Thermal induction of 9t12t linoleic acid: A new pathway for the formation of Conjugated Linoleic Acids

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Abstract
Thermal induction of 9t12t fatty acid leading to the formation of Conjugated Linoleic Acids (CLA) has been demonstrated by subjecting glyceride, and methyl ester of the acid to heat treatment. Fifteen micro liter portions of the glyceride sample containing 9t12t fatty acid (trilinoelaidin) were placed in micro glass ampoules and sealed under nitrogen and then subjected to thermal treatment at 250^{o}C. The glass ampoules were taken out at regular time intervals, and the contents were subjected to composition analysis by gas chromatography. The samples from each ampoule were subjected to derivatisation into their methyl esters. The methyl esters of the isomerised fatty acids were analysed by gas chromatography. The same procedure was repeated with methyl ester samples containing 9t12t fatty acid (methyl linoelaidate). The gas chromatographic analysis was carried out after appropriate dilution in heptane.

The results show that the thermal induction of 9t12t fatty acids from both glyceride molecules and methyl esters give identical Conjugated Linoleic Acid (CLA) profiles. These profiles are identical to the profile obtained by the thermal induction of 9c12c fatty acids.

Introduction
Mono and poly unsaturated fatty acids in edible oils and fats undergo several changes and lead to several different products when subjected to thermal treatment. These changes include isomerisation to *trans* fatty acids, oxidation, intra cyclisation and polymerization (Moreira et al., 1999; Rojo and Perkins, 1987; Fullana et al., 2004; Umano and Shibamoto, 2001; Fujisaki et al., 2002; Christy et al., 2009). Some of the products formed during thermal treatment are toxic and may cause health problems in humans. Similarly, polyunsaturated fatty acids in edible oils can also form toxic products. There are are several polyunsaturated fatty acids in edible oils and the processes leading to these toxic products are very complex. Analysing 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 Trlinoelaidin and methyl linolelaidate during thermal induction in inert atmosphere. Reports involving the nanalysis of products formed from the 9t12t 18:2 fatty acids during thermal induction are not available in the literature. Methyl limolelaidate and trilinoelaidin contain18:2 (9,12) fatty acids with *trans trans* configuration. The acid molcules in methyl linolelaidate and trilinolelaidin are of definitive structure and the products formed should originate from these acid molecules. This will give understanding of the changes taking place and behaviour of the molecules under thermal stress.

Experimental
Samples and methods
Trilinolelaidin and methyl linolelaidate samples were purchased from Sigma Aldrich.

The heating experiments were carried out in one side sealed micro glass ampoules of length 4 cm. The samples were placed in the ampoules under nitrogen atmosphere and sealed using an oxygen-propane flame. The ampoules containing the samples were then placed in a short 5 ml glass vial and placed in a chromatographic oven set at 250^{o}C. Samples were removed at regular time intervals and stored in dark before chemical analysis. At the end of the heating experiments, the glass tubes containing the samples were cut open and the contents were analysed by infrared spectroscopy. After the infrared measurements, the heat treated trilinolelaidin samples were derivatised into their methyl esters (alfred) and analysed by gas chromatography. The heated methyl trilinolelaidate samples were diluted in heptane before gas chromatograpic analysis.

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 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 steps gardients was used. The program started with 1min at 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 remained at 220^{o}C for 30 minutes giving a total running time of 95 minutes for each sample. The peaks in the chromatograms were identified by using the pure methyl ester standards of 9t12t fatty acid and literature references.

Results and discussion

The thermal iduction of 9t12t fatty acid is expected to give 9c12t, 9t12c and 9c12c isomers. In addition to these, Conjugated Linoleic Acid isomers can also be expected.

The gas chromatograms of the FAME of the heat treated trilinoelaidin and methyl linolelaidate are shown in Fig. 1. The concentrations of the isomers from both trilinolelaidin and methyl linolelaidate based on the areas of the peaks in the chromatograms are presented in Fig.2. The FAME profiles are the same for these samples. Isomerisation of 9t12t fatty acids into the 9c12t, 9t12c and 9c12c isomers is very slow. The low concentrations of 9t12c, 9c12t and 9c12c isomers in the heated samples of trilinolelaidin and methyl linolelaidate show the stability of 9t12t double bonds towards isomerisation. Furthermore, the isomer 9t12c is in relatively higher concentrations in all the heat teated samples of trilinolelaidin and methyl linolelaidate. It appears that the isomerisation of the 9t12t double bonds into 9c12t double bonds seem less favourable than into 9t12c double bonds.

