Thermal induced isomerization of Trilinolein and Trilinoelaidin at 250^{o}C: Analysis of products by gaschromatography and infrared spectroscopy

Alfred A. Christy, Department of Science, Faculty of Engineering and Science, University of Agder, Serviceboks 422, 4604 Kristiansand Norway.
e-mail: alfred.christy@uia.no, Tel: +4738141502, Fax: +4738141071

Abstract
The products formed by thermally induced isomerization of trlinolein and trilinoelaidin at 250^{o}C have been studied by infrared spectroscopy and gas chromatography.

The triglycerides of the 9c12c and 9t12t fatty acids, linoleic and linoelaidic acid respectively, were subjected to thermal treatment under nitrogen in glass. The products were removed at regular intervals and analysed by infrared spectroscopy using a single reflectance attenuated total internal reflectance (ATR) crystal accessory. Trans-esterification of the products provided the corresponding fatty acid methyl esters (FAME) which were studied by gas chromatography.

The results show that the samples undergo decomposition and isomerization. Thermally induced 9c12c fatty acid (linoleic acid) molecules in the trilinolein molecules isomerise into 9c12t, 9t12c and 9t12t isomers. Thermally induced 9t12t fatty acid (linoelaidic acid) molecules in the trilinoelaidin molecules isomerise into 9c12t, 9t12c and 9c12c isomers. However, the concentration profiles are different for these two triglyceride samples. The rates of formation of isomers from linoleic acid are higher than the rates of formation of isomers from linoelaidic acid. In addition, these two fatty acids also isomerise into Conjugated Linoleic Acids (CLAs). The profiles of the CLAs are identical in both cases.

Key words: Trilinoelaidin, trilinolein, Conjugated Linoleic Acids, isomerization, Gas Chromatography, infrared spectroscopy.

Introduction
There have been several reports in the literature dealing with *trans* fatty acids in edible oils and fats. This is due to the fact that the *trans* fatty acids in human diet has been linked to the risk of coronary heart disease [1-5]. Partial hydrogenation [6], thermal induction [7,8] and deep frying [9] can form trans fatty acids in mono and poly unsaturated fatty acids in edible oils and fats. During thermal induction and deep frying, other changes also take place resulting in several different products in the heated oil and fats. These changes include oxidation, intra cyclization and polymerization [7, 8, 10-14]. The products formed from mono unsaturated fatty acids can reach up to a concentration of 60% in oils heated at 300 ^{o}C. These products are toxic [12, 14] and may cause health problems in humans. Similarly, polyunsaturated fatty acids also form toxic products, however their identities and concentrations are not fully studied. This is because the processes leading to these products are very complex and identifying the precursors of different products can be difficult because of the number of fatty acids involved. Analyzing such a complex mixture for its total chemical composition is a daunting task.

The intention in this work is to study the chemical changes taking place in glycerides

containing 18:2 fatty acids, namely trilinolein and trilinoelaidin during thermal induction in inert atmosphere. This study would give understanding of the quantities of the isomers formed, the chemistry of isomerisation and the relative stability of the fatty acid molecules towards isomerization. Reports involving the analysis of products formed from the glycerides containing 18:2 fatty acids during thermal induction are scarce in the literature. Furthermore, there are no reports in the literature regarding thermal induction of 18:2 fatty acids with *trans,trans* configuration. The published literature only deals with the chemical analysis of the mixture formed during heating of edible fats in the temperature range 240-265^{o}C [15-17]. The analysis in one of the reports [15] was carried out for the purpose of determining kinetic parameters for the *cis-trans* isomerization of linoleic acid. The acids analyzed were only 9t12t, 9c12t and 9t12c. There was no mention about the other products formed during heating. Here, the assumption was that the formation of 9t12t, 9c12t and 9t12c were purely from 9c12c fatty acid. The second paper [16] reports the appearance of the individual isomers during heat induced isomerization of α–linolenic acid. The effect of temperature and heating time were study parameters in this report. In both reports, the identification of isomers was carried out from the total chromatogram of the fatty acid methyl esters (FAME) of the fatty acids in the samples. The third report [17] deals with the quantification of cis/trans isomerization in triolein, trilinolein, trilinolenin during heat induction. The maximum heating time was 8 hours and the *trans* isomer formation in trilinolein was not significant. Furthermore, there was no attempt to look at the chemistry of the formation of the *trans* isomers in the heated mixture.

When the fatty acids in the glyceride molecules are identical, then analysis of the glyceride for products after heating becomes comparatively easy. Trlinolein and trilinoelaidin are glycerides of 18:2 (9, 12) fatty acids with *cis, cis* and *trans, trans* configurations respectively. The acid molcules in the glyceride backbone are of definitive structure and the products formed should originate from these acid molecules. This will give understanding of the changes taking place and how the molecules behave under thermal stress.

The major products formed during thermal induction of the triglyceride samples were mixtures of isomers with *cis* or *trans* hydrogens on the double bonds in trilinolein and trilinoelaidin. These were analysed by two different techniques namely gas chromatography and infrared spectroscopy. The use of infrared spectroscopy and gas chromatography to determine the *trans* content in edible oils and fats have been extensively investigated and they are standardized by The American Oil Chemists Society (AOCS) [18]. The quantitative determination of *trans* fatty acid concentration in fats and oils is based on the fact that the *trans* isomers of monounsaturated fatty acids and *trans* isomers of the polyunsaturated fatty acids containing isolated *cis-trans, trans-cis* and *trans-trans* double bonds give rise to one specific absorption at 969 cm^{-1} [19-22]. Conjugated Linoleic Acids (CLAs) with *cis, trans* and *trans, cis* configurations give rise to two specific absorptions around 986 and 946 cm^{-1}. These absorptions arise due to the =CH out of plane deformation vibration [23]. The CH out of plane deformation vibration of the *tt* (*trans, trans*) isomers absorb around 982 cm^{-1} and, *cis-cis* isomers do not absorb in this region. This characteristic difference in the absorptions makes it possible to quantify methylene interrupted *trans* isomers and CLA isomers with *cis, trans* and *trans, cis* configurations using infrared spectroscopy. Christy et al. [24] have utilised this fact in simultaneous quantification of isolated *trans,* and CLAs in oils and fats. Furthermore, the presence of absorption peaks at 986 and 946 cm^{-1} of the fatty acids formed

during the isomerization is a clear indication of the formation of CLA isomers containing *cis, trans* and *trans, cis* configurations.

