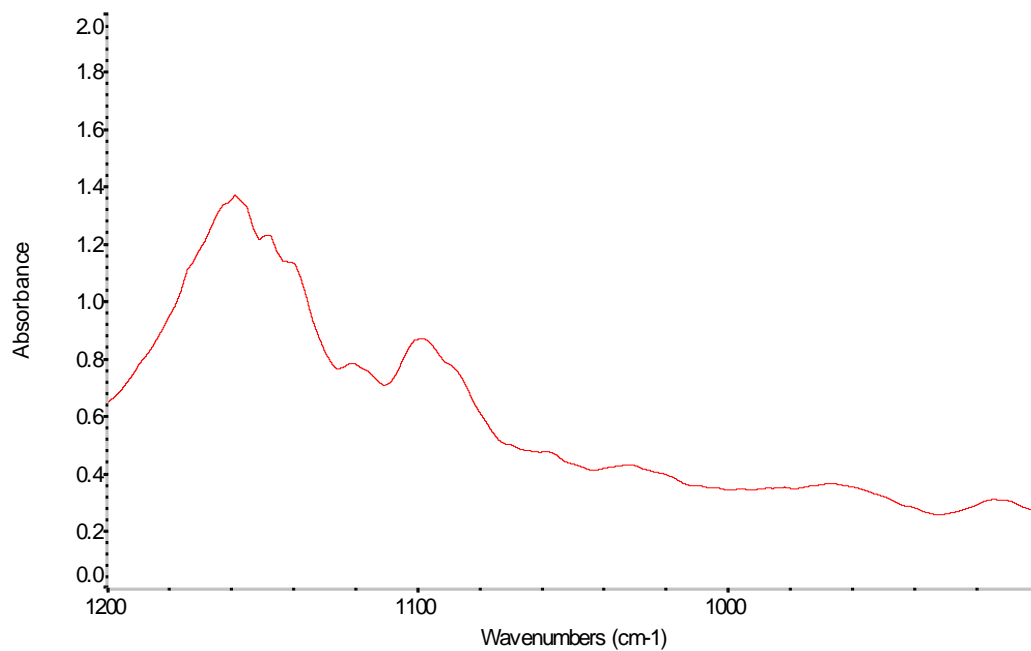


Fig 3.7 FT-IR spectra of Sunflower oil. The oil contains 60-75 percent of linoleic acid . It is widely used for cooking and valued as important component of soft spreads.

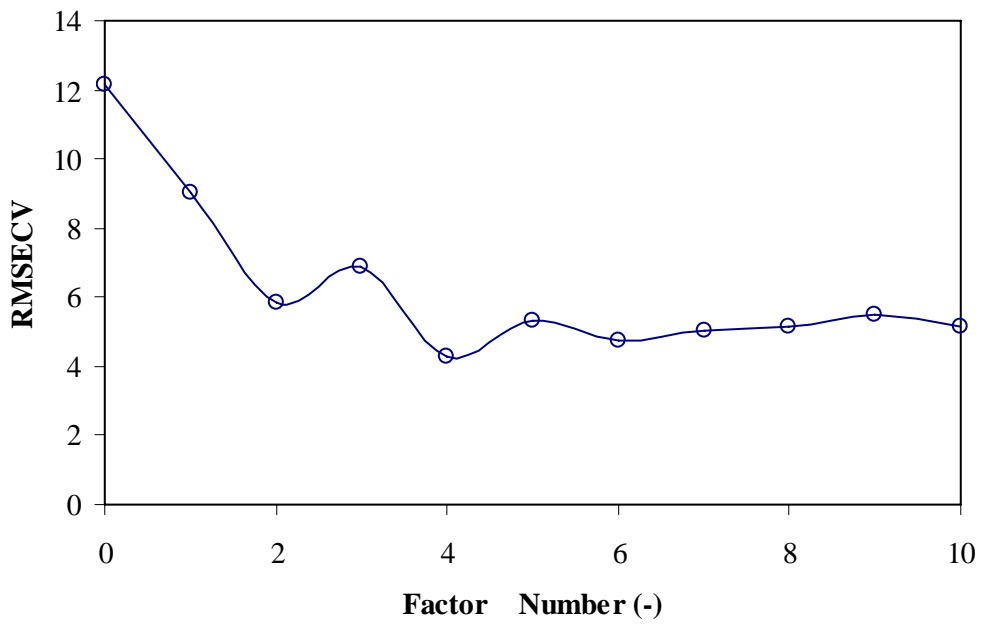


Fig 3.8 Typical PRESS algorithm performed for the evaluation of the model.

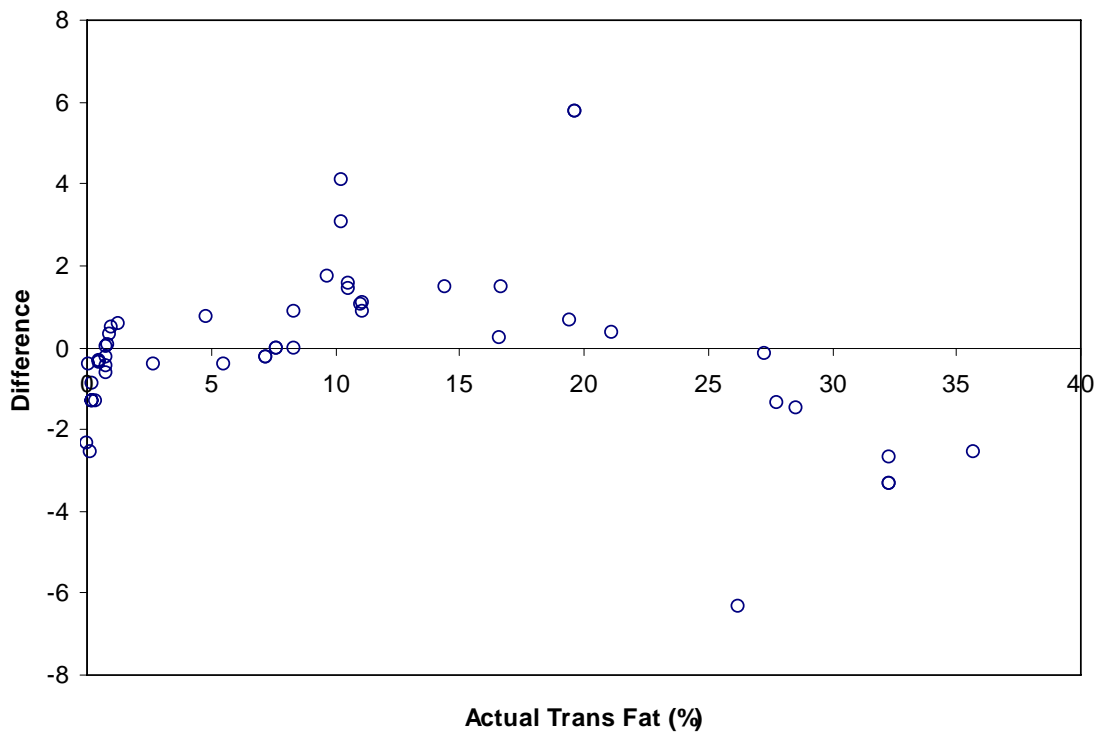


Fig 3.9 Outlier diagram for PLS model.