Apart from positional isomarization, heat induction of trilinoelaidin and methyl linolelaidate can also form Conjugated Linolic Acids. The parts of the chromatograms indicating the area where CLAs are eluted are shown in Fig. 3 for both trilinolelaidin and methyl linolelaidate The CLA profiles produced in these thermal induction reactions are identical. The profiles are also identical to the CLAs formed during thermal induction of trilinolein containing 9c12c

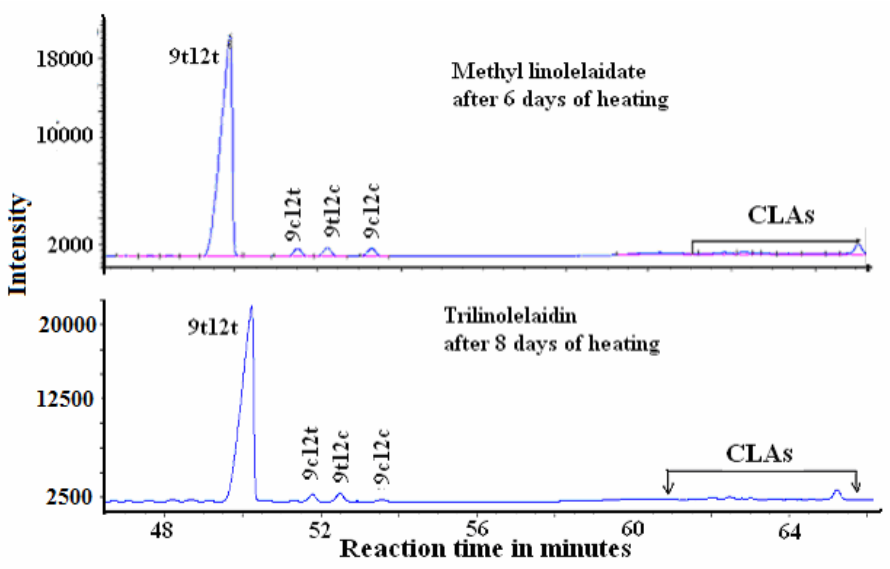

Fig.1. The gas chromatograms of the FAME of the heat treated trilinoelaidin and methyl linolelaidate.

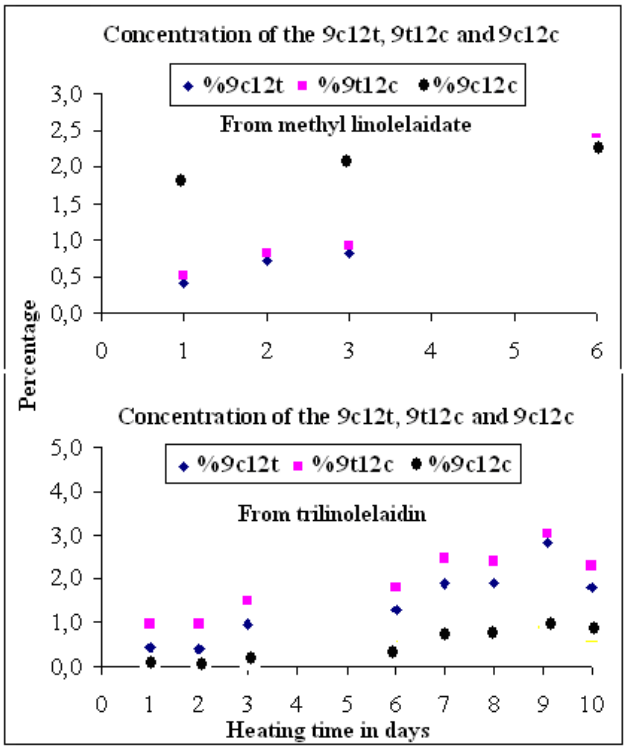

Fig. 2. The concentrations of the isomers 9c12t, 9t12c and 9c12c from heated samples of trilinolelaidin and methyl linolelaidate

fatty acid molecules (Destaillats and Angers, 2005). A report by Eulitz et al. (1999) was used for identifying the elution order of the CLAs. The concentrations of total CLAs and the concentration of *trans, trans* CLAs within the total CLAs are shown in Fig. 4.

The concentrations in the plots shown in Fig. 2 were based on the total fatty acid profiles analysed in the samples and represent relative concentrations of Lilnoleic Acids (LAs) and CLAs in the samples. The relative concentration of CLAs increases with the heating time in methyl linolelaidate and trilinoelaidin. The relative total concentration of CLAs in the heated samples approaches a maximum of around 5%.

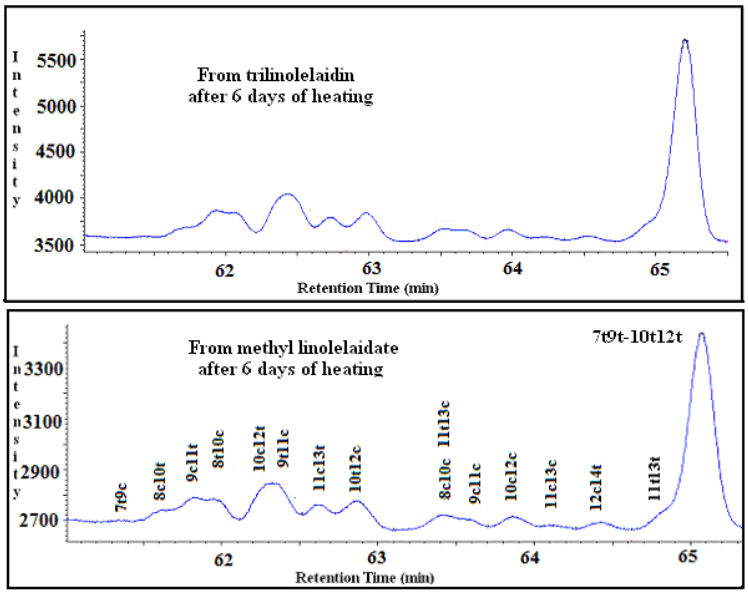

Fig. 3**.** The parts of the chromatograms indicating the area where CLAs are eluted