Gas chromatography involves saponification and derivatization of the oil or fat involved and the separation of the methyl esters using a high resolution capillary column that is suitable for the separation of isomers. Quantification of different isomer components is carried out using Standards of fatty acids methyl esters and literature references [25].

Experimental

Samples and methods

The triglycerides of 9t12t and 9c12c fatty acids and the methyl esters of the same fatty acids were purchased from Sigma Aldrich. The methyl esters containing the 9c11t and 10t12c (99%) fatty acids were purchased from Larodan Chemicals, Sweden.

The heating experiments were carried out in micro glass ampoules of length 4 cm. These were made from glass tubes of 1.5 mm internal diameter and a wall thickness of 1 mm. The triglyceride samples containing the 9c12c fatty acid were injected in the tubes using a plastic syringe and each sample was flushed by a weak nitrogen flow. The open end of the glass tubes were melted and sealed. In the presence of air, the samples undergo oxidation and polymerisation. The ampoules containing the samples were then placed in a short glass vial and placed in a chromatographic oven set at 250^{o}C. Thermally induced samples were removed from the oven at regular intervals, cut open, their infrared spectrum recorded, sealed again by paraffin film and stored in darkness for the analysis by gas chromatography at the end of the thermal induction experiment. The experiments were repeated with the triglyceride of the 9t12t fatty acid.

Infrared spectroscopic measurements

Each of the samples removed from the oven was subjected to infrared measurements A Perkin Elmer Spectrum One FT-IR spectrometer equipped with a Harrick single reflectance ATR accessory and lead glycine sulphate detector was used in measuring the infrared spectra. Each sample was spread on the ATR crystal using a capillary glass tube. The blunt side of the capillary tube was used to pick up a small amount of the sample and spread on the ATR plate. A background spectrum was scanned in the range of 4000-600 cm^{-1} before the application of a sample. A total of 30 scans at a resolution of 4 cm^{-1} were then measured on each sample. The ATR crystal was washed with dichloromethane and acetone after each measurement. The same procedure was repeated for the thermally induced 9t12t LA samples. All the infrared spectra were saved in absorbance format. The spectra were then doubly derivated and used in the quantitative analysis.

Gas chromatographic analysis

The remaining glyceride samples containing 9c12c and 9t12t fatty acids after the infrared analysis were subjected to derivatization. Each glass tube containing the sample was cut just above the liquid mark and crushed inside a 15 mL test tube. To each test tube was then added 2 ml of 0.5 M sodium methanolic sodium hydroxide and placed in a water bath at 60 ^{o}C for 15

min. After cooling, 2 ml portions of BF_3/methanol were added to the solutions in the test tubes and placed in the water bath again for 10 min. To the solutions in each test tube was then added a 2 ml portion of a saturated solution of NaCl followed by a 1 ml portion of heptane and the resultant mixture well shaken to aid separation and dissolution of the FAMEs in the heptane layer. After a few minutes standing, anhydrous magnesium sulphate powder was added to the top heptane layers in the test tubes. The heptane layers from the test tubes were extracted, placed in small brown vials and stored in dark for gas chromatographic analysis.

The GC analysis of the methyl esters of the fatty acids resulting from the thermal induction was carried out by using a Hewlett Packard 5890 gas chromatograph equipped with a 100 m capillary column with 0.25 mm internal diameter coated with 0.20 μm thick 90%-bis-(cyanopropyl)-methyl polysiloxane stationary phase (HP 88). A temperature program involving two step gardients was used. The program started with 1min at an initial temperature of 150^{o}C and followed by a temperature gradient of 5^{o}C/min to reach a temperature of 180^{o}C. After a time of 50 min at 180^{o}C another temperature gradient of 5^{o}C/min was used to bring the final temperature to 220^{o}C. The temperature was held at 220^{o}C for 30 minutes giving a total running time of 95 minutes for each sample.

Results and discussion

Infrared spectroscopy

The thermal induction of 9c12c fatty acid is expected to give 9c12t, 9t12c and 9t12t isomers. In addition to these, decomposition and formation of Conjugated Linoleic Acid isomers can also be expected [26, 27]. The positional isomers containing *trans* configurations are expected to absorb at 969 cm^{-1} in the infrared because of the =CH *trans* bending vibrations. The infrared spectra measured on the thermally induced samples of trilinolein and trilinolelaidin are given in Fig. 1. The figures clearly show that the peak at 969 cm^{-1} increases with time in the case of trilinolein samples and decreases with time in the case of trilinolelaidin samples. The transformation of fatty acid chains is clearly evident in both cases. The trilinolein has a weak absorption around 3004 cm^{-1} because of the =CH stretching modes. This absorption decreases as the fatty acid moieties in the trilinolein molecules isomerise.

The formation of CLAs in the heated samples of 9c12c fatty acid has been demonstrated by Destaillats and Angers [26]. There is also evidence in the infrared spectra of the heated samples for the formation of CLAs. There are humps on both sides of the peak at 969 cm^{-1} which become clearly evident in the fourth derivative spectra of the heated samples of trilinolein (Fig. 2). The peaks at 987 and 946 cm^{-1} are characteristic to CLAs. The formation of CLAs in the heated samples of trilinoelaidin has been shown by Christy [8] in a recent report. The fourth derivative spectrum of the heated sample of trilinoelaidin shows absorptions at 989, 969 and 946 cm^{-1}. The peaks at 989 and 946 cm^{-1} indicate the presence of CLAs in the heated sample. The slight shift in the peak at 989 cm^{-1} indicates the presence *trans,trans* isomers in the mixture of the CLAs formed. The CLAs in the heated sample are a mixture of *c,t/t c* and *t,t* isomers of CLAs with a high percentage of *t, t* isomers. The peak at 969 cm^{-1} indicates the presence of *t,c* or *c,t* or *t,t* LAs or mixture of these isomers.

Gas chromatography

The gas chromatograms of the FAME of the heat treated trilinolein and trilinoelaidin are shown in Fig. 3 and 4 respectively. The concentrations of the isomers from both trilinolein

and trilinoelaidin are presented in Fig. 5. The FAME profiles are very different for these samples. The 9c12c fatty acid readily isomerises to 9c12t and 9t12c isomers. Furthermore, isomerization into 9t12t isomer seems to be very slow. In contrast to this, the isomerization of 9t12t fatty acid into the 9c12t, 9t12c and 9c12c isomers is much slower.