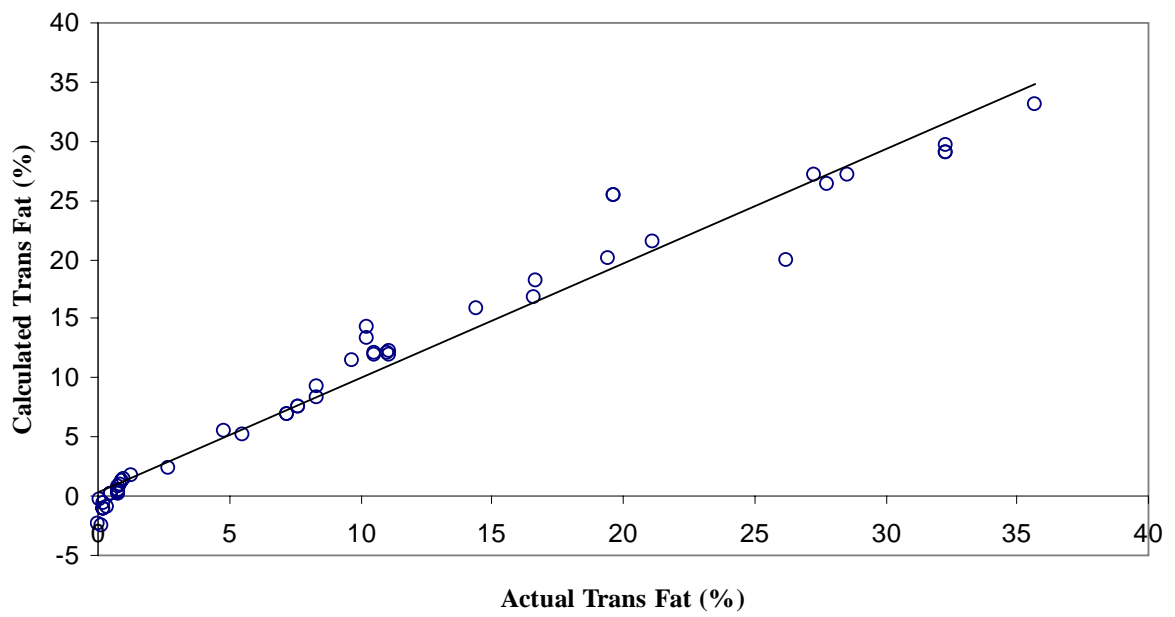


Fig 3.10. Calibration curve for PLS model . R^2 :0.98, RMSEC:2.07

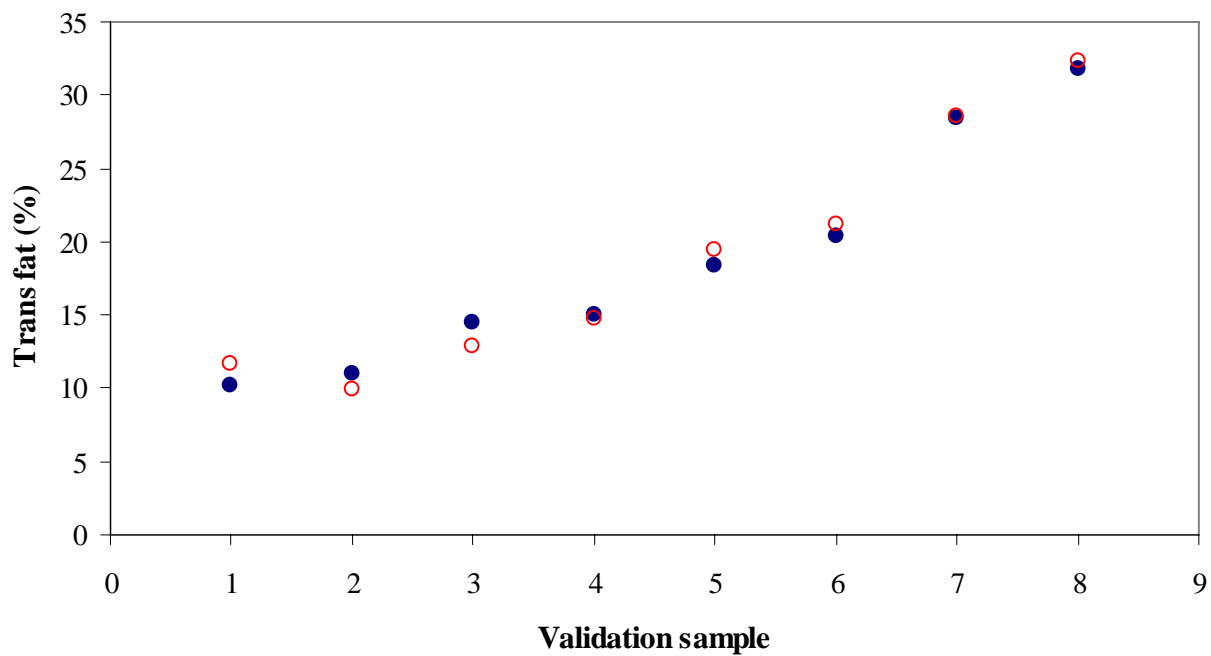


Fig 3.11 Variation in values of validation samples estimated by PLS model . The small dots in the chart are the real values and the circles represent the values estimated by the model.

