

Thermally Induced Cis-Trans Isomerisation in Trilinolein Studied by Infrared Spectroscopy and Gas Chromatography

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ABSTRACT

Trilinolein, a triglyceride containing linoleic acid as the only acid moiety in the glyceride molecules has been isothermally treated at 250 °C, 275 °C and 300 °C in sealed glass ampoules. The products formed during the thermal treatment at each temperature have been analysed both by infrared spectrophotometry and gas chromatography. In infrared spectroscopy the samples were analysed by transmittance technique using a single reflectance attenuated total internal reflectance crystal and a lead glycine sulphate detector. Second derivative profiles of the infrared spectra of the heated samples were obtained for the analysis of the peaks. The fatty acids in remaining triglyceride samples after infrared analysis were subjected to derivatisation into methyl esters. The methyl esters were then subjected to GC analysis. The second derivatives of the infrared profiles of the heated samples show that the thermally induced 9c12c fatty acid undergoes isomerisation. The peak intensity at 969 cm⁻¹ due to =CH trans bending vibration increased with heating time while the peak intensity at 3004 cm⁻¹ due to =CH cis stretching vibration decreased indicating the isomerisation of 9c12c fatty acid. The identification and quantification of 9c12t, 9t12c and 9t12t isomers were carried out by GC and used in the determination of kinetic parameters for the isomerisation reaction. The results of the isomerisation show that the formation of 9c12t and 9t12c are equally probable during thermal induction. Furthermore, the isomerisation reaction followed a first order reaction with an activation energy of 39.00 ± 1.00 kcal mol⁻¹.

KEYWORDS:

INTRODUCTION

Plants, animals and micro-organisms contain fatty acids in the form of triglycerides. The nature and their chemical transformations play very important roles in the biochemical processes in living systems. The fatty acids found in the glycerides of biological systems vary from saturated to mono and polyunsaturated fatty acids ranging from 12 to 20 carbon atoms and found in *cis* configurations. These unsaturated fatty acids isomerise into more stable *trans* configurations during extraction process [1], hydrogenation [2], heating [3,4], deep frying [5], deodorization [4,6,7] and radical catalisation [8]. A review on the formation and properties of *trans* fatty acids has been published by Żbikowska [9].

The impact of the nature of fatty acids on health effects has aroused interest in research on fatty acids and their transformations in different processes and systems. There have been numerous articles published on various aspects of fatty acids in the literature [1,2,5,7,10-22]. However, there has been very little work done on the *cis-trans* isomerisation of linoleic acid. One report published by Leon-Camacho *et al.* [7] and one report published by Mateos *et al.* [4] deal with the study of *cis-trans* isomerisation of linoleic acid during industrial deodorisation and physical refining processes. The reports deal with *cis-trans* isomerisation of linoleic acid and reported a zero order reaction for the isomerisation reaction. Gercar and Smidovnik [3] studied the geometrical

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isomerisation of linoleic and linolenic acid in heated soybean oil and Henon *et al.* [6] studied the geometrical isomerisation of fatty acids during deodorization of vegetable oils. These two studies reported a first order reaction for the geometrical isomerisation.

The determination of kinetic parameters for the *cis-trans* isomerisation of linoleic acids in trilinolein is interesting and will reveal whether the matrix surrounding the linoleic acid moieties play a part in affecting the kinetic parameters for the isomerisation reaction. The parameters determined for the thermally induced isomerization also would give researchers those who are interested in the chemistry and changes taking place with the linoleic acid in chemical reactions some idea on the possible products formed at a particular temperature.

Trilinolein is a triglyceride containing three linoleic acid (9c12c) units and thermal induction of trilinolein leads to the isomerisation of 9c12c fatty acid into 9c12t, 9t12c and 9t12t fatty acids. Apart from these, isomers of Conjugated Linoleic Acids (CLA) and isomers due to the migration of double bonds in linoleic acids can also be formed [14,15]. The study of isomerisation kinetics for the *cis-trans* isomerisation of linoleic acid in trilinolein therefore requires the precise determination of the concentrations of the remaining linoleic acid (9c12c) in the trilinolein samples or concentrations of the 9c12t and 9t12c and 9t12t fatty acid isomers formed in the heated samples.

