

Thermally induced chemical reactions in castor oil studied by infrared spectroscopy and gas chromatography: Determination of activation energy for the dehydration of ricinoleic acid

¹Alfred A. Christy and ²N.R. Fernando

^{1,2}Department of Science, Faculty of Engineering and Science, University of Agder, Serviceboks 422, Kristiansand, Norway

Received 7 June 2016; Accepted 12 September 2016; Available 20 September 2016

Address For Correspondence:

Alfred A. Christy, Department of Science, Faculty of Engineering and Science, University of Agder, Serviceboks 422, Kristiansand, Norway.
Tel: 4792292181; E-mail: Alfred.christyia.no

Copyright © 2016 by authors and American-Eurasian Network for Scientific Information (AENSI Publication).

This work is licensed under the Creative Commons Attribution International License (CC BY).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

ABSTRACT

Castor oil has been thermally treated at three different temperatures (250, 275 and 300 °C) in glass ampoules and the products formed during the thermal induction have been studied by infrared spectrometry and gas chromatography. The gas chromatographic results have been used to study chemical reactions induced by heating and determine the activation energy for the dehydration reaction. Hundred milligram (100 mg) portions of castor oil were placed in micro glass ampoules, sealed using oxygen and propane flame and then subjected to thermal treatment. The glass ampoules were removed at regular time intervals, cut open, and a part of the sample was analyzed by infrared spectroscopy using a Harrick single reflectance attenuated total internal reflectance (ATR) accessory and a DTGS (Deuterated TriGlycine Sulphate) detector. The remainder of the sample was subjected to derivatization into their methyl esters. The methyl esters of fatty acids in the mixture were then analyzed by gas chromatography after appropriate dilution in heptane. The results show that the thermally induced castor oil mainly gave CLA (conjugated Linoleic Acid) isomers ranging from 7t9c to 12t14t. The CLA isomers 9c11t, 8t10c and *trans trans* CLAs were predominating in the mixture. In addition to CLAs considerable quantities of 18:2 LA (Linoleic Acid) isomers (9c12t, 9t12c and 9c12c) were also observed. The activation energy determined for the dehydration reaction was 47±1 kcal/mol.

KEYWORDS: Castor oil, Conjugated Linoleic Acid, Dehydration, Infrared spectroscopy, Gas Chromatography

INTRODUCTION

Castor oil has been known for a long time as industrial oil and also has reputation for its medicinal use. Most of the properties of castor oil come from the key component of ricinoleic acid (12-hydroxy-9-octadecenoic acid). The castor oil comprises 86-90% of ricinoleic acid [1]. Castor oil is inedible, but it has been an article of commerce mainly due to the versatility of the oil [2-4]. The hydroxyl group, double bond and ester linkages in ricinoleic acid provide reaction sites for the preparation of many useful compounds for industrial and medical purposes. Especially, hydroxyl group can be eliminated by dehydration leading to the formation of new double bond in the fatty acid chain of ricinoleic acid. The thermal dehydration and subsequent isomerization reactions taking place in the mixture lead to the formation of CLAs and LAs.

To Cite This Article: Alfred A. Christy and N.R. Fernando., Thermally induced chemical reactions in castor oil studied by infrared spectroscopy and gas chromatography: Determination of activation energy for the dehydration of ricinoleic acid. *Advances in Natural and Applied Sciences*. 10(13); Pages: 146-156

The Conjugated Linoleic Acid (CLA) is a collective term used to describe the mixture of positional and geometrical isomers of octadecadienoic (linoleic) acid with two conjugated double bonds (C18:2). The double bonds of CLA may be in the positions of C 7,9 ; 8,10; 9,11; 10,12 or 11,13 with the combination of *cis* and or *trans* three dimensional configurations. In recent years, there have been considerable interests in conjugated linoleic acids, owing to their unique biological properties. It is known that the CLA isomers have a wide range of biological activities including reduction in body fat, enhanced bone mineralization, antioxidative, anticarcinogenic, antiatherosclerotic, antidiabetic, and effect on immune function and diabetes [5-19].

Production of CLAs via dehydration of the castor oil (ricinoleic acid) is an attractive research topic because castor oil is inexpensive and simple starting material. Lin Yang and co-workers [20] have reported a new method to produce CLAs from the dehydration of ricinoleic acid (source castor oil) in the presence of KOH or DBU (1, 8-diazabicyclo-(5.4.0)-undec-7-ene) as catalyst. But the DBU is an expensive dehydrating agent. The KOH catalyzed dehydration yielded 72% 9c11t and 26% 9c11c, while DBU catalyzed dehydration yielded 78% 9c11t and 16% 9c11c [20]. Jafari *et al.* [3] also obtained 9c11t CLA isomer in high concentration by dehydration of castor oil in the presence of KOH as catalyst. They investigated the reaction by using response surface methodology (RSM) [3]. Nezihe and co-workers [4] have reported suitable reaction parameters under micro wave irradiation (reaction time, reaction temperature, catalyst ratio, and pressure) for obtaining dehydrated castor oil from raw castor oil. There are no reports in the literature dealing with the thermally induced dehydration of castor oil without catalyst.