The 9c12t and 9t12c isomers in heat treated trilinolein are equal in concentration. This fact proves that the isomerization of the 9,12 double bonds in 9c12c fatty scids are equally probable. However, heat treated trilinoelaidin gives different concentration profiles for 9c12t and 9t12c isomers with relatively higher concentrations of 9t12c compared to 9c12t isomers in all the heated samples of trilinoelaidin. Formation of different concentrations of 9t12c and 9c12t isomers imply that the double bonds in the 9t12t molecules are perturbed by the neighbouring molecules or groups. The absolute lower concentrations of 9t12c, 9c12t and 9c12c isomers in the heated samples of trilinoelaidin show the stability of 9t12t double bonds towards isomerization.

These results enable the depiction of an energy diagram for the isomerization (Fig. 6). The isomerization of 9t12t fatty acid into 9c12t and 9t12c isomers requires higher activation energy. Furthermore, the isomerization of 9t12t fatty acid into 9t12c requires relatively lower activation energy compared to the isomerization into 9c12t. It is not clear why the isomerization of 9t12c requires lower activation energy. A theoretical approach is necessary to resolve this question.

Apart from positional isomarization, heat induction of trinlinolein and trilinoelaidin also forms Conjugated Linolic Acids [8, 26, 27]. The CLA profiles produced in these thermal induction reactions are identical [8]. The top parts of the Figures 3 and 4 show the CLA isomers produced in trilinolein and trilinoelaidin during thermal induction. The concentrations of total CLAs and the concentration of *trans, trans* CLAs within the total CLAs are shown in Fig. 7. The bar diagrams show the increase in concentration of CLAs during thermal induction of trilinolein and trilinoelaidin. The relative total concentration of CLAs in the heated samples approaches a maximum of around 5%. When the degradation of the trilinoelaidin (Fig. 8) is taken into account, the concentration of the total CLA in the heated trilinoelaidin amounts to around 2.2%. At the same time when the degradation of trilinolein (Fig. 8) is taken into account, the concentration of CLAs in the heated glyceride samples of 9c12c fatty acids amounts to around 3.3%. The relative concentration of *t,t* CLA isomers which elute together amounts to around 60% in all the heated samples.

The mechanism of formation of CLAs from heat induced 9c12c fatty acids has been described in detail elsewhere [26]. The same mechanisms can also be applied to the formation of CLAs from heat induced isomerization of 9t12t fatty acids [8]. Conjugated 9t11t and 10t12t fatty acids are formed as primary products through either 1) A free radical chain reaction mechanism or 2) An intramolecular [1,3] sigmatropic rearrangement mechanism. All the orther isomers of the CLAs are formed from these primary products through a series of positional isomerization and [1,5] sigmatropic rearrangements [8].

Gas chromatography and infrared spectrometry

As mentioned earlier, the acid molecules in the trilinolein and trilinolelaidin undergo

degradation and a part of the molecules will not be available for isomerization reaction. The remaining trilinolelaidin can be quantitatively determined by using pure trinlinolelaidin standard. The use of peak at 969 cm^{-1} in quantifying the concentrations of rest trilinoleladin in the mixtures is reasonable. Heat treatred trilinolelaidin yields around 6-7% 9c12t and 9t12c isomers and therefore the error in the determination of rest trilinolelaidin will be in that range. In the case of trilinolein, the formation of 9t12t is around 3% and therefore the total concentration of 9c12t and 9t12c in each sample can be determined by comparing the peak height at 969 cm^{-1} with trielaidin peak height at the same wavenumber. Rest trilinolein can then be calculated by relative comparison of the percentages.

The concentration of rest trilinoelaidin and trilinolein in the heat treated samples determined by infrared spectrometry and gas chromatography are given in Fig.8. The plots clearly show the difference between the concentrations determined by GC and infrared spectroscopy. The analysis of fatty acids in the thermal induced mixtures was carried out after derivatization into fatty acid methyl esters. This derivatization reaction involves only the acidic components formed during themal induction in the mixture and excludes all the other organic components formed from the thermal degradation. That is the concentrations of fatty acids determined as FAME profiles by gas chromatography are overestimated. The calculations show that both trilinolein and trilinoelaidin decompose during heating in addition to isomerization. Around 50% of trilinoelaidin decomposed after 10 days of heating and at the same time around 30% trilinolein decomposed after 10 days of heating. Difference in the decomposition characteristics is not difficult to understand. The concentration profiles of the products formed during the heat induction of trilinolein suggest that the activation energy for the isomerization is low and most of the fatty acid molecules in the molecule isomerise before decomposition into various products. In the case of trilinoelaidin, the molecule is very stable and the activation energy for the isomerization is very high and decomposition of trilinoelaidin may take place under thermal stress. These characteristics support the energy diagram shown in Fig. 6.

The decomposition products are mostly saturated and unsaturated aldehydes and shorter fatty acids [7]. Some of these aldehydes are carcinogenic [28]. These products were not analyzed for their chemical composition. However, there is evidence in the infrared spectra of the trilinolein and trilinoelaidin for oxidation during thermal induction. Infrared spectra of the heated sample of trilinolein and trilinoelaidin are given in Fig. 9. The absorption at 3470 cm^{-1} is due to the overtone of the –C-O stretching in the glyceride molecules [29]. As the sample is being heated, some molecules are oxidised and hydroperoxide molecules formed . These hydrperoxide groups give a broad band which overlaps with the –C-O overtone. Several aldehydes are formed depending on the position of the peroxide group in the molecules [30,31]. A detailed mechanism for the formation of different aldehydes during the oxidation process can be found in reference 31.

Conclusion

In this paper, we have shown the transformation of trilinolein and trilinoelaidin under thermal stress. The fatty acids in the triglyceride molecules undergo decomposition and isomerization. The concentration profiles of the positional isomers and conjugated linoleic acids have been acquired. These profiles give clear indications of the behaviour of the molecules under thermal stress. The 9c12c fatty acids lead to isomerization easily compared to the 9t12t fatty

acids. These findings confirm the stability of the 9t12t isomer compared to 9c12c.

The thermal decomposition of 9c12c and 9t12t also follow different patterns. Because of the molecular stability of the 9t12t fatty acid, the molecule undergoes decomposition easily instead of isomerization. Almost 50% of the trilinioelaidin decomposes in 10 days and at the same time 30% of trilinolein decomposes in 10 days. The products of the decomposition include peroxides and aldehydes.

The 9c12c and 9t12t fatty acids isomerise also into conjugated linoleic acids. The maximum concentration of CLAs in the heated samples reaches around 5% both in trilinolein and trilinoelaidin. All the *trans,trans* isomers except 11t13t co-elute under a large peak which comprises about 60% of the total CLAs in the sample. The concentration of CLAs formed from the 9t12t fatty acid is almost 66% of that yielded by 9c12c fatty acid. This again reaffirms the stability of 9t12t fatty acid toward transformations.