Both infrared spectrometry and gas chromatography were used in achieving this. IR spectroscopy has been the method of choice for the determination of total *trans* content in food fats. Quantification of the total *trans* content in isolated *trans* fatty acids was based on the intensity of absorption of the C-H deformation vibration at 969 cm^{-1} [21,16]. The use of infrared spectroscopy in determining the concentrations of *trans* and CLA fatty acids can be found in Christy *et al.* [12] and Christy [15].

Gas chromatography involves saponification and derivatisation of the fatty acids in the thermally induced trilinolein samples and separation of the methyl esters using a high resolution capillary column that is suitable for the separation of *cis* and *trans* isomers.

2. Experimental:

2.1 Sample preparation and heating:

Trilinolein was purchased from Sigma-Aldrich. Triglycerides of the 9c11t and 10t12c were purchased from Larodan AS, Sweden.

The heating experiments were carried out at $250\text{ }^{\circ}\text{C}$, $275\text{ }^{\circ}\text{C}$ and $300\text{ }^{\circ}\text{C}$ in glass ampoules. Several glass ampoules of length 4 cm were made from glass tubes with 0.5 mm internal diameter and a wall thickness of 1 mm. Propane-oxygen flame was used to melt one of the edges of the glass tubes. Fifteen glass tubes were injected with about 0.5 mL portions of trilinolein using a plastic syringe. The other end of the glass ampoules were melted and sealed by using propane-oxygen flame. The sealed glass tubes were then kept in a metal beaker and placed in a chromatographic oven set at a temperature of $250\text{ }^{\circ}\text{C}$. The metal beaker was placed deep inside the oven. The glass tubes were removed at regular time intervals and the cooled samples were used in the infrared and gas chromatographic analysis. The same procedure was followed for samples heated at $275\text{ }^{\circ}\text{C}$ and $300\text{ }^{\circ}\text{C}$.

2.2 Infrared spectroscopic measurements and data treatment:

Each glass tube was cut open at one end and a portion of the sample was used to measure the representative infrared spectrum. A PerkinElmer Spectrum One FT-IR spectrometer equipped with a Harrick single reflectance attenuated total internal reflectance (ATR) accessory and lead glycine sulphate detector was used in measuring the infrared spectra. The accessory requires only a thin layer of sample on the crystal to acquire the infrared spectrum.

Each sample was spread on the ATR crystal using the blunt side of a capillary glass tube. A back ground spectrum was scanned in the range of $4000\text{-}600\text{ cm}^{-1}$ before the application of the sample. A total of 10 scans at a resolution of 4 cm^{-1} were then measured on each sample. The ATR crystal was washed with acetone and dried after each measurement. The same procedure was repeated for all samples heated at $275\text{ }^{\circ}\text{C}$ and $300\text{ }^{\circ}\text{C}$. The sample tubes were resealed and kept in dark for derivatisation and further analysis by gas chromatography.

All the infrared spectra measured on the samples were saved in absorbance format. Each infrared absorbance spectrum was transformed into their second derivative profiles by using the software of the infrared instrument and saved for further analysis. Second derivative profiles of the infrared spectra of the heated samples in the region 1000 cm^{-1} to 900 cm^{-1} were used for the identification and quantitative analysis of the *trans* and conjugated linoleic acids in the heated samples.

2.3 Derivatisation:

The trilinolein samples remaining in the test tubes after infrared analysis were subjected to derivatisation. Each glass tube containing the sample was cut open at both ends and the sample was pressed using an air flow into a pre weighed test tube. Then the total weight of the test tube with the sample was measured. The test tube

was then added 1 mL of magaric acid (hepta decanoic acid) in methanol (0.25 mg/ mL) as internal standard and 1 mL of 0.5 M methanolic NaOH solution. The test tubes containing the mixtures were then placed in a water bath set at a temperature of 65 °C for ten minutes. After cooling, each test tube was added 1 mL of BF₃-methanol and kept in the water bath again for 5 minutes at 65 °C. Again after cooling each of the test tubes was added 1 mL of n-heptane and 1 mL of saturated NaCl solution. Then the tubes were shaken well to aid separation and dissolution of the Fatty Acid Methyl Esters (FAMES) in the heptane layers. Each of the test tubes was then added more saturated NaCl solution until the heptane layer came up in the test tube. The test tubes were allowed to stand for a few minutes and the heptane layers in each of the test tubes were added a little amount of anhydrous MgSO₄. The heptane layers in the tubes were carefully withdrawn by using plastic syringes and filtered through 45 µm acetate filters into 1mL gas chromatographic vials. The samples were diluted using heptane and the vials were kept in a dark place before gas chromatographic analysis.

