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Research Article

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An Insight into the Isomerization Chemistry of Methyl Linolenate

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ABSTRACT

Methyl linolenate has been thermally induced in glass ampoules at 225, 250 and 275°C to isomerize into cis, trans isomers of linolenic acid. The isomer mixtures were separated by gas chromatography using a 120 m capillary column. All the 8 isomers of linolenic acid, 9c12c15c, 9c12c15t, 9c12t15c, 9t12t15c, 9t12c15t, 9c12t15t and 9t12t15t of the linolenic acid were observed in the heated mixtures. The formation of isomers followed a distinct pattern in all the heated samples. The trans bond formation at C-9 or C-15 seems to take place first in the molecules leading to the formation of 9t12c15c and 9c12c15t isomers. The formation of 9c12t15c isomer seem to be high energetic compared to 9t12c15c and 9c12c15t formation and appear at a later stage in the thermal induction. This difficulty in the formation of trans configuration also explains the difficulties in the formation of 9t12t15c and 9c12t15t isomers during thermal induction. Their concentrations are smaller than the 9t12c15t isomer in the prolonged heated mixtures.

Keywords: Methyl linolenate; Trans-isomers; Isomerization; Thermal induction

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INTRODUCTION

Frying and deep frying in the cooking process of food is universe and has become one of the important methods in preparing food in all cultures. The process is carried out usually edible oils such as olive, coconut, soya, sunflower, peanut oil etc. The compositions of edible oils vary widely. For example, olive oil has high content of oleic (18:1, 9c) acid (65-80%), sunflower seed oil has high content of linoleic (18:2 9c12c) acid (45-75%) and soya oil has relatively high content of linolenic (18:3 9c12c15c) acid (5-11%) compared to other edible oils. All these fatty acids undergo isomerization, oxidation, polymerization and decomposition during heating and the degree of transformation through these processes depend on the stability of the unsaturated fatty acids in the glyceride molecules. The oleic acid (18:1, 9c) isomerizes to 18:1, 9t, linoleic acid (18:2, 9c12c) isomerizes into 9c12t, 9t12c and 9t12t and linolenic acid (18:3, 9c12c15c) isomerizes into 9t12c15c, 9c12t15c, 9c12c15t, 9t12t15c, 9c12t15t, 9t12c15t and 9t12t15t. These transformations and other processes taking place during the frying process produce several different chemical compounds that can be toxic or unhealthy [1-5]. These are consumed with fried food and some of these can accumulate in the body and can cause serious health problems. It is also obvious that the intake of food fried in oil and food prepared with heated oil contain *trans* fatty acid isomers and these increase the risk of coronary heart disease [6-11]. The double bonds in all the naturally occurring unsaturated fatty acids are in *cis* configurations. Linolenic acid has three double bonds and it forms seven different *trans* isomers during heating. Some of these isomers are formed easily in high concentration compared to other *trans* isomers. A survey in the literature shows that very little has been done on the study of isomerization chemistry of linolenic acid isomers.

My intention in this article is to study the *cis-trans* isomerization in heat induced isomerization of methyl linolenate, isomerization pathways taken by these isomers during heating process and understand the stability of these isomers during heating.

EXPERIMENTAL SECTION

Materials and Methods

The methyl isomer of the linolenic acid was chosen to the purpose of stability analysis of the *trans* isomers. Methyl linolenate (99% purity) and the analytical standard of methyl derivatives of linolenic acid isomers (100 mg) was purchased from Sigma. Methyl linolenate was used as received. The reference standard was diluted in 1.5 ml heptane. The composition of the reference standard is given in Table 1. 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. A small amount (250 ml) was injected in the ampoule using a plastic syringe with needle. The ampoule was then sealed after removing air in the ampoule by blowing with a stream of nitrogen gas. Fifteen ampoules were placed in a glass beaker and placed in an oven set at 225°C. The ampoules were removed at regular time intervals until all the 15 were exhausted. The ampoules were then cut open and the contents were poured into a test tube for dilution with heptane (1.5 ml). The diluted solution of the heated methyl linolenate was placed in a gas chromatographic vial for GC analysis. The above experiment was repeated in the same manner at 250°C and 275°C respectively. The samples heated at 250°C were removed at 4 hour intervals and samples heated at 275°C were removed at ½ hour time intervals.

Gas Chromatographic Analysis

GC analyses of Methyl linolenate isomers formed during the thermal induction were carried out by using Perkin Elmer, auto XL system 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 150°C with 2 minutes' equilibrium time, a temperature gradient of 0.5°C/min up to 170°C with 50 minutes holding time, then a temperature gradient of 1°C/min up to 190°C with 10 minutes holding time was used. The total running time was 122 minutes. The peak identification and elution order was carried out using the the gas chromatogram of the reference standard and literature references [2].

Table 1: Composition of the methyl linolenate isomers

Compound	Composition (%)
9c12c15c	3
9t12c15c	7
9c12t15c	7
9c12c15t	7
9t12t15c	15
9t12c15t	15
9c12t15t	15
9t12t15t	30

RESULTS AND DISCUSSION

A gas chromatogram of the reference standard is shown in Figure 1. A collection of GC chromatograms obtained with samples heated at 275°C is shown in Figure 2. The concentration profiles of the isomers formed during thermal treatment of methyl linolenate at 225, 250 and 275°C are shown in Figure 3.