These findings suggest that linoleic acids in oils and fats transform into other products in the same way as the mono unsaturated fatty acids. Because of these changes, oils and fatts turn into a mixture of complex organic molecules that may have implications for human health.

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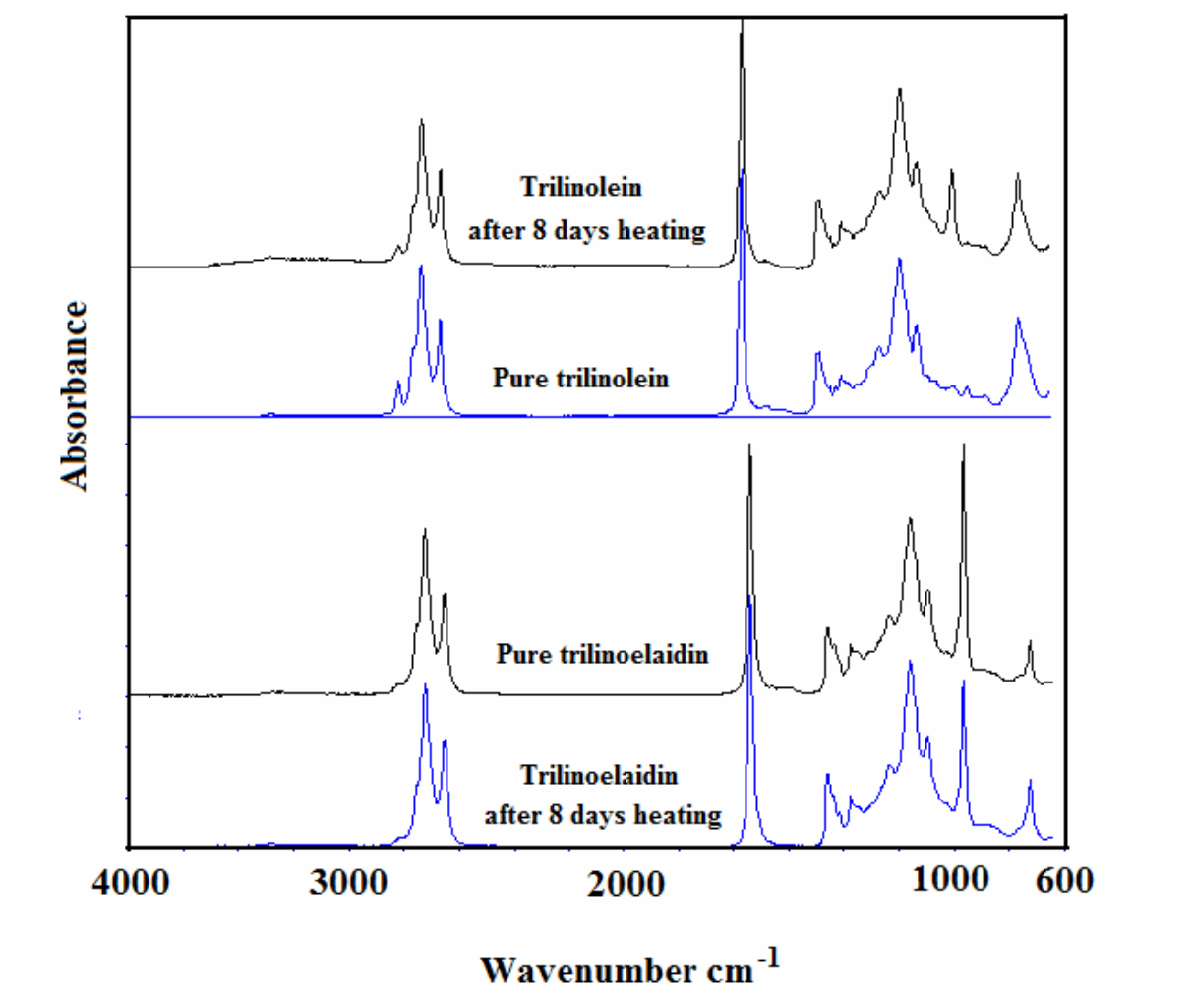

Fig. 1. Infrared spectra of heated samples of trilinolein and trilinoelaidin. The spectra are scale adjusted for clarity.