The objective of the present study was to follow the dehydration process of castor oil without using any catalyst and compare the isomeric distribution of CLAs and LAs produced at three different temperatures. It was also our interest to look at the mechanistic pathways leading to the different products in the thermal induction process and to determine the activation energy for the dehydration reaction.

Experimental:

1.1 Samples and methods:

All the chemicals were obtained from commercial suppliers and used without any further purification. Boron trifluoride in methanol, heptadecanoic acid and heptane were purchased from Sigma. Methanol for liquid chromatography and sodium hydroxide were obtained from Merck (Germany). Anhydrous magnesium sulfate was obtained from Fluka. Castor oil was bought from the local market. The heating experiments were carried out in micro glass ampoules. Ampoules were 4 cm long, 1.55 mm internal diameter and 1 mm wall thickness. Each ampoule was sealed at one end using propane, oxygen and air flame. Castor oil samples (≤ 100 mg) were injected into the ampoules using plastic syringes and sealed in the same manner as above [21].

The first set of sealed glass ampoules were placed in a gas chromatograph oven at 250 °C. Samples were removed at 12h time intervals until 11 samples were exhausted. The experiment was repeated in the same manner with second and third set of samples at 275 °C and 300 °C respectively. The samples heated at 275 °C were removed at 2h intervals and samples heated at 300 °C were removed at 0.5h time intervals. The compositions of the samples were determined by infrared spectroscopic analysis and Gas chromatography.

1.2 Infrared measurements and data treatment:

A Perkin Elmer Spectrum One FT-IR spectrometer equipped with a zinc selenide Harrick single reflectance ATR crystal accessory was used in measuring the infrared spectra. The accessory requires only a thin layer of sample on the crystal to measure the infrared spectrum. Each glass ampoule cut open and a small portion of sample was spread on the ATR crystal using the blunt side of a capillary glass tube. A background spectrum was scanned in the range of 4000 – 600 cm^{-1} before introducing the sample. A total of 32 scans were made on each sample at a resolution of 4 cm^{-1} . The infrared spectra measured on the series were saved as absorption spectra. Each infrared absorbance spectrum was transformed to second derivative and used for further analysis.

1.3 Derivatisation and GC Analysis:

The remaining heated samples of castor oil in the glass ampoules were subjected to derivatisation. Each glass tube containing the sample was cut just above the liquid mark and crushed inside a 15 mL test tube. Each of the test tubes was each added 1 mL (1.5 mg/mL) heptadecanoic acid and 1 mL of 0.5 M methanolic NaOH. The test tubes were then placed in a water bath at 65 °C for 10 minutes. After cooling, each test tube was added 1 mL of BF_3 /methanol and placed in the water bath again for 4 minutes at 65 °C. The test tubes were then cooled and each of them was added 1 mL of heptane and 3 mL of saturated sodium chloride solution. The test tubes were shaken well to get better separation and dissolution of fatty acids methyl esters in the organic layer (heptane). The test tubes were then allowed to stand for a few minutes and the top layer in each of the test tubes was added anhydrous magnesium sulfate. After a few minutes standing the heptane layers in each of the test tubes was carefully removed using a syringe and filtered through a 0.45 μm filter and placed in a small brown GC vial and kept in dark until GC analysis [21].

The GC analysis was carried out by using Perkin Elmer Auto system XL gas chromatograph. A 120 m capillary column with 0.25 mm internal diameter coated with 0.25 μm thick, 70% cyanopropyl(equiv) polysilphenylene-siloxane stationary phase used. A temperature program with initial temperature of 170 $^{\circ}\text{C}$ with 2 minute equilibration time, a temperature gradient of 10 $^{\circ}\text{C}/\text{min}$ up to 180 $^{\circ}\text{C}$ with 20 minutes holding time, then a temperature gradient of 1 $^{\circ}\text{C}/\text{min}$ up to 190 $^{\circ}\text{C}$ with 15 minutes holding time and finally a temperature gradient of 10 $^{\circ}\text{C}/\text{min}$ up to 250 $^{\circ}\text{C}$ with 20 minute holding time was used for the GC analysis. The total running time was 83 minutes.

The peak identification was carried out by comparing with the retention times of the standard fatty acid methyl esters, methylated 9c11t and 10t12c CLA isomers and reported conjugated linoleic acid profiles [22]. The gas chromatographic peaks were integrated and the peak areas were obtained as percentages.

RESULTS AND DISCUSSION

2.1 Infrared spectroscopy:

Infrared spectra of pure castor oil and isomer mixtures obtained after the heat treatment at three different temperatures are shown in Fig. 1. The infrared band assignments are given in table 1. The partial second derivative profiles of the heated samples at 250, 275 and 300 $^{\circ}\text{C}$ are shown in Fig. 2. The peaks at 946 cm^{-1} and 986 cm^{-1} indicate the presence of CLAs in the heated samples while peak at 967 cm^{-1} indicates the presence of *t,c* or *c,t* or *t,t* LAs or mixture of these isomers [6, 23-26]. The CLAs with *cis-trans* and *trans-cis* configurations give rise to two specific absorption at around 946 cm^{-1} and 986 cm^{-1} because of the =CH out-of plane deformation vibrations. The out of plane deformation vibration of *trans-trans* CLA isomers absorb around 982-988 cm^{-1} , and *cis-cis* isomers do not absorb in this region.