2.4 Preparation of reference standard:

A mixture of fatty acid methyl esters ranging from C16-C18 was prepared from the methyl esters of fatty acids. Then a mixture of Methyl esters of Conjugated Linoleic Acids was prepared by a procedure described in Christy [14,15]. These two mixtures were mixed and a total reference standard of fatty acids was prepared.

2.5 Gas chromatographic analysis:

The gas chromatographic analysis of the samples was carried out by using a Perkin Elmer gas chromatograph with an auto injector. A 120 m TRACE TR-FAME, capillary column with 0.25 mm internal diameter coated with 0.25 µm thick 70 % cyanopropyl polysilphenylene-siloxane stationary phase was used in the separation of the methyl esters of the fatty acid isomers.

A temperature program with initial temperature 170 °C with equilibration time of 2 minutes, then temperature gradient 1 °C / min up to 180 °C with equilibration time of 4 minutes and temperature gradient 1 °C / min were used to bring the final temperature to 200 °C. The temperature was held at 200 °C for 30 minutes. Each sample had 66 minutes run time.

The peak identification was carried out by comparing the retention times of the FAMES in the reference standard. The chromatographic peaks in the 30-50 min time interval were integrated and the peak areas were used in the calculation of kinetic parameters.

2.4 Methodology of the Isomerisation kinetics:

The kinetics parameters were determined by considering the isomerisation as a single reaction of the type A → B + C where A is the linoleic acid and B and C are *trans* isomers formed during the thermal induction. For an nth order chemical reaction the reaction rate k can be given as the rate of change in the concentration of the reactant.

$$d[A]/dt = -k[A]^n \quad (1)$$

If the concentrations of the reactant during the thermal induction can be determined at a time t from the start of the reaction, then the equation can be integrated for concentrations [A]₀ to [A]_t.

And the result becomes

$$\{[A]^{(1-n)}\}_{A_0}^{A_t} / (1-n) = -k[t]_0^t \quad (2)$$

$$\{[A_t]^{(1-n)} - [A_0]^{(1-n)}\} / (1-n) = -kt \quad (3)$$

The result is valid for 2nd and higher order reactions. If the reaction is of 1st order then the integration of equation 1 gives the relationship

$$\ln\{[A]_t / [A]_0\} = -kt \quad (4)$$

and for a reaction of zeroth order the result becomes

$$[A]_t - [A]_0 = -kt \quad (5)$$

The equations clearly show that the determination of the kinetic parameters require the precise determination of the concentrations of the reactant at reaction time t. For a reaction of first order the reaction rate for the reaction at temperature T_n can be determined by graphics using equation 4. Once the rates of the reaction k₁, k₂ and k₃ are determined for three different temperatures T₁(250 °C), T₂(275 °C) and T₃(300 °C) then the activation energy for the reaction can be determined by graphics using the Arrhenius equation (6).

$$k = A \exp(-E_a/RT) \quad (6)$$

Here A is the Arrhenius constant, E_a is the activation energy for the reaction, R is the gas constant and T is the temperature of the reaction.

$$\ln k = \ln A - E_a/RT \quad (7)$$

$$\ln k = \ln A - (E_a/R) (1/T) \quad (8)$$

During thermal induction 9c12c fatty acid moieties in trilinolein isomerise into triglycerides containing 9c12t, 9t12c, 9t12t fatty acids and the relationship between the concentration of the 9c12c fatty acid in the unreacted fraction and time can be investigated to determine the rate and activation energy for the isomerisation reaction.