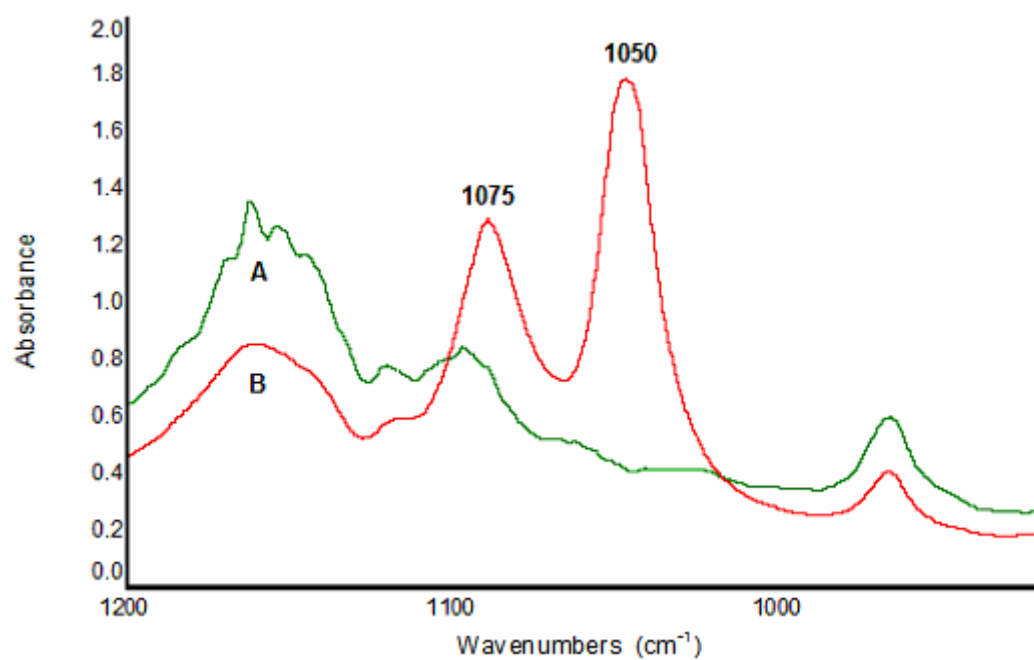


Fig 3.12 FT-IR spectra of Margarine. (A. Pure margarine B. Margarine treated with nano carbon)

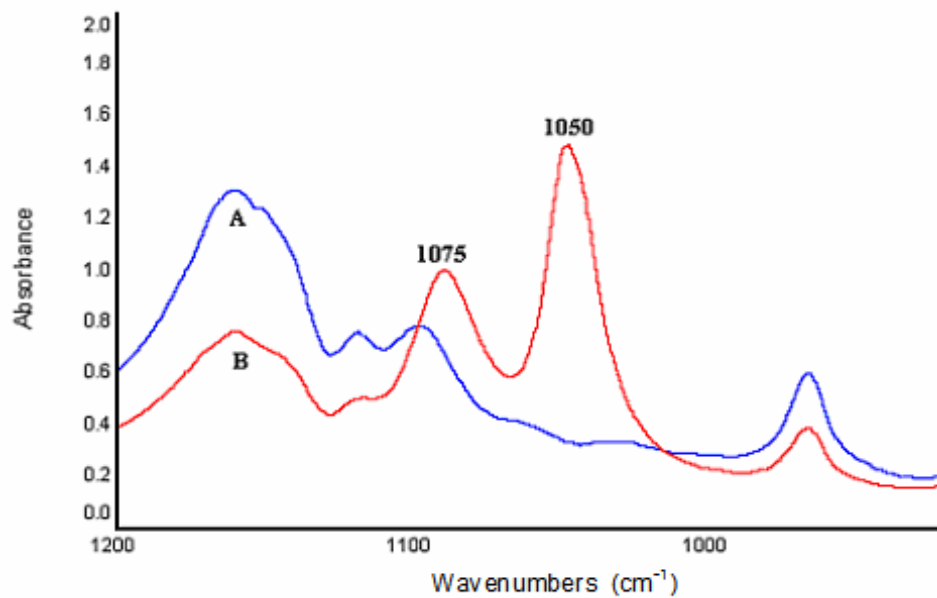


Fig 3.13 FT-IR spectra of Spread. (A. Pure Spread B. Spread treated with nanocarbon)

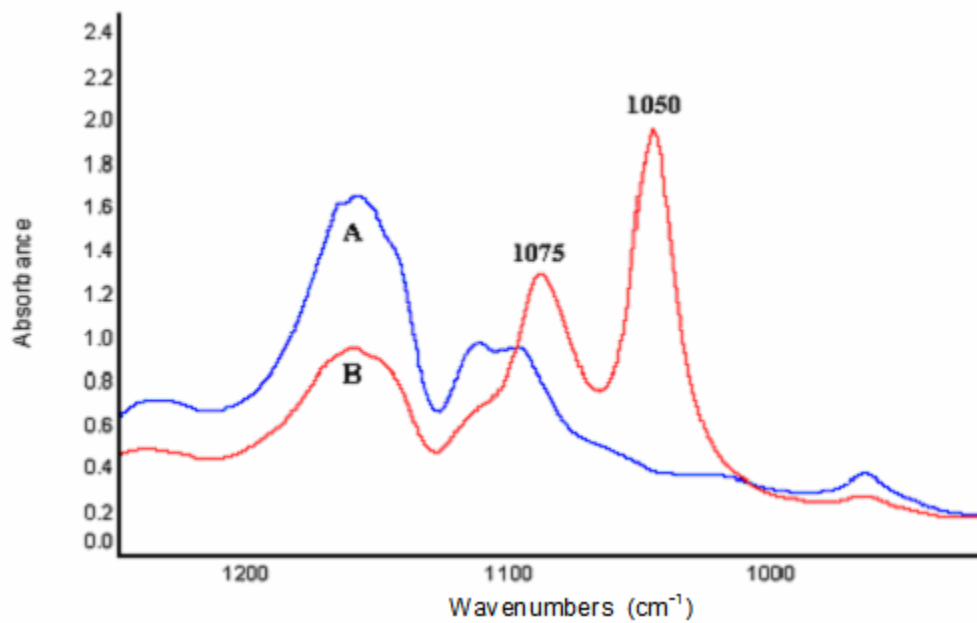


Fig 3.14 FT-IR spectra of Butter. (A. Pure Butter B. Treated with nanocarbon)