Table 1: Infrared peak assignments for castor oil

Frequency (cm^{-1})	Functional group and mode of vibration
3600-3100	OH stretching Hydrogen bonded
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

The second derivative profiles are clearly indicating the changes in the absorptions with heat treatment. The spectra at three different temperatures show that the peaks at 946 cm^{-1} and 986 cm^{-1} increase in absorption intensity and reach a maximum and then deflect with the heat treatment time. The peak at 967 cm^{-1} decreases in intensity first and then increases with increasing heating time.

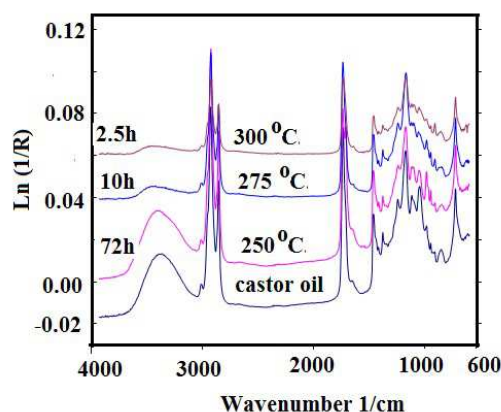


Fig. 1: Infrared spectra of castor oil and heat treated castor oil samples

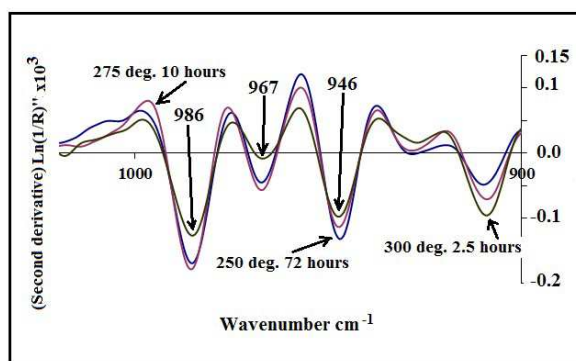


Fig. 2: Second derivative profiles of the heated castor oil samples shown in Fig.1

The concentrations of trans and CLAs can be simultaneously quantified by multivariate calibration and prediction [27]. However, an approximate quantification of CLAs can be made by measuring the intensities of absorptions of the peaks at 946 cm^{-1} and 986 cm^{-1} and comparing with the infrared absorption intensities (from second derivative profiles) of triglyceride containing 9c11t fatty acid. Similarly, the 18:2 trans fatty acids in the heated castor oil can be approximately quantified by comparing the peak at 967 cm^{-1} with intensity of absorption of tri-glyceride containing pure 18:1 trans fatty acid. Plots between the absorption intensities of the peak at 967 cm^{-1} and the combination of the intensities of the peaks at 946 cm^{-1} and 986 cm^{-1} against time are shown in Fig. 3. The maximum concentrations of CLAs were approximately 30% after 66 h heating at $250\text{ }^{\circ}\text{C}$ while 26% after 10 h heating at $275\text{ }^{\circ}\text{C}$ and 22% after 3 h heating at $300\text{ }^{\circ}\text{C}$. However, infrared spectroscopy cannot reveal the identities of the different isomers of trans LAs and CLAs. This can only be done by gas chromatographic separation of the fatty acids in the mixtures.

2.2 Gas chromatography:

The concentration of ricinoleic acid methyl ester (RAME) decreases with heat treatment at all three different temperatures, because of the dehydration of ricinoleic acid. The dehydration process leads to the formation of different isomers of Conjugated Linoleic Acids and methylene interrupted Linoleic Acids. The total chromatograms of FAMES of raw castor oil and of one of the heated sample are shown in Fig. 4. The raw castor oil sample had 92 % ricinoleic acid, 0.04 % CLA isomers including 9c11t, 10c12t and 9t11t and 0.24 % of 18:2 LA isomers including 9c12t and 9c12c.