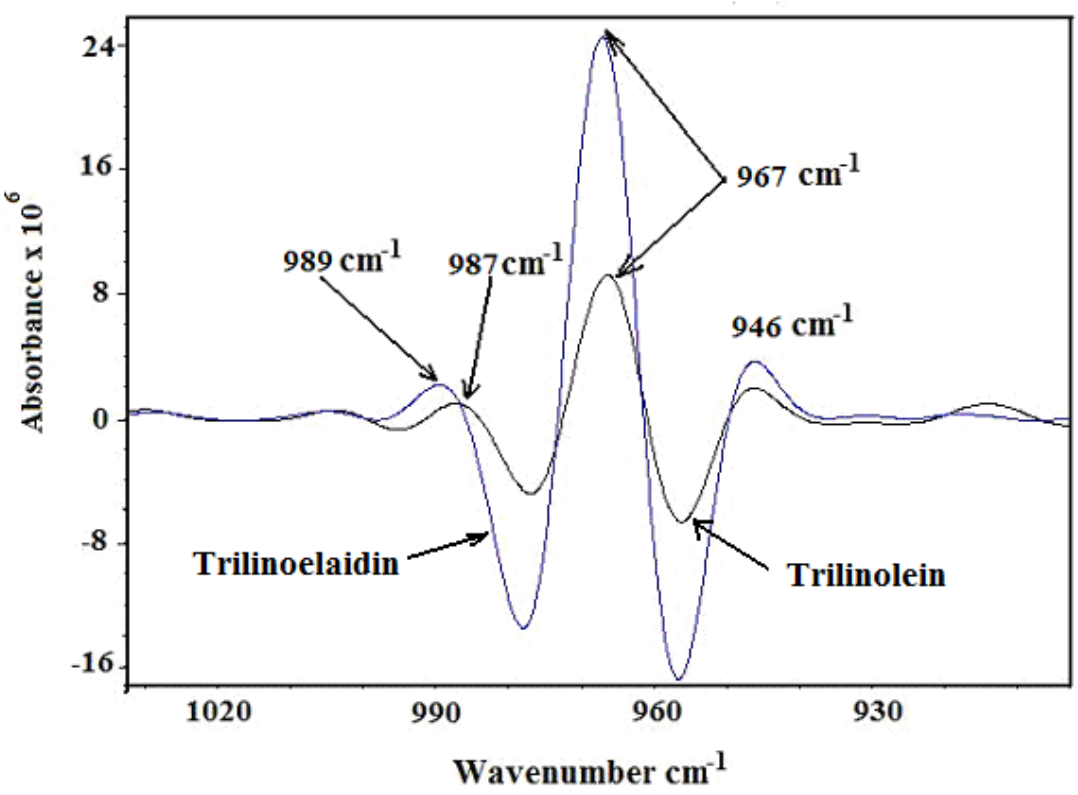

Fig. 2. Fourth derivative spectra of trilinolein and trilinoelaidin

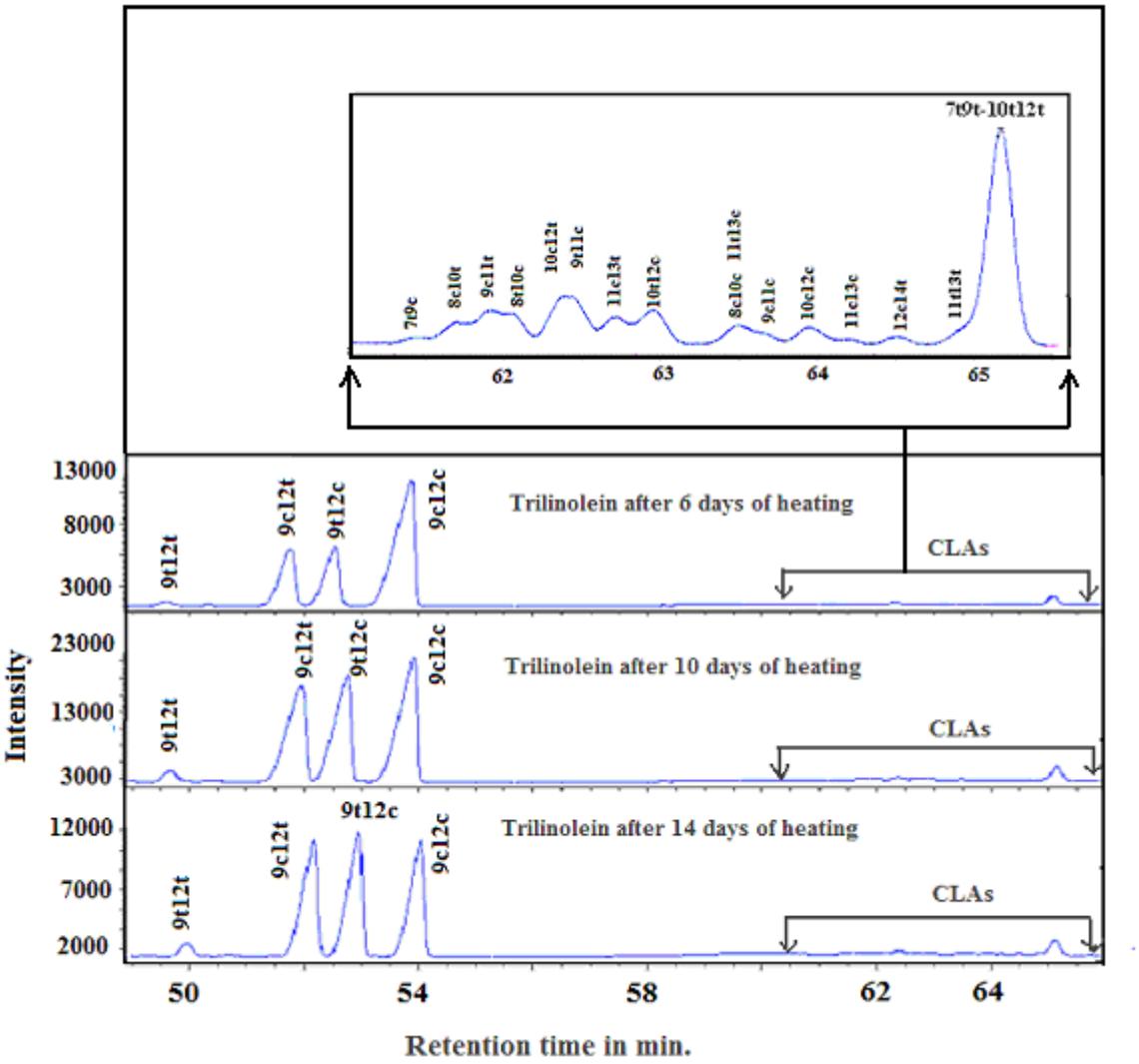

Fig. 3. Gas chromatograms of FAMEs of the heated trilinolein samples. The top window shows the elution order of CLAs formed during heating (Reprinted from Chemistry and Physics of Lipids, 161, A. A. Christy, "Evidence in the formation of Conjugated Linoleic Acids from thermally induced 9t12t linoleic acid: A study by Gas Chromatography and infrared spectroscopy", 86-94.,2009 with permission from Elsevier)