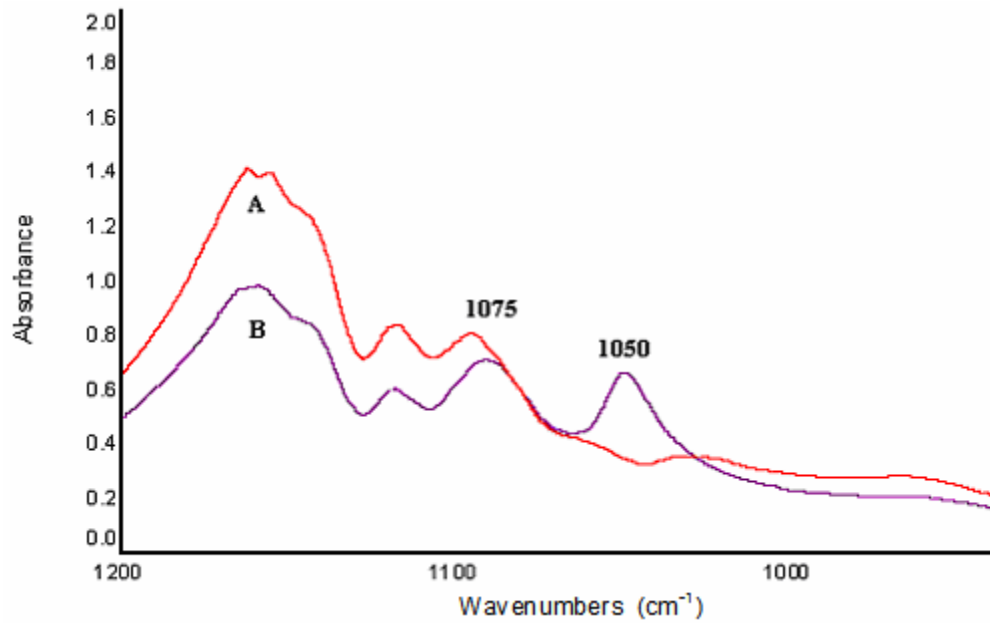


Fig 3.15 FT-IR spectra of Olive oil. (A. Pure olive oil B. Olive oil treated with nanocarbon)

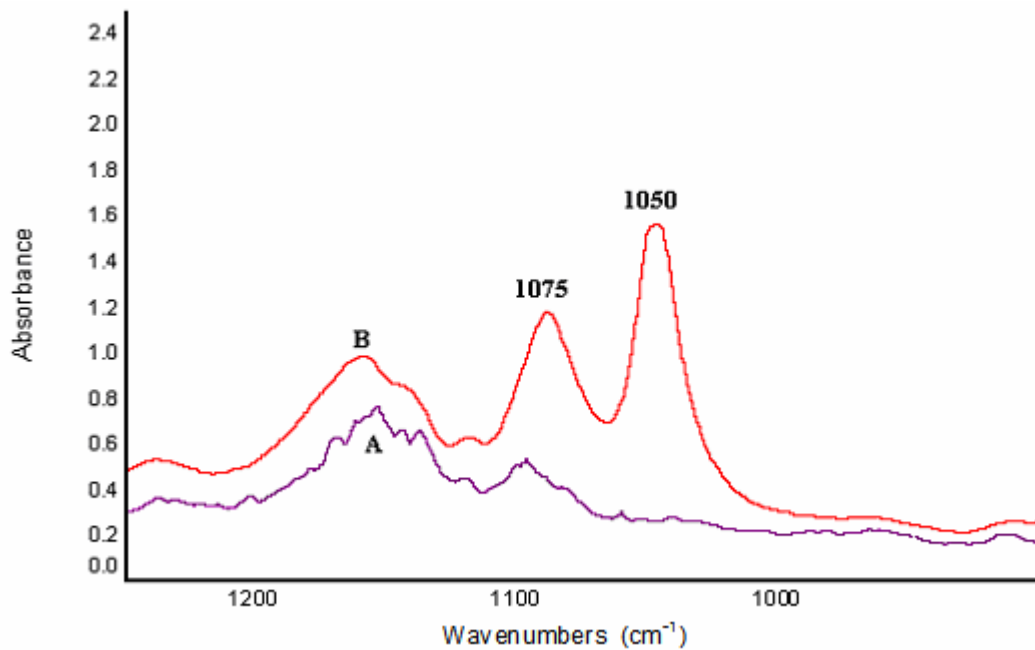


Fig 3.16 FT-IR spectra of Sunflower oil. (A. Pure sunflower oil B. Oil treated with nanocarbon)

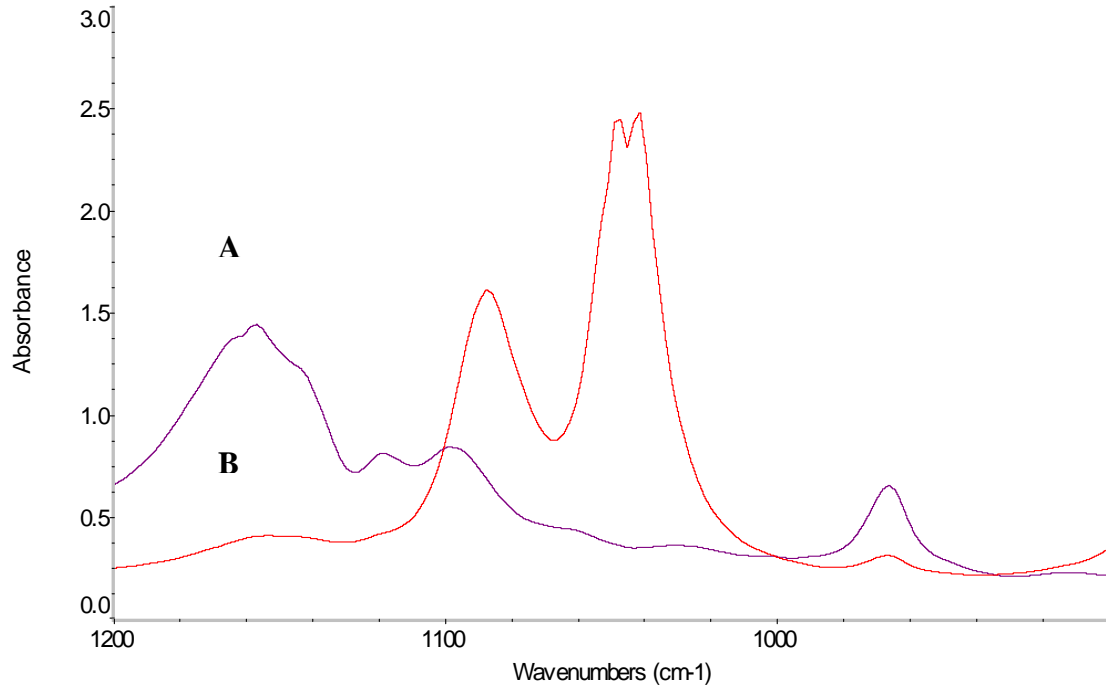


Fig 3.17 FT-IR spectra of ZnO treated Margarine (A. Pure Margarine B. Margarine treated with nanoZnO)