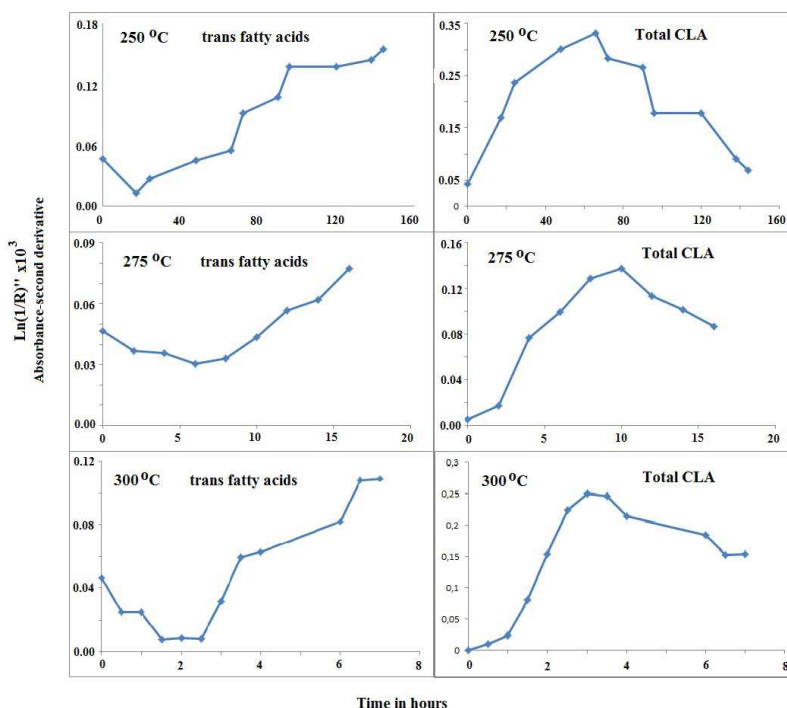


Fig. 3: Plots showing the relationships a) between absorbances (at 967 cm^{-1}) of LAs against heating time in the heated castor oil samples and b) between absorbances (total absorbances at 947 and 982 cm^{-1}) of CLAs against time

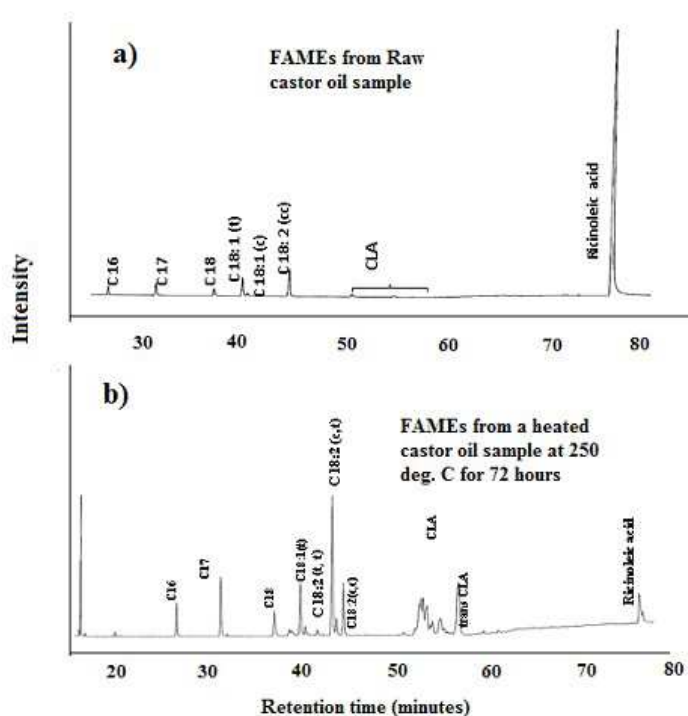


Fig. 4: Total chromatograms of the methyl fatty acids of a) raw castor oil b) a heated castor oil sample

At a dehydration temperature of 250 °C for 66 hours, the concentration of ricinoleic acid decreased to 0.79% while the concentrations of CLAs and LAs increased to 30 and 32.5% respectively.

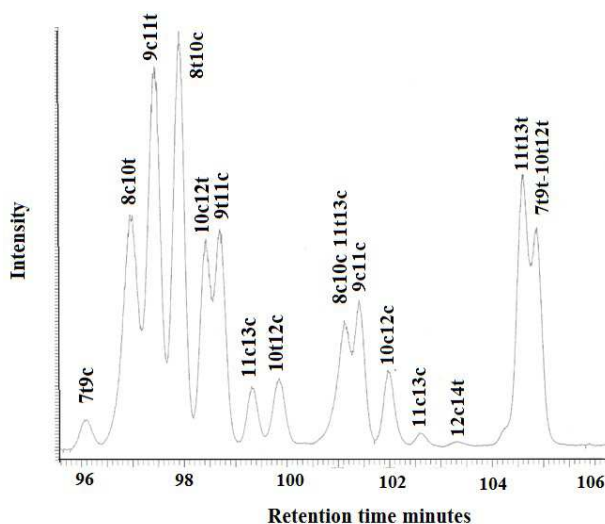


Fig. 5: Gas chromatogram of the methylated CLAs in a heated castor oil sample

In the beginning of heat treatment few CLA isomers identified at all three temperatures, but after prolonged heating various isomers of CLAs appeared. The elution order of CLA isomers was determined as described in Kramer et al. [22] who reported the complete elution order first in 1999. The same was also used in the identification of CLAs by Christy [28]. Most of the CLA isomers identified are shown in Fig. 5. Major isomers of CLA were the 9c11t, 8t10c and *trans trans* CLAs. In addition to CLA isomers methylene interrupted C18:2 linoleic acid isomers 9c12t, 9t12c and 9c12c were also formed.