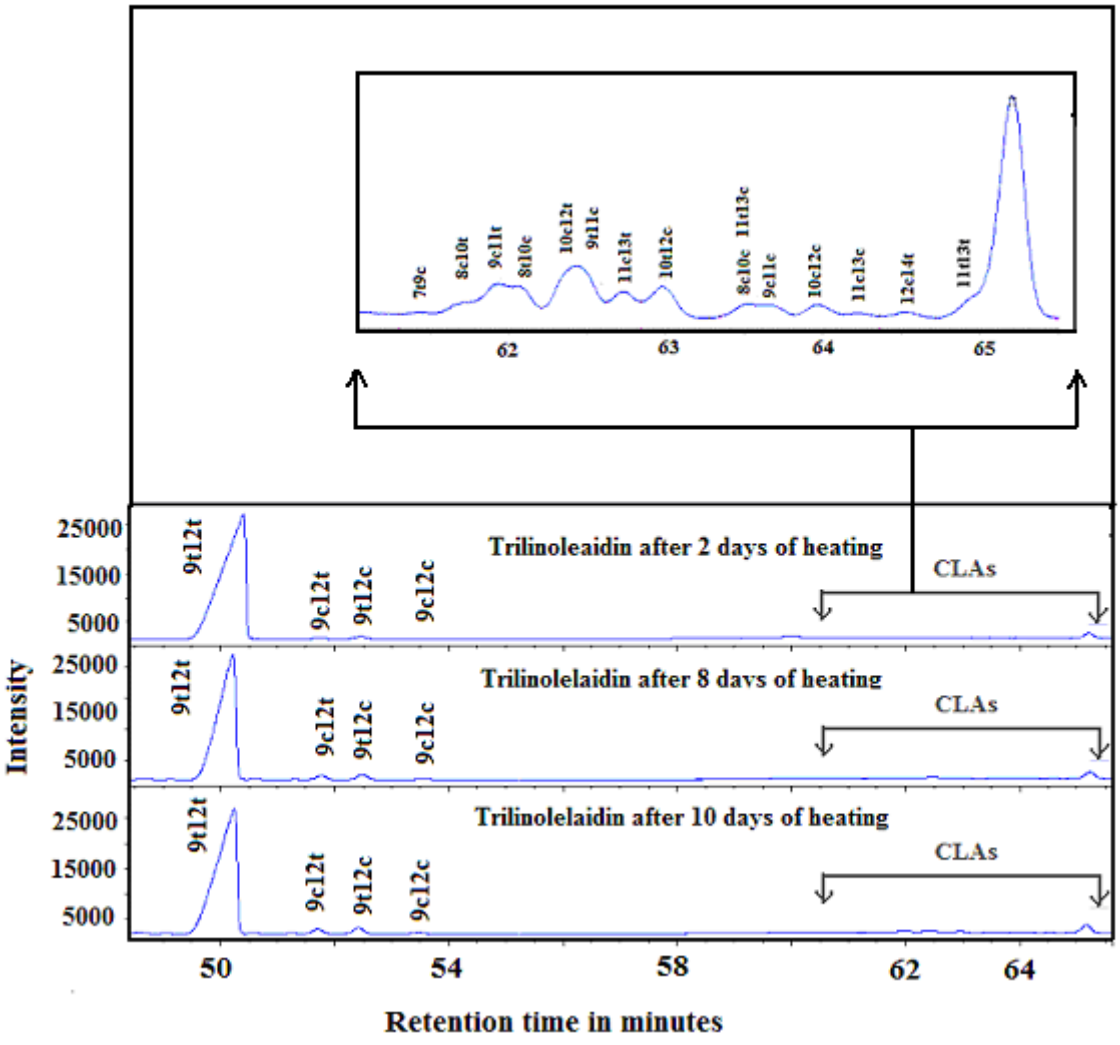

Fig. 4. Gas chromatograms of FAMEs of the heated trilinoelaidin samples. The top window shows the elution order of CLAs formed during heating (Reprinted from Chemistry and Physics of Lipids, 161, A. A. Christy, "Evidence in the formation of Conjugated Linoleic Acids from thermally induced 9t12t linoleic acid: A study by Gas Chromatography and infrared spectroscopy", 86-94.,2009 with permission from Elsevier)

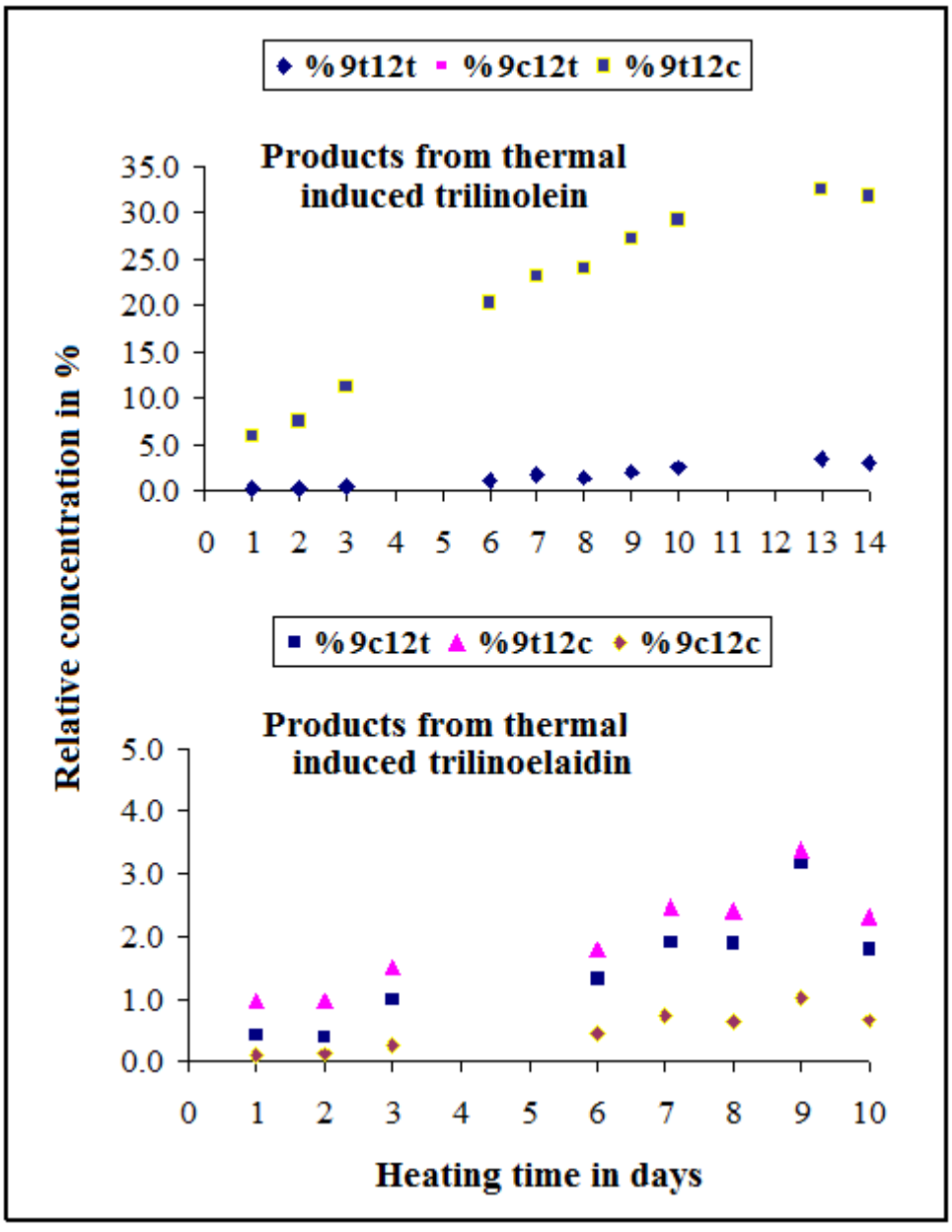
%9t12t
%9c12t
%9t12c
Products from thermal induced trilinolein
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
%9c12t
%9t12c
%9c12c
Products from thermal induced trilinoelaidin
5.0
4.0
3.0
2.0
1.0
0.0
0 1 2 3 4 5 6 7 8 9 10
Relative concentration in %
Heating time in days