The Gas chromatograms shown in Figure 2 and the concentration profiles shown in Figure 3 clearly illustrate the isomerization taking place and increase in the concentration of *trans* isomers 9t12c15c, 9c12t15c, 9t12t15c, 9c12c15t, 9t12c15t and 9c12t15t with respect to the 9c12c15c isomer. The isomerization into 9t12t15t isomer seems to be very slow. The methyl linolenate sample heated at all three temperatures contains all the *trans* isomers except 9t12t15t, because the activation energy for the formation of 9t12t15t isomer is very high. There is an interesting feature in the chromatograms is that the isomers 9t12t15c and 9c12c15t co-elute and form a single peak at the retention time of 86 minutes. The proportions of these isomers formed during heating are very different. The Formation of different proportions of isomers implies that the double bonds in the 9c12c15c molecules are perturbed by the neighboring molecules or groups.

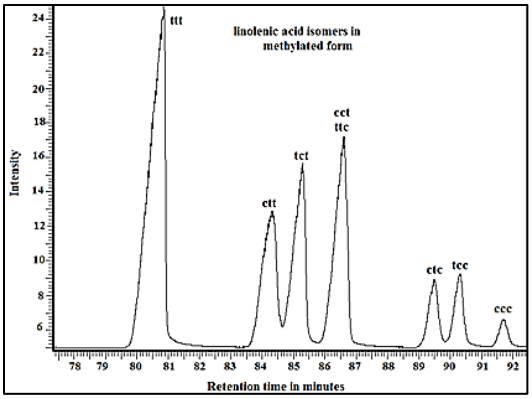

Figure 1: Gas chromatogram of the reference standard of methyl linolenate isomers

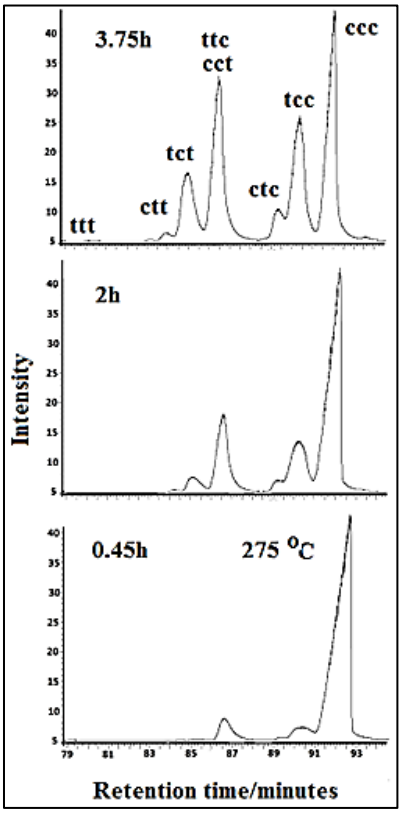

Figure 2: A collection of chromatograms of the isomers formed during thermal treatment of methyl linolenate

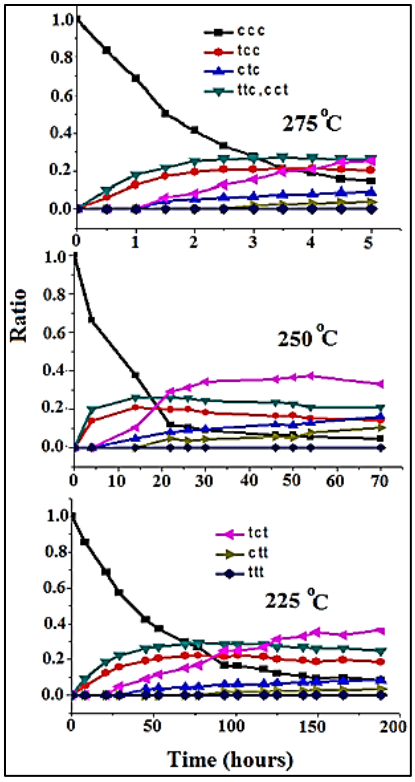

Figure 3: Concentration profiles of the isomers formed during thermal treatment of methyl linolenate

The Mechanism of Formation of Mono-Trans Isomers

The thermal induction of an unsaturated fatty acid leads to the *cis-trans* isomerization in the molecule. The activated complex formed during the activation involves opening of the double bond and the formation of *trans* isomer involves rotation of the bond in the activated complex. The activation energy needed for the opening of the double bond and ease of free rotation of the bond between the two carbon atoms containing the double bond decide the variations in the concentrations of the isomers formed in the mixtures (Figure 4).