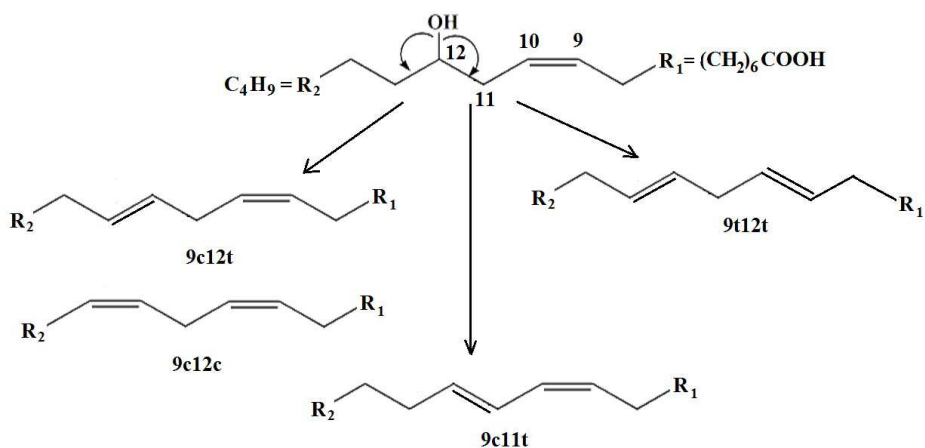


Fig. 6: Dehydration mechanisms of ricinoleic acid in castor oil

2.3 Mechanism and Isomerization:

The dehydration mechanism of ricinoleic acid is shown in Fig. 6. The formation of a double bond directly between 11th and 12th carbon atoms or between 12th and 13th carbon atoms of ricinoleic acid is possible. The configuration of the LAs depends on the preferential dehydration reaction during thermal induction. In the dehydration process the 9c12c isomer was predominant in the beginning with no 9t12t isomer present. However, during thermal induction the concentration of 9c12c decreased and the concentrations of 9c12t and 9t12c increased. Three partial chromatograms shown in Fig. 7 confirm this. However, the formation of 9c12t is preferred during the heating compared to 9t12c isomer. This can be easily explained by considering the formation of double bond between 12th and 13th carbon atoms. The planar nature of the molecule resulting from the formation of the double bond between 12th and 13th carbon atoms tends to assume *trans* configuration to ease from steric hindrance of the remaining carbon chains both sides of the double bond.

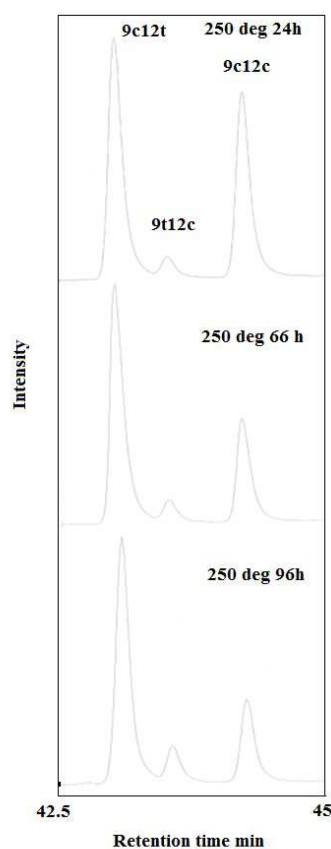


Fig. 7: The formation of linoleic acid isomers during the thermal induction

Similarly, the CLA isomer is 9c11t is formed when the dehydration takes place between 11th and 12th carbon atoms of ricinoleic acid. The 9c11t CLA undergoes a [1,5] sigmatropic transformation and forms 8t10c CLA (Fig. 8). This explains the domination of the 9c11t and 8t10c CLAs in the thermally induced castor oil in the beginning. The formation of other CLA isomers during prolonged heating of the castor oil are due to a series of [1,5] sigmatropic rearrangements [29-31]. This is illustrated in Fig. 9. When the heating is prolonged the 9t11c can form 10c12t through [1,5] sigmatropic rearrangement and this can isomerize to give CLAs such as 10t12c, 10c12c and 10t12t. Further [1,5] sigmatropic rearrangement of 10t12c can lead to the formation of 11,13 CLA isomers. These begin to form at the latter part of the dehydration and heating process.