Fig. 5. Concentration profiles of the isomers formed during heating. The concentrations of 9t12c and 9c12t are identical in the heat induced trilinolein

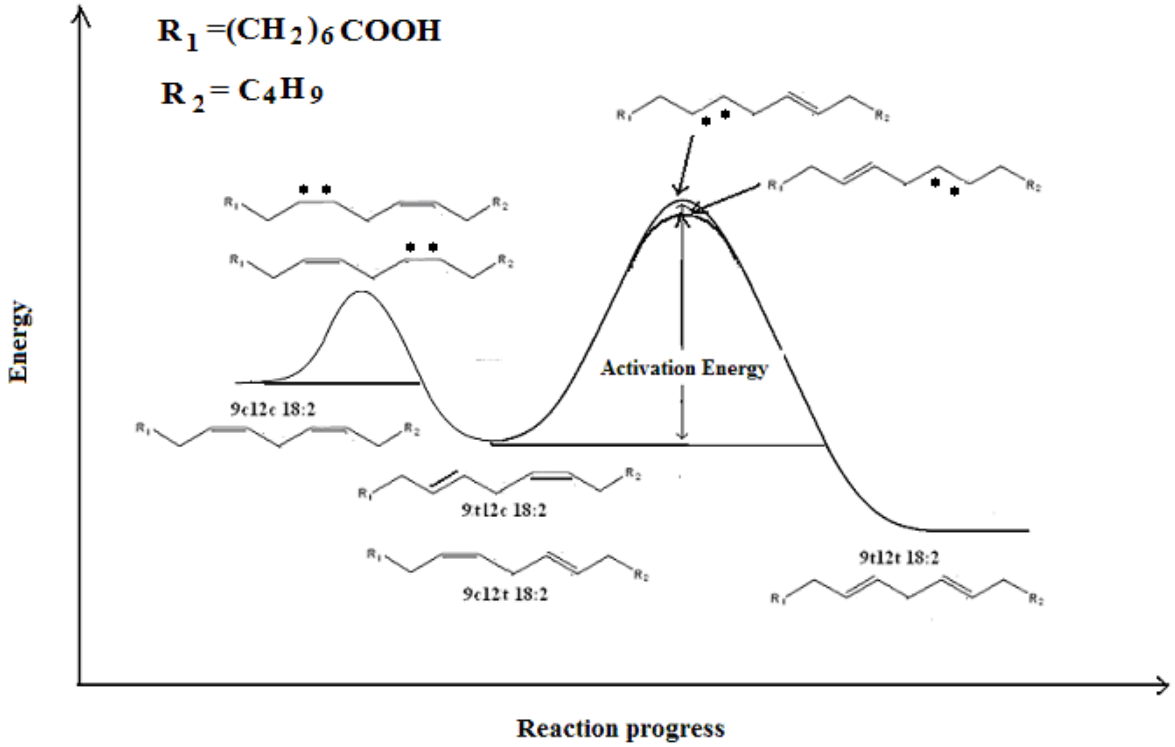

Fig. 6. Energy level diagram for the isomerisation into mono *trans* isomers following a diradical mechanism

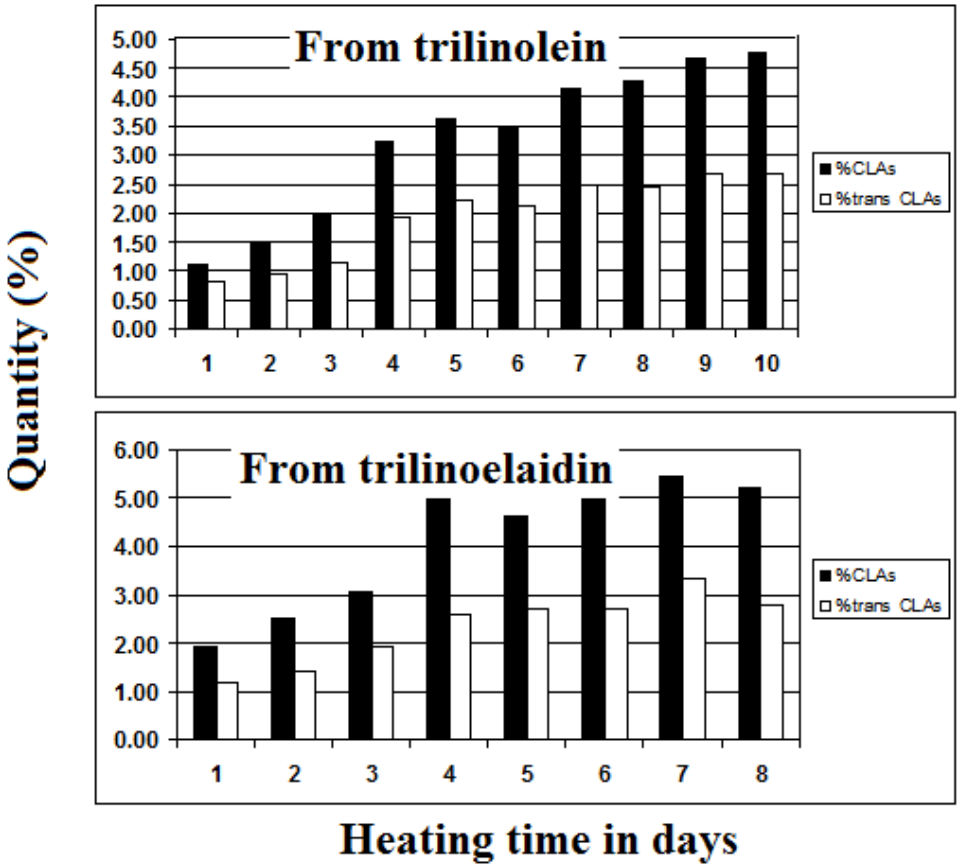

Fig. 7. Concentration profiles of Conjugated Linoleic Acids in the heated trilinolein and trilinoelaidin