RESULTS AND DISCUSSION

3.1 Infrared spectroscopy of fatty acids:

General band assignments for the infrared absorptions in triglycerides are given in Table 1. Infrared spectra of glycerides containing unsaturated fatty acids are similar except small changes in the *cis* =CH stretching absorption in the region 2960- 3010 cm^{-1} and *trans* =CH bending absorption in the region 930-1010 cm^{-1} . Changes in these two absorptions indicate the isomerisation taking place in the fatty acid molecules. The identification and quantification of the isomerisation in fatty acids have been discussed in the articles by Christy *et al.* [12,13].

Table 1: Some of the infrared band assignments of triglycerides.

Frequency (cm^{-1})	Functional group and mode of vibration
3025	=CH <i>trans</i> stretching
3004	=CH <i>cis</i> stretching
2953	CH (CH_3) asymmetric stretch
2924	CH ($-\text{CH}_2-$) asymmetric stretch
2854	CH ($-\text{CH}_2-$) symmetric stretch
1746	-C=O ester Fermi resonance
1653	-C=C- <i>cis</i> stretching
1465	-CH ($-\text{CH}_2-$, CH_3) bending
1377	-CH (CH_3) symmetric bending
1238	-C-O, $-\text{CH}_2-$ stretching, bending
1161	-C-O, $-\text{CH}_2-$ stretching, bending
1118, 1097	-C-O stretching
967	=CH <i>trans</i> bending
944, 984	Conjugated =CH bending for all <i>c,t</i> and <i>t,c</i> isomers
988	Conjugated =CH bending for all <i>t,t</i> isomers

Some mid infrared spectra and the second derivative profiles of the infrared spectra acquired with the samples subjected to thermal induction of trilinolein at 250 $^{\circ}\text{C}$ are shown in Figures 1 and 2 respectively. The changes in intensities at 3004 cm^{-1} and 967 cm^{-1} are not very clear in the raw spectra shown in Fig. 1. However, these changes are amplified and become clearer in the second derivative spectra shown in Fig. 2. Similar observations were made with samples heated at 275 and 300 $^{\circ}\text{C}$. The changes taking place in the regions above clearly indicate the isomerisation process and provide an easy and quick way to check on the progress of isomerisation. Kinetic parameters determined for the isomerisation reaction in this work have been based on the GC data.

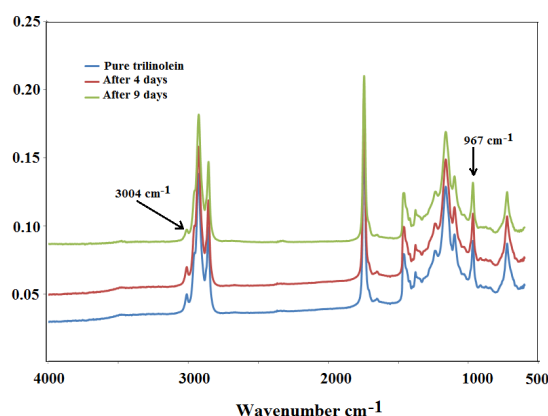


Fig. 1: Infrared spectra of three trilinolein samples heated at 250 $^{\circ}\text{C}$.

3.2 Gas chromatography:

Gas chromatograms obtained with trilinolein samples thermally treated at 250 and 300 $^{\circ}\text{C}$ are shown for three different heating times in Figures 3 and 4 respectively. The gas chromatograms clearly show the formation and increase in the concentration of *trans* isomers 9c12t and 9t12c with respect to the 9c12c isomer. Formation of a small amount of 9t12t is also evident. There is another interesting feature in the chromatograms is that the almost equal formation of the 9c12t and 9t12c *trans* isomers during heating. However, one can easily see that the 9t12c isomer is slightly high in concentration. This was observed in each and every chromatogram of the

samples analysed after heating in this work. This was also observed in the mixture of 9c12t and 9t12c isomers formed during the thermal induction of trilinoelaidin a triglyceride containing 9t12t fatty acid. Reason for this very small difference in concentration is not very clear. It is possible that steric hindrance may be a factor in this. The formation of almost equal concentrations of the *trans* isomers indicate that the opening of the double bonds at position 9 or position 12 in the radical mechanistic pathway have equal probabilities (Fig. 5). The formation of 9t12t takes place either from the isomerisation of isomer 9t12c or 9c12t. Here, the opening of the double bond at position 12 in 9t12c or at position 9 in 9c12t isomer. The isomerisation can take place through a di-radical mechanism leading to 9t12t isomer (Fig. 5 and 6).