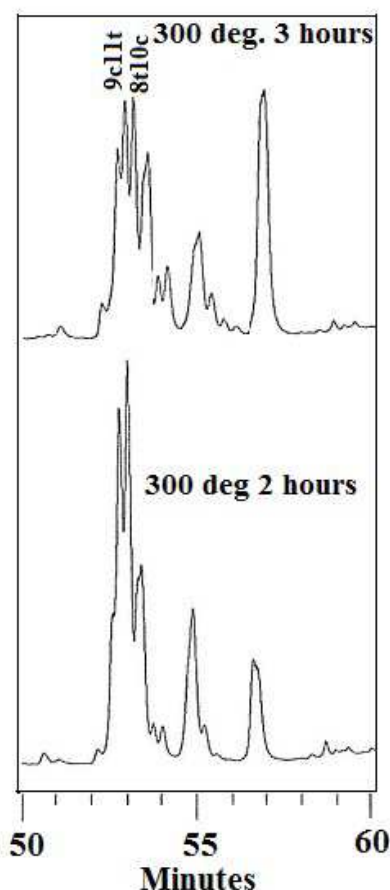


Fig. 8: FAMES of CLAs in two heated samples

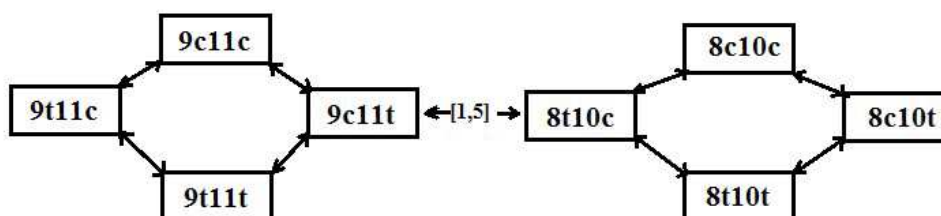


Fig. 9: Isomerization sequence of the dehydrated fatty acid in castor oil

Dehydration Kinetics:

The kinetic parameters for the dehydration were determined using an approach similar to the one described in references [21,30]. The concentration profile of the dehydrated castor oil was obtained from the gas chromatography. The peak representing the methylated ricinoleic acid was integrated and obtained as percent remaining (Fig.10).

The dehydration reaction was considered as a single reaction of the type $A \rightarrow B + C + D$ where A is ricinoleic acid and the equation 1 describes the relationship between rate of change in concentration of A and reaction rate k and reaction order n. If the dehydration reaction is first order the concentration of ricinoleic acid at time t can be given by equation 2.

$$d[A]/dt = -k[A]^n \tag{1}$$

$$\ln\{[A]_t/[A]_0\} = -kt \tag{2}$$

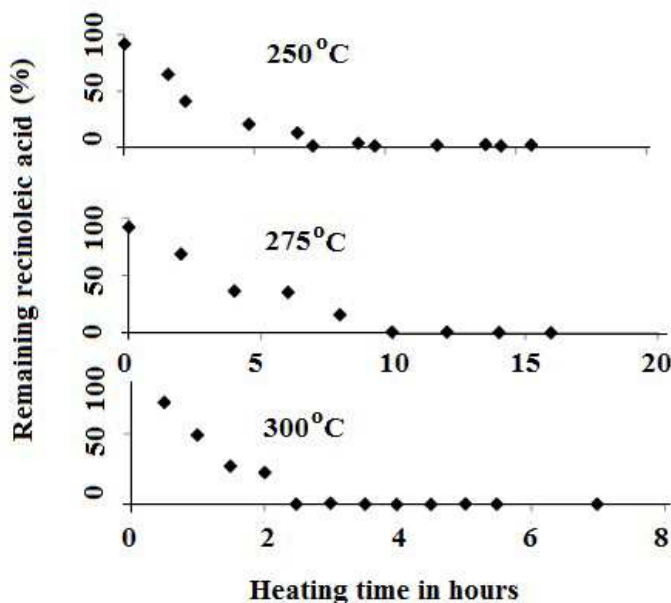


Fig. 10: Plots showing the relationships between the remaining ricinoleic acid and heating time in the heated castor oil samples

With a three temperature approach, the dehydration reaction rates k_{250} , k_{275} and k_{300} were determined for three different temperatures $T_1(250\text{ }^\circ\text{C})$, $T_2(275\text{ }^\circ\text{C})$ and $T_3(300\text{ }^\circ\text{C})$. The activation energy for the reaction can then be determined by graphics using the Arrhenius equation 3. The equation can be used to establish correlation between $\ln k$ and $1/T$ and the slope of the graph in the plot is used to calculate the activation energy for the dehydration of ricinoleic acid.

$$k = A \exp(-E_a/RT) \tag{3}$$

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

$$\ln k = \ln A - (E_a/R) (1/T) \tag{4}$$

The correlation plots between $\ln(A_t/A_0)$ and heating time are shown in Fig. 11. All the three plots results into linear plots and confirms first order dehydration reaction of ricinoleic acid. The three temperature approach results into linear plots between $\ln k$ and $1/T$ (Fig. 12).

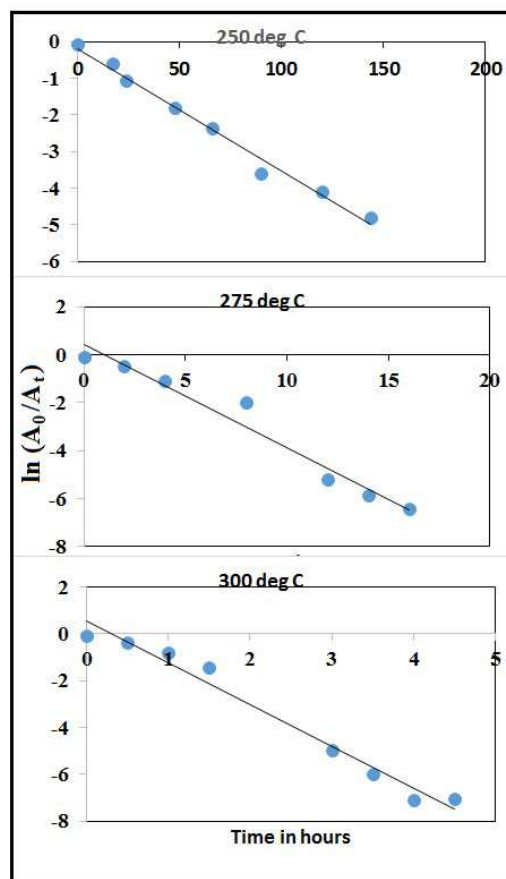


Fig. 11: Correlation plots between $\ln(A_t/A_0)$ and heating time

The kinetic parameters determined for the dehydration reaction are given in Table 2. The activation energy determined for the dehydration reaction of ricinoleic is 47kcal/mol. It is higher than the activation energy determined for the dehydration reactions of ricinoleic in the presence of catalysts.