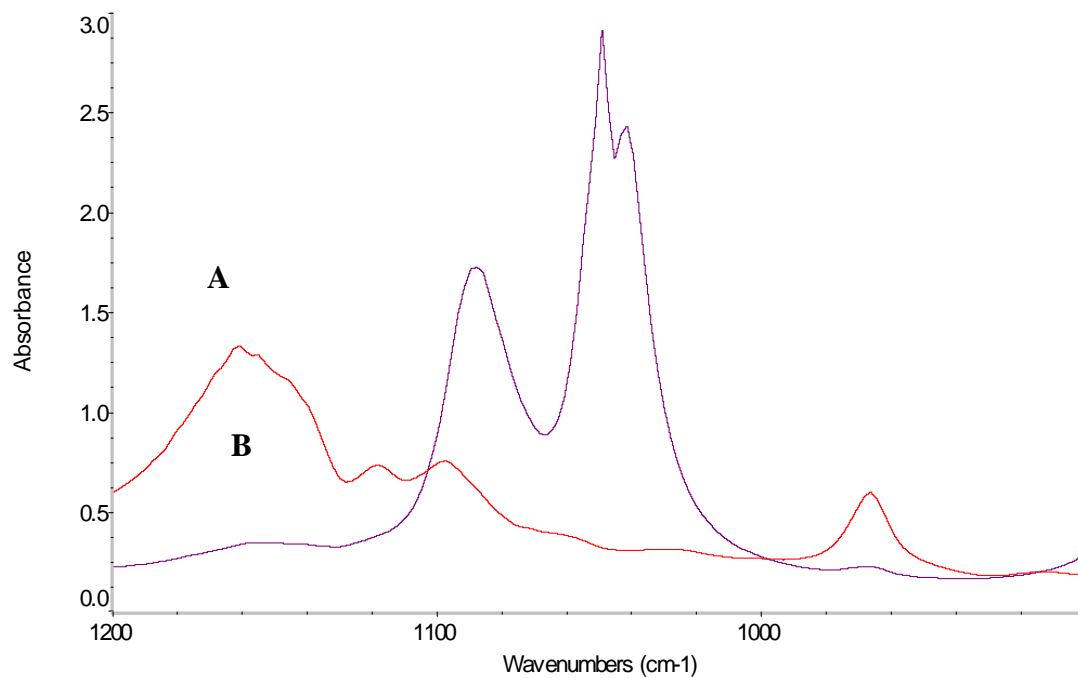


Fig 3.18 FT-IR spectra of Spread. (A. Pure spread B. Spread treated with nano ZnO)

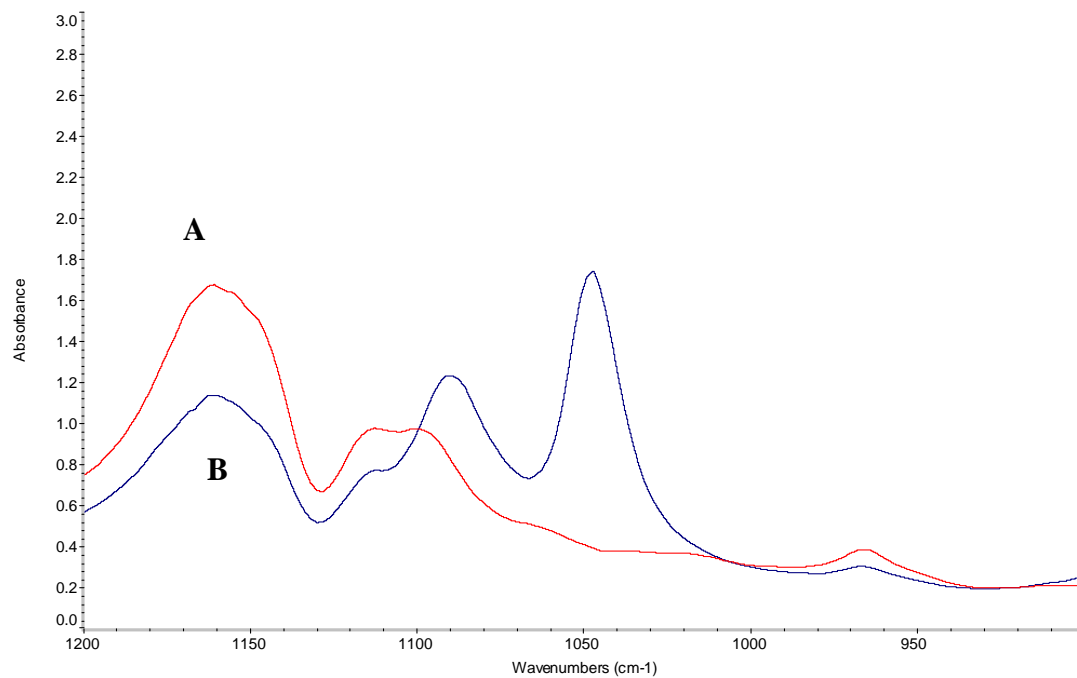


Fig 3.19. FT-IR spectra of ZnO treated Butter. (A. Pure Butter B. Butter treated with nano ZnO)

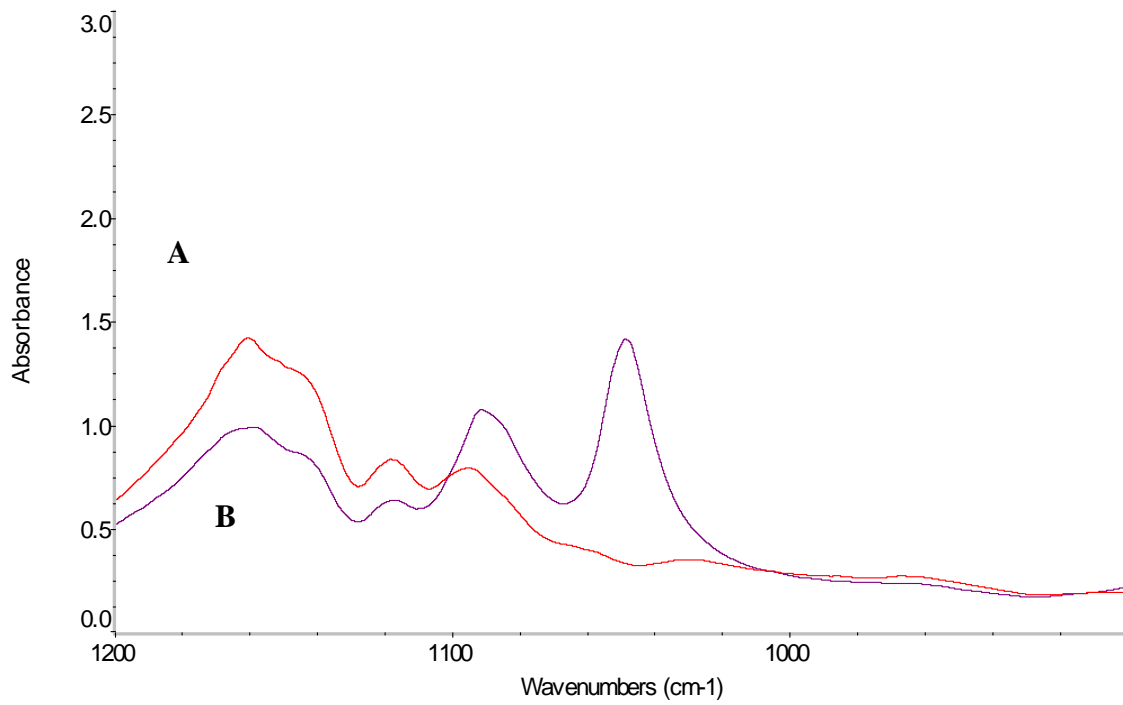


Fig 3.20 FT-IR spectra of nano ZnO treated Olive oil. (A. Pure olive oil B. Oil treated with nano ZnO)