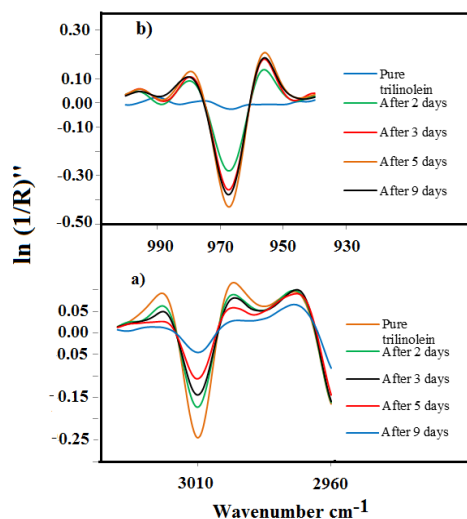


Fig. 2: Second derivative profiles of the spectra shown in Fig. 1 in the regions 1010-930 and 3010-2960 cm^{-1}

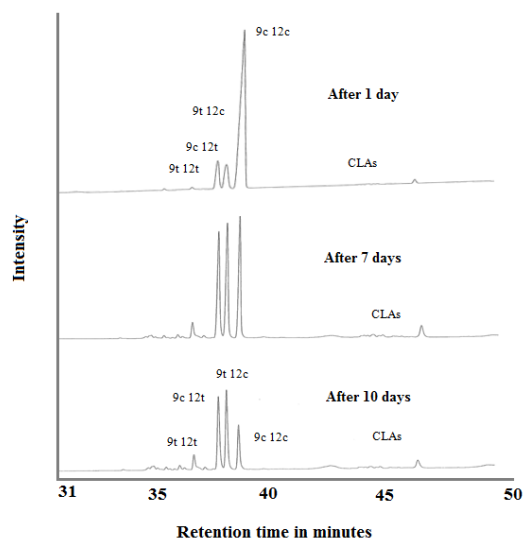


Fig. 3: Gas chromatograms of fatty acid methyl esters in the heated trilinoelmin samples at 250 °C.

The peak areas in the chromatograms were integrated and the percentages of the individual isomers were calculated. The percentages of the remaining trilinoelmin in heated samples were used to construct the concentration plots used in the determination of kinetic parameters.

The concentration evolution plots for heated trilinoelmin samples are shown in Fig. 7. The relationships show that the reaction is not a zeroth order reaction. Plots for $\ln(A_t/A_0)$ against time are shown for the same in Fig. 8. Linear correlations indicate that the reaction is a first order reaction. The reaction rates determined from the correlations in Fig. 8 were used in determining the activation energy for the isomerisation reaction. A plot showing the correlation between $\ln k$ and $1/T$ is shown in Fig. 9. Excellent linear correlation leads to a relatively precise activation energy for the isomerisation reaction.

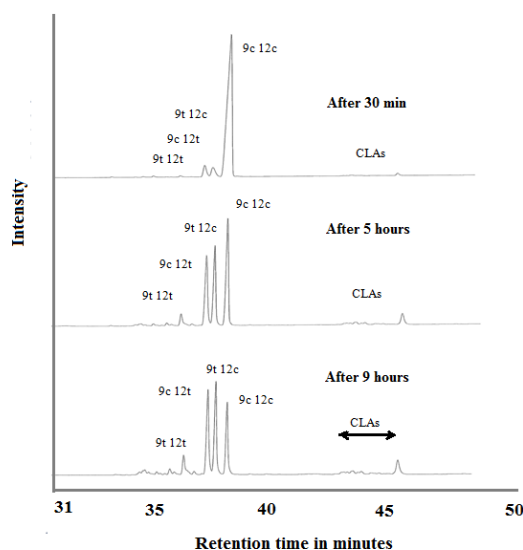


Fig. 4: Gas chromatograms of fatty acid methyl esters in the heated trilinolein samples at 300 °C.