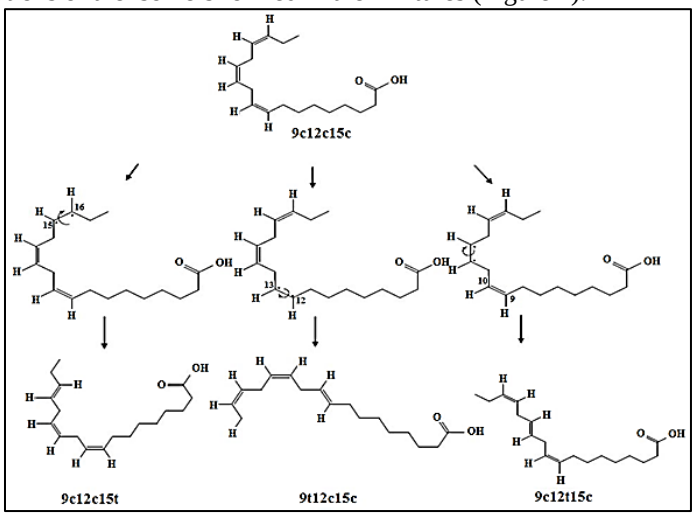

Figure 4: Mechanism of formation of mono-trans isomers in methyl linolenate

During the initial stages of heat treatment of methyl linolenate (9c12c15c), three isomers 9c12c15t, 9t12c15c and 9c12t15c are observed. These isomers have different concentrations of 9c12c15t, 9t12c15c and 9c12t15c. Furthermore, the mixtures contain higher proportion of 9c12c15t compared to 9t12c15c and 9c12t15c. Even though,

the 9c12c15t isomer co-elutes with 9c12t15t isomer, the initial formation rate suggests that the mixture contains mostly 9c12c15t isomer. Reason for this difference in proportion is the relatively easy free rotation of the C-C bond between carbon number 15 and 16. The carbon number 16 in the methyl linolenate molecule contains small - CH_2CH_3 group compared to C-9 which contains a long chain of methylene groups. The free rotation of the C-C bond between carbon number 15 and 16 is easy compared to free rotation of the C-C bond between carbon number 9 and 10. This leads to the formation of relatively higher concentration of 9c12c15t compared to the isomer 9t12c15c. It is difficult to observe this from the chromatograms because of the co-elution of 9c12c15t and 9t12t15c. However, I believe that the 9c12c15t isomer contributes to a very high proportion in the mixture. The concentration of 9c12t15c is very much low compared to the concentrations of 9t12c15c and 9c12c15t. This is because of the difficulty in rotation of the C-C bond between carbon numbers 12 and 13. Both carbons have larger groups and free rotation about the bond is difficult. Therefore, the concentrations of the *mono trans* isomers follow the order 9c12c15t > 9t12c15c > 9c12t15c.

The Mechanism of Formation of *Di-Trans* Isomers

The appearance of the *di-trans* isomers is delayed and it suggests that the di-trans isomers are formed from the already formed mono-trans isomers. The same argument should lead to explain the formation of 9t12c15t from 9c12c15t and 9t12c15c. The concentrations of 9c12t15t and 9t12t15c are low compared to 9t12c15t. The isomer 9c12t15c forms both 9t12t15c and 9c12t15t isomers with higher proportion of 9c12t15t isomer. The formation of isomers during heating of methyl linolenate can be illustrated by an isomerization cube (Figure 5). The isomers 9c12c15c and 9t12t15t are at the opposite corners and the reaction pathways leading to the different isomers are clear. The formation of tri *trans* isomers is not prominent in the reaction mixtures. The *tri-trans* isomer can be formed from the isomers 9t12t15c or 9c12t15t or 9t12c15t. It appears that the formation of 9t12t15t is not favored from any of these isomers.

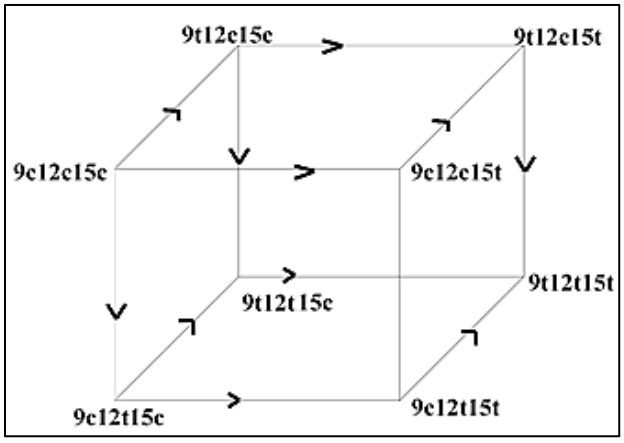

Figure 5: Isomerization cube for the isomerization reactions in methyl linolenate

CONCLUSION

Methyl linolenate forms all the 7 isomers during thermal induction at 275°C. After heating of the sample for 3 hours and 45 minutes the mixture contained all the 7 isomers. The formation of *mono* and *di-trans* isomers and their concentrations can be easily understood by considering their molecular structure. The ease of free rotation along the bond containing the double bonds during heating clearly contributes to the different concentrations of different isomers in the mixture. At this point it is very difficult to say anything about the activation energies needed for the formation of different isomers from 9c12c15c. Determination of activation energies require complicated mathematical treatment with several equations. Until then it is difficult to say whether activation energies for the formation of isomers or the variation in frequency factors decide the variations in concentrations.

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