Table 2: Kinetic parameters for the dehydration reaction of castor oil

Temperature	k, Reaction Rate(1/m)	Activation Energy (kcal mol ⁻¹)
250 °C	0.033	
275 °C	0.429	47.56±0.50
300 °C	1.780	

Conclusion:

In this paper, we have investigated dehydration products of castor oil by heat treatment at three different temperatures as 250 °C, 275 °C and 300 °C. The resulting major products were the CLA and LA isomers. In the early stages of dehydration 9c11t and 8t10c CLAs were dominating. These acids isomerize through [1,5] sigmatropic transformations into other CLA isomers under prolonged heating of the castor oil. Among the linoleic acids 9c12t was increasing in concentration. The formation of all these isomers has been justified by known mechanistic approaches.

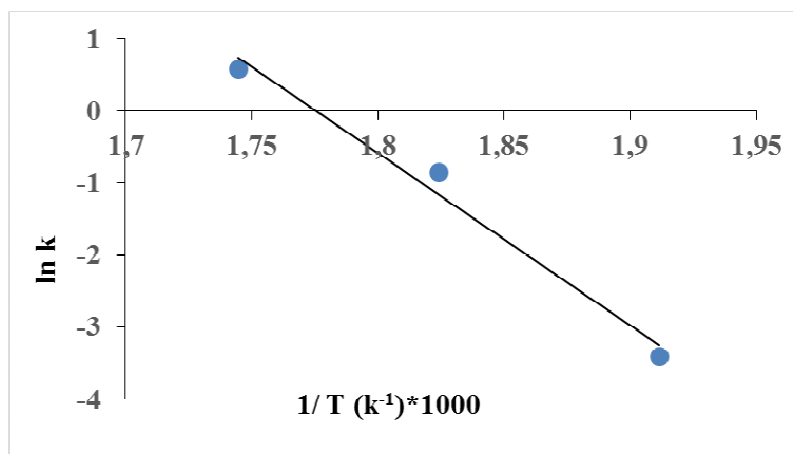


Fig. 12: Correlation plot between $\ln k$ and $1/T$

The thermal induction of castor oil produced more LA isomers specially 9c12t than the CLAs. The thermally induced dehydration produced 32% LA isomers in 72 h heating at 250 °C. At the same time the concentration of CLAs was 30%. At 300 °C temperature the concentration of LAs was 30% and the concentration of CLAs was 19%. It appears that the thermal induction of ricinoleic acid prefers the dehydration leading to the formation of linoleic acid at higher temperatures. The finding is interesting because one can understand the differences in the thermal induction with and without catalysis.

The kinetic parameters for the thermally induced dehydration without a catalyst has been determined for the first time. The activation energy for the dehydration reaction is 47 kcal/mol, which seems almost twice that of *cis-trans* isomerization reactions. This higher value of dehydration may also indicate the difficulty in eliminating a water molecule in a sterically challenged molecule. Furthermore, the dehydration reaction without a catalyst requires higher activation energy for the elimination of water from the molecule.

REFERENCES

1. Top-Notch Technology in Production of Oils and Fat. <http://www.chempro.in/fattyacid.htm> Accessed 20 Feb 2016
2. Huang, Y., Q.W. Han, Y.C. Zhen, 2002. Production of conjugated linoleic acids through KOH catalyzed dehydration of ricinoleic acid. *Chem Phys Lipids*, 119: 23-31.
3. Jafari., M., M. Kadivar, S.A.H. Goli, 2013. Response surface-optimized synthesis of *cis*9,*trans*-11-octadecanoic acid through dehydration of castor oil. *Int Food Res J*. 20(4): 1997-2003.
4. Nezihe, A., D. Elif, Y. Ozlem, E.A. Tuncer, 2001. Microwave heating application to produce dehydrated castor oil. *Ind Eng Chem Res.*, 50: 398-403.
5. Chin, S.F., J.M. Storksson, W. Liu, K.J. Albright, M.W. Pariza, 1994. Conjugated linoleic acid (9,11- and 10,12-octadecadienoic acid) is produced in conventional but not germfree rats fed linoleic acid. *J Nutr* 124: 694-701.
6. Dutten, H.J., 1974. Analysis and monitoring of *trans*-isomerization by IR ATR spectrometry. *J Am Oil Chem Soc.*, 51: 406-409.
7. DeLany, J.P., D.B. West, 2000. Changes in body composition with conjugated linoleic acid. *J Am Coll Nutr*, 19: 487S-493S.
8. DeLany, J.p., F. Blohm, A.A. Truett, 1999. Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake. *Am J Physiol.*, 276: R1172-R1179.
9. West., D.B., J.P. Delany, P.M. Camet, F. Bloh, A.A. Truett, J. Scimeca, 1998. Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. *Am J Physiol.*, 275: R667-672.
10. Park, Y., K.J. Albright, W. Liu, J.M. Storkson, M.E. Cook, M.W. Pariza, 1997. Effect of conjugated linoleic acid on body composition in mice. *Lipids*, 32: 853-858.
11. Ryder, J.W., C.P. Portocarrero, X.M. Song, L. Cui, M. Yu, 2001. Isomer-Specific Antidiabetic Properties of Conjugated Linoleic Acid, Improved Glucose Tolerance, Skeletal Muscle Insulin Action, and UCP-2 Gene Expression. *Diabetes*, 50: 1149-1157.
12. Kapoor, K., M. Reaney, N.D. Westcot, 2005. Bioriginal Conjugated Linoleic Acid. Oils, food and Science corp. Saskatoon, Saskatchewan, Canada
13. Cesano, A., S. Visonneau, J.A. Scimeca, D. Kritchevsky, D. Santoli, 1998. Opposite effects of linoleic acid and conjugated linoleic acid on human prostatic cancer in SCID mice. *Anticancer Res.*, 18: 1429-1434.