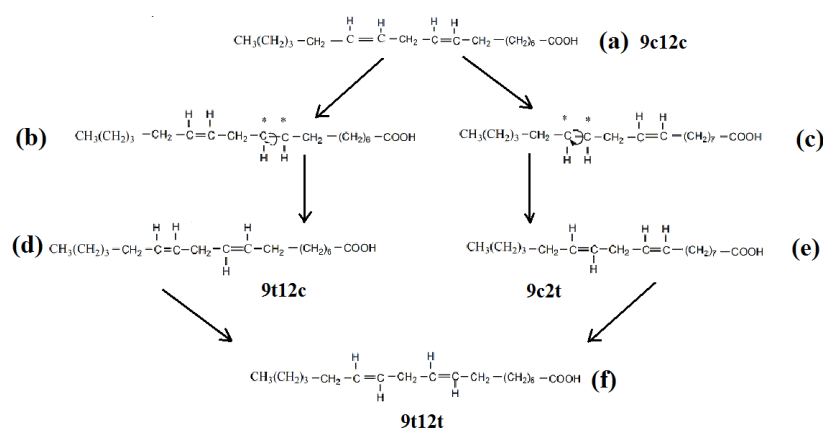


Fig. 5: A di-radical mechanistic pathway for the formation of trans isomers from linoleic acid.

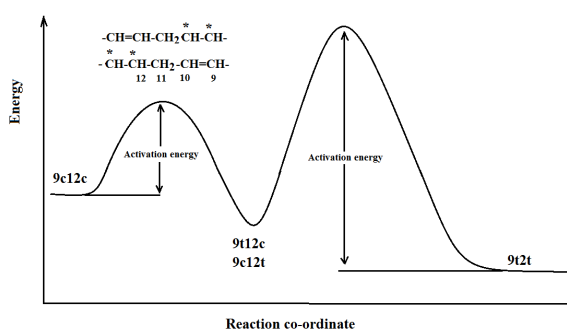


Fig. 6: Activation energy diagram for the reactions shown in Fig. 5.

The kinetic parameters determined in this work are presented in Table 2. The order of the isomerisation reaction is in contrast to the reaction order for the isomerisation reaction of 9c12c fatty acids in edible oils reported by Leon-Camacho *et al.* [7] and Mateos *et al.* [4]. However, the result is in agreement with the reports of Hennon *et al.* [6] and Gercar and Smidovnik [3]. The activation energy determined is in the same range reported by Leon-Camacho *et al.* [7] and Gercar and Smidovnik [3].

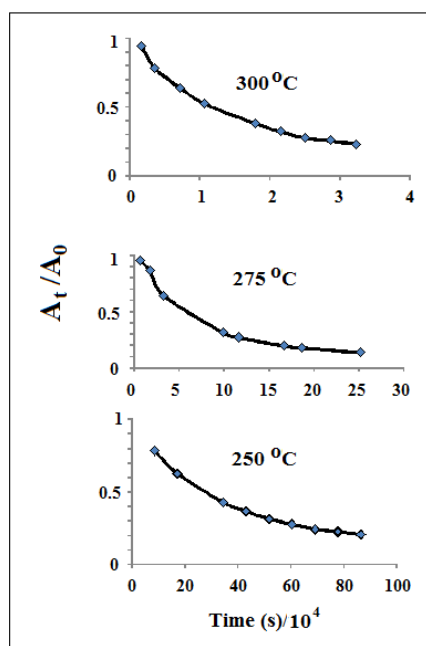


Fig. 7: Concentration ratio of linoleic acid in the heated samples plotted against time.

Table 2: Kinetic parameters for the isomerisation reaction.