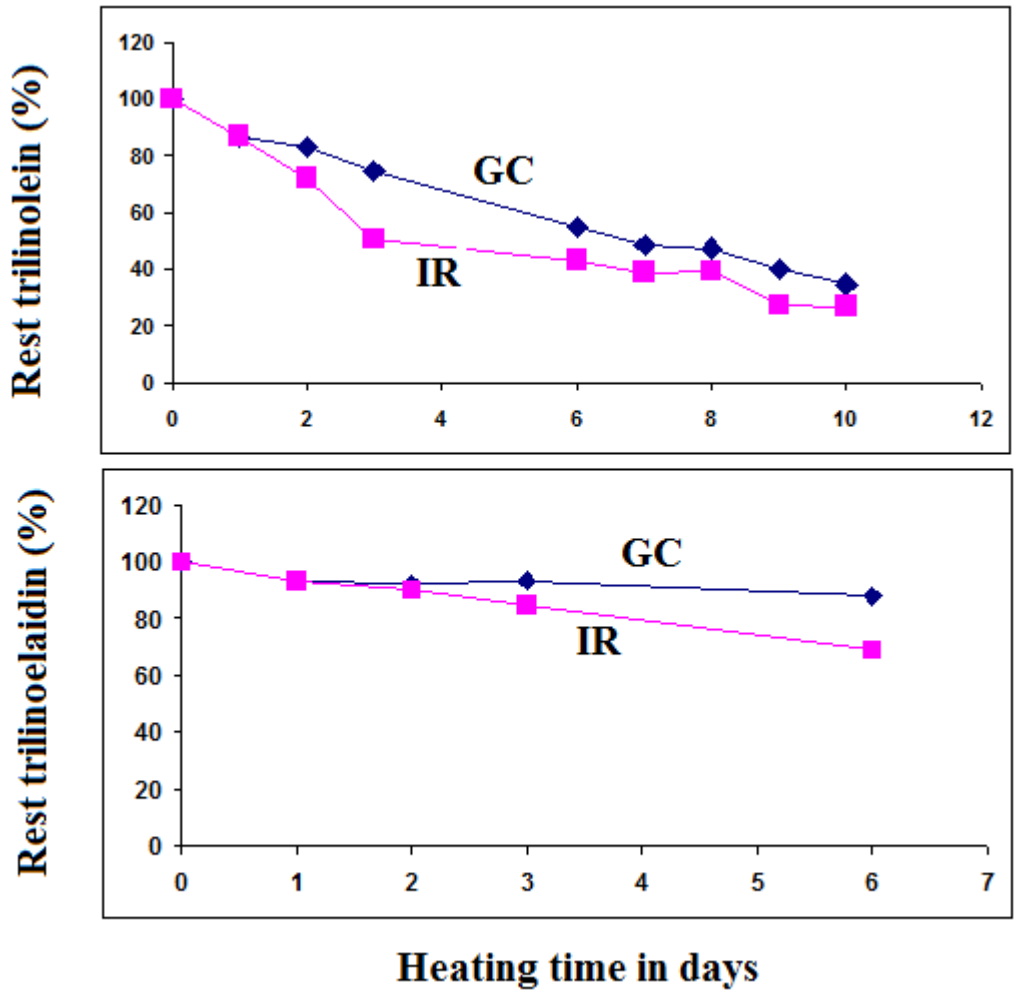

Fig. 8. Concentration of remaining trilinolein and trilinoelaidin in the heated samples as estimated by gas chromatography and infrared spectroscopy

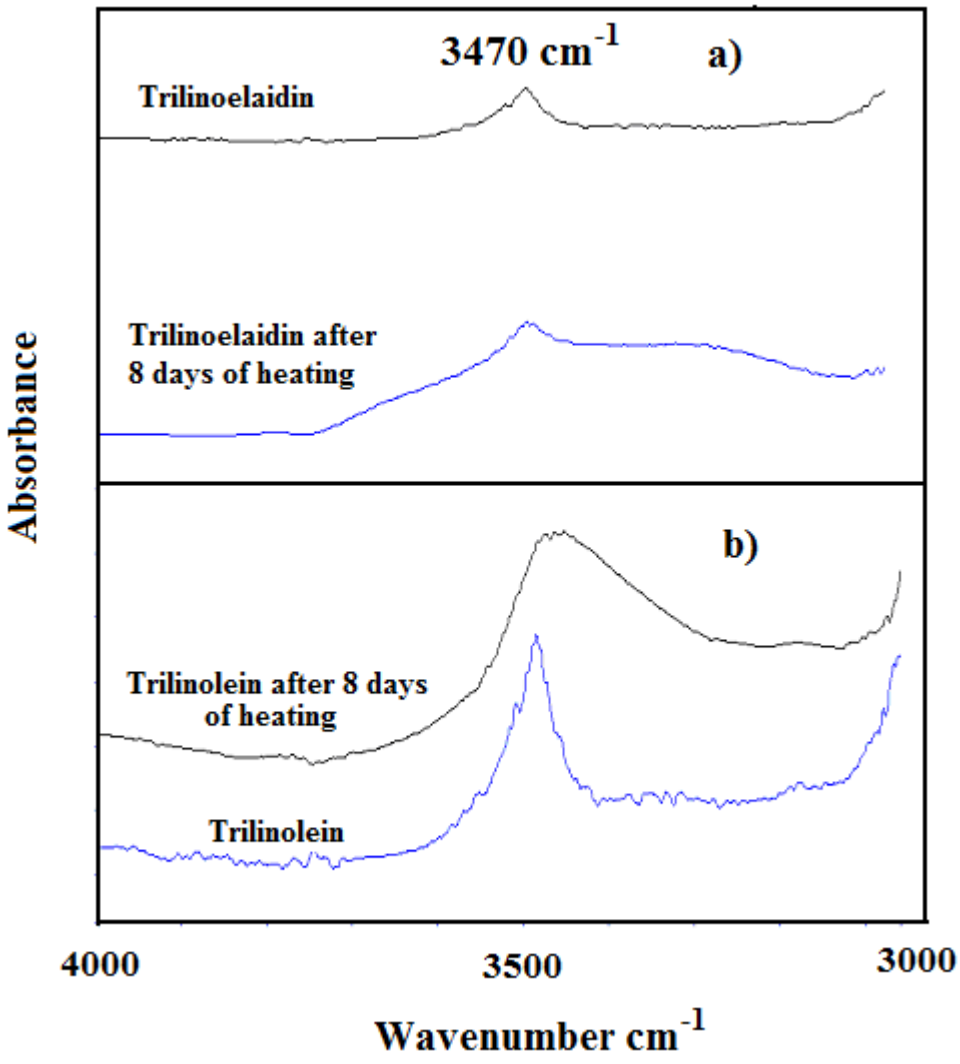

Fig. 9. Infrared spectra of heat treated trilinolein and trilinoelaidin samples in the region 4000-3000 cm^{-1}.