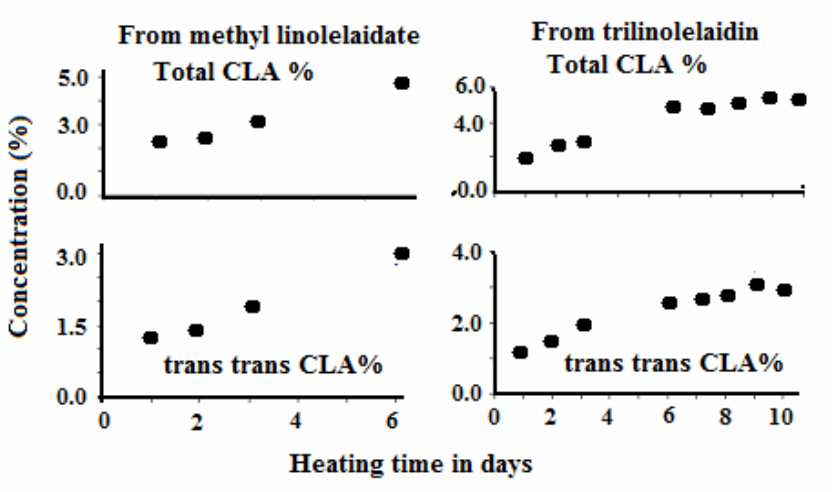

Fig. 4. The concentrations of total CLAs and the concentration of *trans, trans* CLAs.

Infrared absorption of the samples at 969 cm^{-1} was used to quantify the remaining methyl linolelaidate and trilinolelaidin in the samples. The concentrations determined by infrared

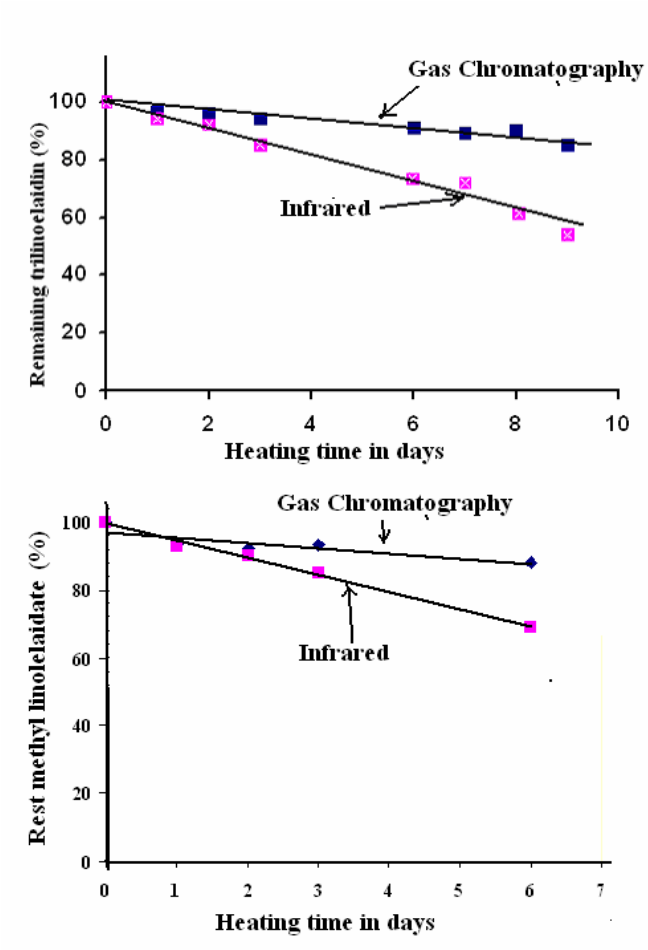

Fig. 5. Rest methyl linolelaidate and trinolelaidin in heated samples

spectroscopy and gas chromatography (Fig. 5) shows that the samples undergo degradation under thermal stress. When the degradation of the trilinoelaidin is taken into account, the concentration of the total CLA in the heated sample amounts to around 2.2%. Out of these the *t,t* CLA isomers which elute at the same time amount to around 60% in all the heated samples.

Conclusion

It has been demonstrated that the thermal induction of methyl linolelaidate and trilinolelaidin containing 9t12t fatty acid moieties undergo isomerisation into their positional isomers and Conjugated Linoleic Acids. The positional isomerisation appears to be slow and indicates the relative stability of the molecules containing 9t12t fatty acids.

The total concentration of CLAs formed in the mixtures amount to 2.2%. This is almost the same concentration of CLAs formed from the thermal induction of molecules containing 9c12c fatty acids (Triliolein). The trans,trans isomers comprise about 60% of the CLAs.

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