Isomer	Temp.(^o C)	k, Reaction Rate(1/s) $k \cdot 10^{-4}$	Activation Energy (kcal mol ⁻¹)
18:2(9c12c)	250 ^o C	0.017	
18:2(9c12c)	275 ^o C	0.083	39.00±1.00
18:2(9c12c)	300 ^o C	0.459	

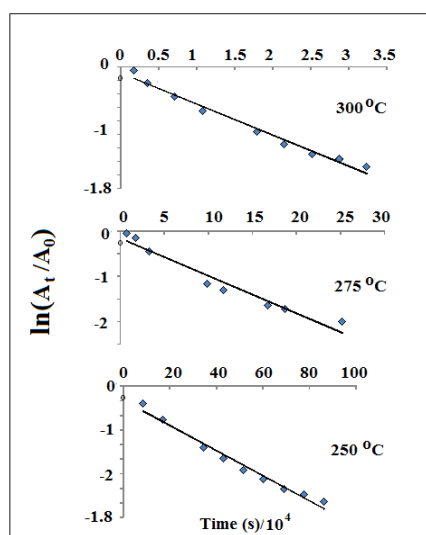


Fig. 8: A plot of $\ln(A_t/A_0)$ vs time.

Conclusion:

Kinetic parameters for the thermally induced isomerisation of 9c12c fatty acids in trilinolein have been determined in this work and reported for the first time.

The isomerisation reaction follows a first order kinetics as in the thermally induced isomerisation of 9c fatty acids in triolein [13]. The order of the reaction seems to differ from the work reported by Leon-Camacho *et al.* [7] and Mateos *et al.* [4]. However, the first order reaction kinetics of the isomerisation is in agreement with the reports of Hennon *et al.* [6] and Gercar and Smidovnik [3].

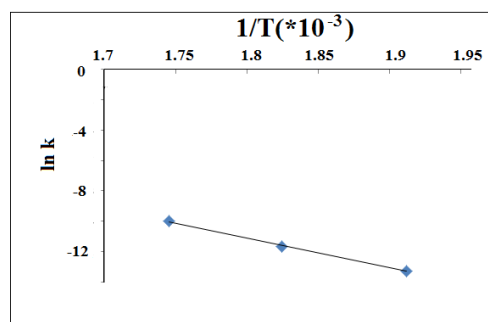


Fig. 9: A plot of $\ln k$ vs $1/T$.

The activation energy for the *cis-trans* isomerisation of 9c12c fatty acid in trilinolein is 39.00 ± 1.00 kcal mol⁻¹. The activation energy determined in this work is in the same range as the values reported by Leon-Camacho *et al.* [7] and Gercar and Smidovnik [3].

The double radical mechanism that describes the isomerisation of 9c12c fatty acids clearly shows the equal formation probabilities of 9t12c and 9c12t isomers. The formation of 9t12t requires higher activation energy and this will be reported in the future.

Infrared spectroscopy provides an easy way to check up on the isomerisation progress by monitoring the peak at 967 cm⁻¹. Quantification of total *trans* and CLA isomer concentrations based on the peaks in the region 900-1000 cm⁻¹ has been already reported by Christy *et al.* [12].