14. Banni, S., E. Angioni, G. Carta, J. McGinley, H. Thompson, D. Barbano, D. Bauman, 1999. Conjugated linoleic acid enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. *J. Nutr.* 129: 2135-2142.
15. Aydin, R., 2005. Conjugated Linoleic Acid: Chemical Structure, Sources and Biological Properties. *Turk J Vet Anim Sci.*, 29: 189-195.
16. Song, H.J., I. Grant, D. Rotondo, I. Mohede, N. Sattar, S.D. Heys, K.W. Wahle, 2005. Effect of CLA supplementation on immune function in young healthy volunteers. *Eur J Clin Nutr.*, 59(4): 508-517.
17. Sugano, M., A. Tsujita, M. Yamasaki, M. Noguchi, K. Yamada, 1998. Conjugated linoleic acid modulates tissue levels of chemical mediators and immunoglobulins in rats. *Lipids.*, 33: 521-527.
18. Lee, K.N., D. Kritchevsky, M.W. Pariza, 1994. Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* 108: 19-25.
19. Gavino, V.C., G. Gavino, M. Leblanc, B. Tuchweber, 2000. An isomeric mixture of conjugated linoleic acids but not pure cis- 9, trans-11- octadecadienoic acid affects body weight gain and plasma lipids in hamsters. *J. Nutr.*, 130: 27-29.
20. Yang, L., Y. Huang, H.Q. Wang, Z.Y. Chen, 2002. Production of conjugated linoleic acids through KOH-catalyzed dehydration of ricinoleic acid. *Chem Phys Lipids.*, 119: 23-31.
21. Christy, A.A., Z. Xu, P.B. Harrington, 2009. Thermal degradation and Isomerization Kinetics of Triolein studied by Infrared Spectrometry and GC-MS combined with Chemometrics. *Chem phys Lipids*, 158: 22-31.
22. Kramer, J.K.G., N. Sehat, J. Fritsche, M.M. Mossoba, K. Aulitz, M.P. Yurawecz and Y. Ku, 1999. Separation of Conjugated Fatty Acid Isomers in: *Advances in Conjugated Linoleic Acid Research*, Volume 1. (Editors: M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M. W. Pariza, and G.J. Nelson). AOCS Press, Champaign, IL.
23. Mossoba, M.M., R.E. McDonald, D.J. Armstrong, S.W. Page, 1991. Identification of minor C18 triene and conjugated diene isomers in hydrogenated soybean oil and margarine by GC-MI-FT-IR spectroscopy. *J Chromatogr Sci.*, 29: 324-330.
24. Mossoba, M.M., M.P. Yurawecz, R.E. McDonald, 1996. Rapid determination of total trans content of neat hydrogenated oils by attenuated total reflection spectroscopy. *JOACS* 73: 1003-1009.
25. Belton, P.S., R.H. Wilson, H. Sadehgi-Jorabegi, K.E. Peers, 1998. 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
26. Lancer, A.C., E.A. Emkem, 1988. Comparison of FTIR and capillary GC methods for quantification of trans unsaturation in fatty acids methyl esters. *Ibid.*, 65: 1483-1487.
27. 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.
28. 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.
29. Destailats, F., P. Angers, 2002. Evidence for [1,5] sigmatropic rearrangement of CLA in heated oils. *Lipids.*, 30(4): 435-438.
30. Christy, A.A., 2010. Thermally induced isomerization kinetics of the 9c11t and 10t12c Conjugated Linoleic Acids in Triacylglycerols as studied by FT-IR spectrometry aided by Gas Chromatography. *TOSPECJ* 4: 41-48.
31. Christy, A.A., 2010. Isomerization of 9c11t/10t12c CLA in triacylglycerols. *Lipids.*, 45: 733-741.