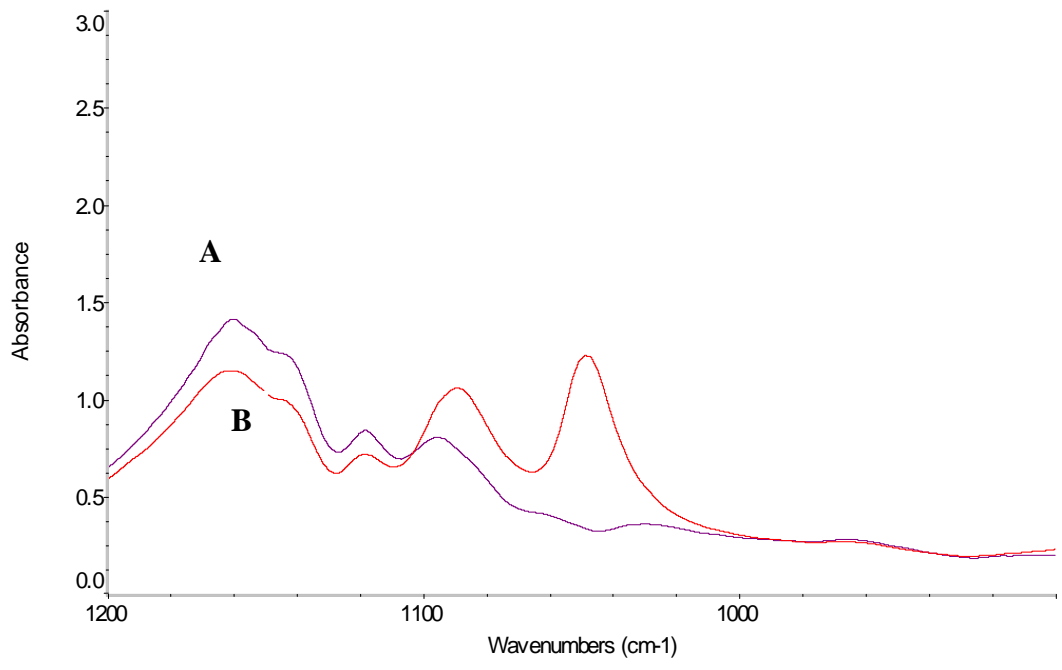


Fig 3.21 FT-IR spectra of nano ZnO treated Sunflower oil. (A. Pure Sunflower oil B. Oil treated with nano ZnO).

CHAPTER 4. SUMMARY AND CONCLUSIONS

FT-IR with the aid of PLS model was found to be a rapid technique to detect and quantify the *trans* fats in shortenings, thus avoiding tedious chromatographic techniques. The PLS model diminish the error in the quantification of the *trans* fat content in the samples when consider the specific area of 990-940 cm^{-1} , that when it is correlated with the peak height or peak area in the infrared spectra, also if the range in the wavenumber change for the PLS model , then the error in the measurement increase.

Enhancement of spectral peaks was observed in the lipids treated with nanomaterials for the C-O interaction around 1050 cm^{-1} , though significant improvement could not be achieved for enhancement of spectral peak corresponding to *trans* fats. The formation of additional peak and sharpening of peak at different wave numbers, as a result of treatment of lipids with nanomaterials showed the potential application of nanomaterials in detection of functional group / stretch, which could not be observed through FT-IR by traditional sample preparation methods.

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APENDICES

Appendix A.
EXPERIMENTAL FOOD PRODUCTS

Spread

Kroger quality. 65 % vegetable oil spread .Partial hydrogenated soybean oil. 9 grams of vegetable fat per serving. Serving size 14 g.

Butter

Land O Lakes. Sweet milk cream salted

Margarine

Great value. Vegetable oil blend (Partial hydrogenated soybean oil, liquid soybean oil).

Sunflower oil

Loriva. Sunflower oil supreme. 100% pure American. The label had declared that this oil was processed in a plant which process also peanut and sesame.

Olive oil

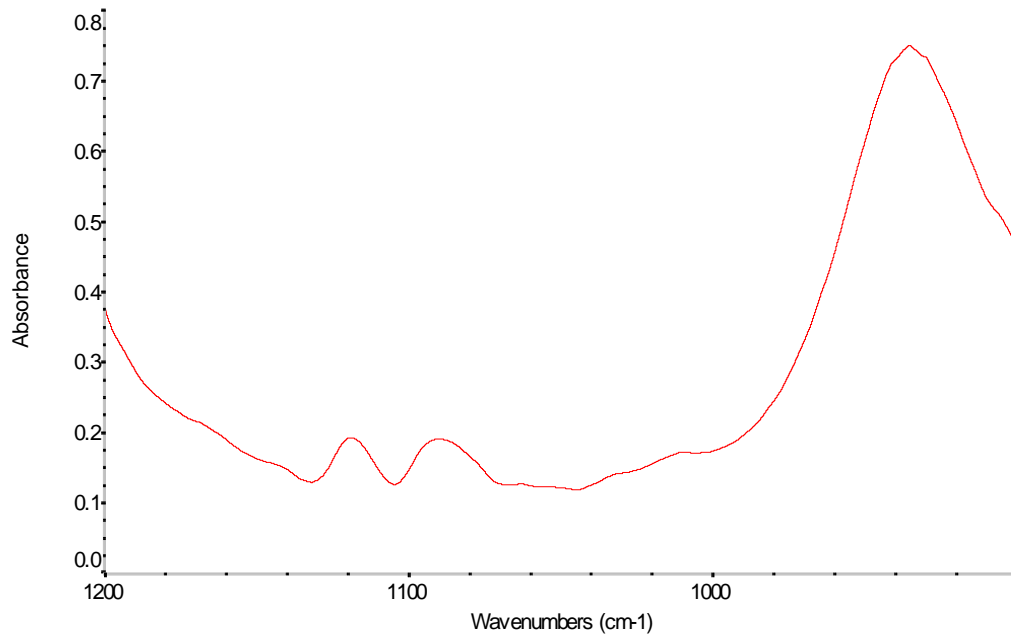
Racconto. Extra virgin olive oil. Naturally pressed. No more that 1% of free oleic acid.