REFERENCES

1. Eder, S.R., 1982. The formation of Artifacts during Deodorization of fats and oils. *Fette Seifen Anstrichmittel*, 84: 136-141.
2. Dutten, H.J., 1979. Geometrical and Positional Fatty Acid Isomers. In: E. A. Emken and H. J. Dutten (Ed) American Oil Chemists' Society, Champaign.
3. Gercar, N., A. Smidovnik, 2002. Kinetics of geometrical isomerization of unsaturated FA in soybean oil, *J. Am. Oil. Chem. Soc.*, 79: 495-500.
4. Mateos, L.E., G.M. Tonetto, G.H. Crapiste, 2010. Isomerization of Fatty Acids in Sunflower oil During Heat Treatment. *Lat. Am. appl. res.*, 40(3): 213-217.
5. Beatriz, M., P.P. Oliveira, M.A. Ferreira, 1996. Capillary Gas Chromatographic Evaluation of *trans*-Fatty Acid Content of Food Produced under the Traditional Conditions of Semi-Industrial Frying. *J. High Resol. Chromatogr*, 19: 180-182.
6. Hénon, G., Z. Kemény, K. Recseg, F. Zwobada, K. Kovari, 1999. Deodorization of Vegetable Oils. Part I: Modelling the Geometrical Isomerization of Polyunsaturated Fatty Acids. *J. Am. Oil Chem. Soc.*, 76:73-81.
7. Leon-Camacho, M., M. Victoria, R. Mendez, M. Graciani-Constante, E. Graciani-Constante, 2001. Kinetics of the *cis-trans* isomerisation of linoleic acid in the deodorization and/or physical refining of edible fats, *Eur.J.Lipid Sci.Tech.*, 103: 85-92.
8. Tzeng, Y.Z., C.H. Hu, 2014. Radical-Induced *Cis-Trans* Isomerization of Fatty Acids: A Theoretical Study, *J. Phys. Chem. A*, 118(25): 4554-4564.
9. Żbikowska, A., 2010. Formation and Properties of Trans Fatty Acids – A Review, *Pol. J. Food Nutr. Sci.*, 60(2): 107-114.
10. Belton, P.S., R.H. Wilson, H. Sadehgi-Jorabegi, K.E. Peers, 1988. A Rapid Method for the Estimation of Isolated *trans* double bonds in Oils and fats Using FTIR combined with ATR. *Lebensm. Wiss. Technol.*, 21: 153-157.
11. Chipault, J.R., J.M. Hawkins, 1960. Conversion of conjugated *cis-trans* Octadecadienoates to the *trans-trans* isomers, *JAOCs*, 37(4): 176-182.
12. Christy, A.A., P.K. Egeberg, E.T. Østensen, 2003. Simultaneous Quantitative determination of isolated *trans* fatty acids and conjugated linoleic acids in oils and fats by Chemometric analysis of the infrared profiles, *Vib. Spectrosc.*, 33: 37-48.
13. Christy, A.A., Z. Xu, P.B. Harrington, 2009. Thermal Degradation and Isomerisation Kinetics of Triolein studied by Infrared Spectrometry and GC-MS Combined with Chemometrics. *Chem. Phys. Lipids*, 158: 22-31.
14. Christy, A.A., 2009. Evidence in the formation of Conjugated Linoleic Acids from thermally induced 9t12t linoleic acid: A study by gas chromatography and infrared spectroscopy. *Chem. Phys. Lipids*, 161: 86-94.

15. Christy, A.A., 2009. Thermally induced isomerisation of Trilinolein and Trilinoelaidin at 250 °C: Analysis of products by gas chromatography and infrared spectroscopy, *Lipids*, 44: 1105-1112.
16. Christy, A.A., 2012. Thermally induced isomerisation kinetics of the 9c 11t and 10t 12c conjugated linoleic acids in triacylglycerols as studied by FT-IR spectroscopy aided by gas chromatography, *TOSPECJ*, 4: 41-48.
17. Destailats, F., P. Angers, 2005. Thermally induced formation of conjugated isomers of linoleic acid, *Eur. J. Lipid Sci. Tech.*, 107: 167-172.
18. Litchfield, C., J.E. Lord, A.F. Isbell, R. Reiser, 1963. Cis-trans isomerisation of oleic, linoleic and linolenic acids, *JOACS*, 40: 553-557.
19. Tsuzuki, W., R. Nagata, R. Yunoki, M. Nakajima, T. Nagata, 2008. Cis-trans isomerisation of triolein, trilinolein and trilinolenin induced by heat treatment, *Food Chem.*, 108: 75-80.
20. Eulitz, K., M.P. Yurawecz, M.P.N. Sehat, J. Fritsche, J.A.G. Roach, M.M. Mossoba, J.K.G. Kramer, R.O. Adlof, Y. Ku, 1999. Preparation, separation and confirmation of the eight geometrical cis-trans conjugated linoleic acid isomers 8,10-through 11,13-18:2 lipids, 34(8): 873-877.
21. Lancer, A.C., E.A. Emkem, 1988. Comparison of FTIR and capillary GC methods for quantitation of *trans* Unsaturation in fatty acids methyl esters, *Ibid*, 65: 1483-1487.
Li, A., B. Yuan, W. Li, F. Wang, Y. Ha, 2013. Thermally induced isomerisation of linoleic acid in soya bean oil, *Chem. Phys. Lipids*, 166: 55-60.
22. Tsuzuki, W., R. Nagata, R. Yunoki, M. Nakajima, T. Nagata, 2008. Cis-trans isomerisation of triolein, trilinolein and trilinolenin induced by heat treatment, *Food Chem.*, 108: 75-80.