Appendix B.
COMPOSITION OF EXPERIMENTAL FOOD PRODUCTS

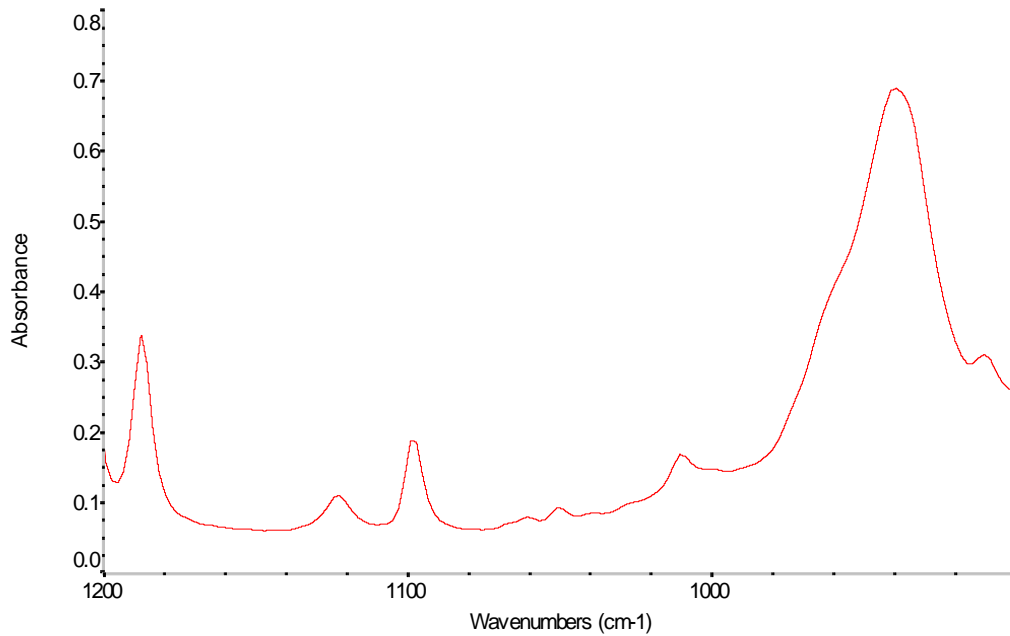
Food product					
Size of the serving: 14 grams					
Component	Spread	Margarine	Sunflower	Olive oil	Butter
Calories	80	100	120	120	100
Total Fat (g)	9	11	14	14	11
Saturated fat (g)	2	2	2	2	7
Trans fat (g)	2.5	3	0	0	0
Polyunsaturated Fat (g)	2	2.5	3	2	-
Monounsaturated Fat (g)	2.5	3.5	9	10	-

Appendix C. Additional spectra.

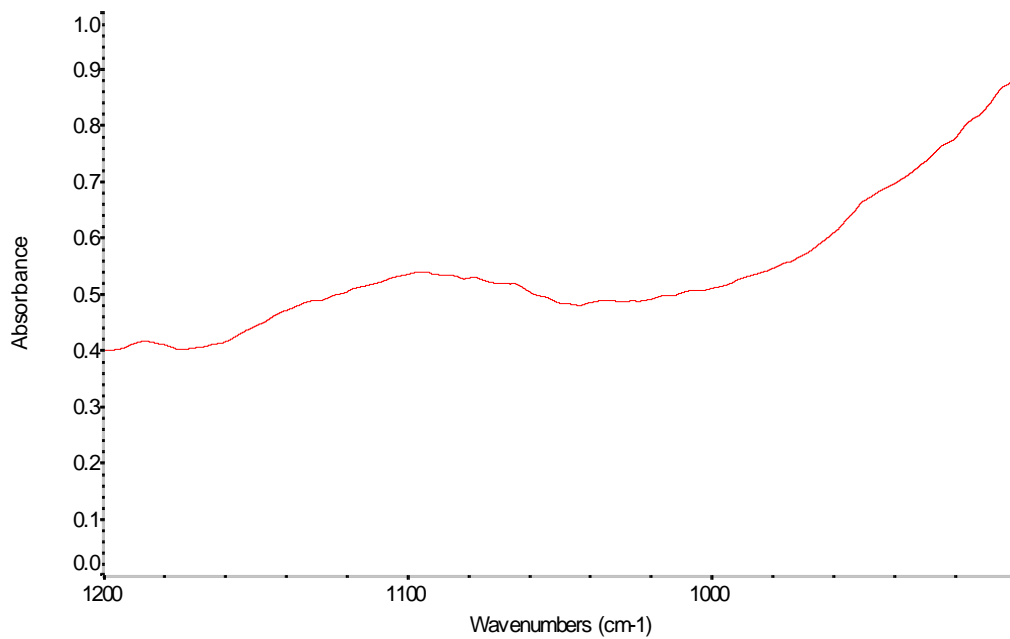
The additional spectra are a saturated fat (palmitic acid) and a monoene acid (oleic acid). For the saturated fat (C2) is founded a peak at 1100 cm^{-1} and a shoulder at 1120 cm^{-1} wavenumber.



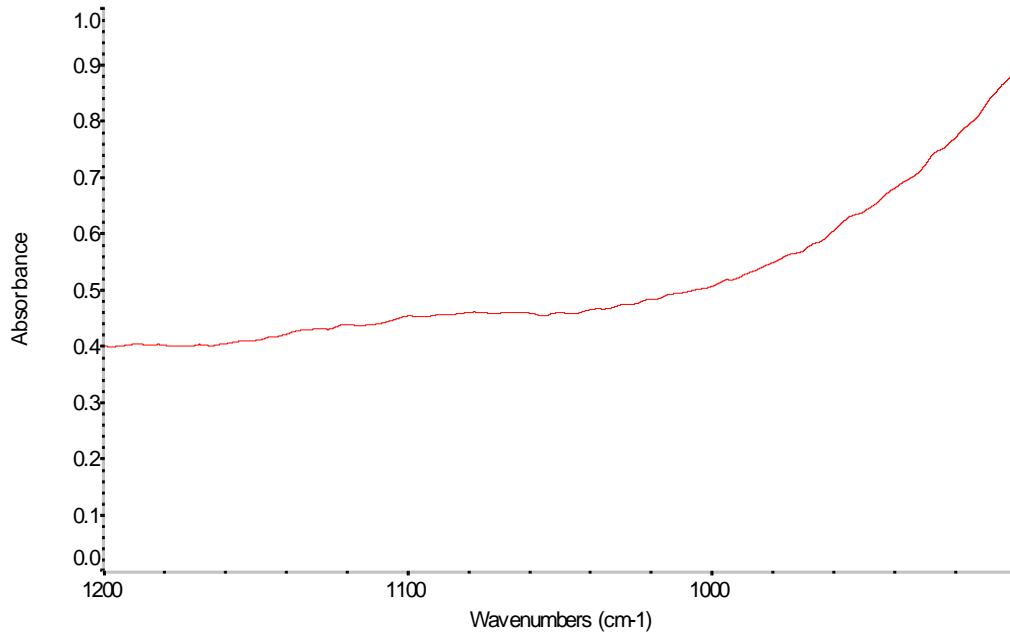
C1. Oleic Acid FTIR spectra. A monoene acid with *cis* configuration present in all natural oils such as olive oil (60-80%) and almond oil (60-70%).



C2. Palmitic acid FTIR spectra. Palmitic acid (hexadecanoic acid) is one of the most common of all the saturated fatty acids. It is present in fish oils (10-30%), in the milk and body fats of most animals (up to 30%).

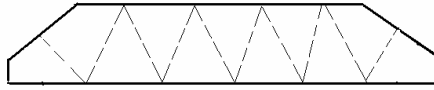


C3. FTIR spectra of Trixton-X 100. The concentration of the surfactant is 4% in weight.

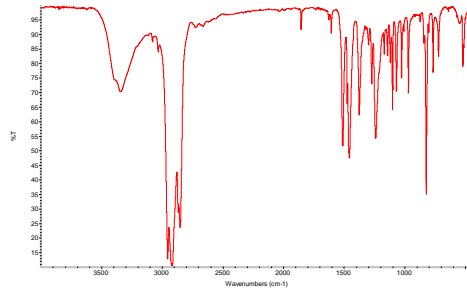


C5.FTIR spectra of ZnO in Triton X-100

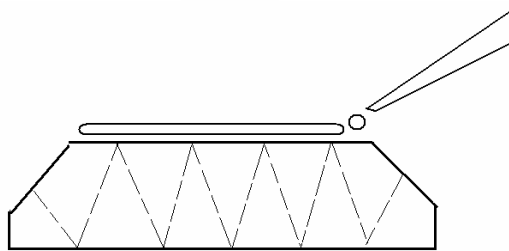
Appendix D.
DIAGRAM OF FTIR SAMPLING METHOD



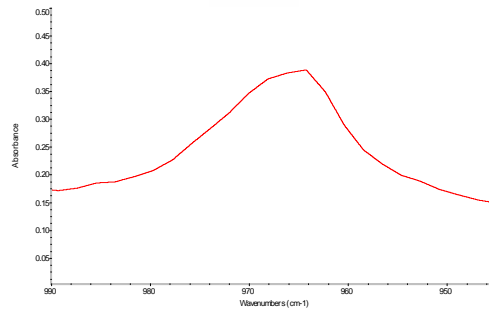
Step 1. Electron beam



Step 2. Collection of the background



Step 3. Direct application of sample



Step 4. Sample spectra

Appendix E.
CALCULATION VALUES FOR PRESS ALGORITHM

trans fat by PLS E:\the method\The_final_method.qnt
Revision: 1 Last saved on: Sat Jun 02 15:55:26 2007
Printed on: Wed Jun 06 19:53:36 2007

PRESS Table - trans fat

Factor	PRESS	RMSECV
0	5165.063	12.14797
1	2837.294	9.00364
2	1203.921	5.86496
3	1656.674	6.87994
4	635.0726	4.25969
5	1001.825	5.3501
6	783.0751	4.73007
7	881.4868	5.0185
8	933.6758	5.16493
9	1058.301	5.49883
10	932.8021	5.16251

Appendix F.

CALCULATION VALUES FOR PLS METHOD

trans fat by PLS E:\the method_4\TFA_method.qnt

Revision: 1 Last saved on: Wed Jun 27 13:53:15 2007

Printed on: Fri Jun 29 12:23:33 2007

2 factors used

Calibration Results Table - trans fat

Index	Spectrum Title	Actual	Calculated	Diff. x Path
1		2 32.301	28.988	-3.313
2		3 19.647	25.411	5.764
3		5 7.626	7.593	-0.033
4		6 7.193	6.966	-0.227
5		7 8.328	9.205	0.877
6	1a	9.697	11.452	1.755
7	2a	4.83	5.571	0.741
8	3a	0.918	1.228	0.31
9	4a	0.847	0.899	0.052
10	5a	0.755	0.518	-0.237
11	6a	0.8	0.178	-0.622
12	7a	0.81	0.347	-0.463
13		8 0.501	0.117	-0.384
14		9 0.224	-1.091	-1.315
15		9 0.224	-1.091	-1.315
16		10 0.374	-0.916	-1.29
17		11 0.215	-0.65	-0.865
18		12 0.117	-2.426	-2.543
19		14 0.064	-0.343	-0.407
20	validation_2	32.32	29.656	-2.664
21	validation_3	28.576	27.109	-1.467
22	validation_4	21.131	21.503	0.372
23	validation_5	19.458	20.11	0.652
24	validation_6	16.63	16.871	0.241
25	validation_7	14.422	15.89	1.468
26	validation_8	11.015	12.083	1.068
27	validation_9	10.248	13.33	3.082
28	s1	10.507	12.07	1.563
29	s2	2.716	2.302	-0.414
30	s3	35.711	33.172	-2.539
31	s4	26.207	19.869	-6.338
32	s6	1.251	1.812	0.561
33	s7	0.997	1.5	0.503
34	s8	0.489	0.17	-0.319
35	s9	0.81	0.818	0.008
36	blend_2c	5.54	5.149	-0.391
37	blend_3a	8.33	8.33	0
38	triolein_2	0	-2.319	-2.319
39		2 32.301	28.988	-3.313
40		3 19.647	25.411	5.764

41		5	7.626	7.593	-0.033
42		6	7.193	6.966	-0.227
43	L1		27.77	26.422	-1.348
44	M1		27.27	27.133	-0.137
45	f1		10.533	11.964	1.431
46	k2		10.248	14.333	4.085
47	blend_4a		11.1	11.99	0.89
48	blend_4b		11.1	12.203	1.103
49	blend_6a		16.662	18.136	1.474