Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2018, 10(8): 17-24

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Effect of UV Irradiation on Conjugated Linoleic Acid (9c 11t and 10t 12c)

Alfred A Christy*, PRUSK Peramune and KDMM Dissanayake

Department of Science, Faculty of Engineering and Science, University of Agder, Kristiansand, Norway

---

ABSTRACT

This study was carried out to examine the effects of UVA irradiation on the Conjugated Linoleic Acid (CLA) isomers; 9c11t CLA and 10t12c CLA. CLA 9c11t free fatty acid (90%) and 10t12c-Octadecadienoic acid methyl ester (90%) samples in UV transparent cuvettes sealed under nitrogen gas were subjected to UVA irradiation at 365 nm. About four drops of each sample were taken out at regular intervals. These irradiated samples were analyzed using IR spectroscopy and Gas Chromatography (GC). The second derivative profiles of the IR spectra of UVA irradiated 9c11t CLA and 10t12c CLA show the decrease in intensity of the peaks at wavenumbers at 944 and 986. Furthermore, a slight increase in the intensity of the peak at around 967/cm and a slight blue shift of the peak at 984 to 988/cm are also observed. The GC peaks corresponding to tt CLA isomers increase in intensity showing the isomerization of methylene interrupted 18:2 linoleic acid to tt isomers of Conjugated Linoleic Acids. Furthermore, formation of oleic acid (!8:1 9c) in the irradiated sample of 9c11t free fatty acid and formation of oleic and elaidic acid (18:1 9t) in the irradiated sample of 10t12c methyl ester also observed. The results clearly indicate the depletion of conjugated linoleic acids during UV irradiation.

Keywords: UVA irradiation; Conjugated linoleic acid (CLA); IR spectroscopy; Gas chromatography (GC)

INTRODUCTION

Linoleic acid is a polyunsaturated fatty acid of 18 Carbon atoms and with 2 double bonds; both of cis (c) configuration. The double bonds are at the ninth and the twelfth positions counted from the carboxylic acid end of the carbon chain. As the first double bond counted from the methyl end is at the sixth position of the carbon chain, linoleic acid is grouped as an omega-6 (ω-6) fatty acid [1]. Animals cannot produce linoleic acid within the body, and therefore, linoleic acid should be consumed from food. Due to this reason, linoleic acid is categorized as an essential fatty acid [2]. The most common sources of linoleic acid are plant oils such as sunflower oil, sesame oil, soybean oil, canola oil and safflower oil and some nuts and peanuts [3].

"Conjugated linoleic acids" (CLAs) is the collective term used to introduce the geometric and positional conjugated dienoic isomers of linoleic acid. Unlike linoleic acid; which has the double bonds at the ninth and twelfth positions of the carbon chain both in cis configuration, CLAs can have the double bonds in either cis or trans or both configurations in the same chain of carbon [4]. CLAs are formed as a by-product during the bacterial conversion of linoleic acid to oleic acid in ruminants. Therefore, CLAs are of animal origin [5]. The major geometrical CLA isomer found in food is the 9c11t CLA. In addition, other isomers such as 7t 9c CLA, 11c13t CLA, 8c10t CLA and 10t12c CLA can also be found [6]. The predominant dietary sources of CLAs are dairy products such as milk, cheese, butter and meat products like beef, lamb etc. [7]. Many research works have been conducted to study the health benefits of CLAs. Several researchers have noted the tumor suppression effect of CLAs, and therefore are considered effective in the modulation of stomach cancer, mammary cancer, prostate cancer and skin cancer. CLAs are also prone to have anti-atherogenic effects, anti-obesity effects, immune enhancing effects, promotion of bone formation, anti-diabetic effects and anti-oxidant properties etc. [8]. However, the health benefits of CLAs are isomer specific. For an

example, 10t12c CLA has more anti-obesity effects on mice than other isomers [9]. Nevertheless, these research results should be further tested for their increased reproducibility.
According to literature, CLAs absorb UV light at ≥230 nm [10]. It was this character of CLAs that made possible of their usage as markers of fat metabolism, due to natural body fats absorbing very little at the wavelength range that CLAs absorb. Several research works have been conducted to increase the content of CLAs in soybean oil by photoisomerization; by UV irradiation [11]. However, research work on the effects of UV irradiation of CLA isomers is scarce. Therefore, the objective of this research was to study the effects of UVA irradiation on 9c 11t and 10t 12 c CLA isomers.

EXPERIMENTAL SECTION

Samples
CLA 9c11t free fatty acid (90%) and 10t12c-Octadecadienoic acid methyl ester (90%) were purchased from Larodan Fine Chemicals, Sweden.

UVA Irradiation
CLA 9c11t free fatty acid (90%) and 10t12c-Octadecadienoic acid methyl ester (90%) samples in UV transparent cuvettes sealed under nitrogen gas were subjected to UVA irradiation at 365 nm. Four drops from each UVA- irradiated sample were taken out at regular time intervals. A thin layer of the sample was used, for IR analysis. The remaining irradiated CLA 9c 11t free fatty acid samples (90%) were stored, for derivatisation. Raw and irradiated samples of 10t12c-Octadecadienoic acid methyl ester (90%) were diluted with heptane and stored in GC vials for gas chromatographic (GC) analysis.

Infrared (IR) Spectroscopic Analysis
PerkinElmer Spectrum One FT-IR spectrometer equipped with a Harrick single reflectance attenuated total internal reflectance (ATR) accessory was used, for the IR analysis of each UVA- irradiated sample. The ATR crystal was washed with dichloromethane, followed by acetone. A background spectrum was scanned in the range of 4000-600/cm prior to the application of the sample. A total of 10 scans at 4/cm resolution were measured on each background and sample. A thin layer of the UVA-irradiated sample was spread on the ATR accessory using a capillary tube and an absorbance spectrum was collected using the Spectrum V5.3.1 software. The absorbance spectra were converted into second derivative profiles. The region 1000-920/cm was used for the qualitative analysis and identification of the variation of CLA upon UVA-irradiation.

Derivatisation
The UVA- irradiated samples of 9c11t free fatty acid were subjected to derivatisation. An aliquot of 1 mL of 0.5 M methanolic NaOH was added into each centrifuge tube containing UVA- irradiated 9c11t free fatty acid and placed in a water bath for 10 min at 65 0C. After cooling, 1.5 mL of BF3/methanol was added to each centrifuge tube and placed in a water bath for 3 min at 65 0C. When the centrifuge tubes had reached room temperature, 1 mL of heptane was added and shaken using a test tube shaker. An aliquot of 4 mL of saturated NaCl was added and shaken well for a better separation and to dissolute fatty acid methyl esters (FAME) in the heptane layer. The test tubes were allowed to stand for few minutes and a small amount of anhydrous MgSO4 was added to the heptane layer. The heptane layers in the tubes were extracted using a syringe, filtered through a 0.45 µm CHROMAFIL disposable syringe filters and stored in small GC vials.

Gas Chromatographic (GC) Analysis
The gas chromatographic analysis of FAMEs of 9c11t and 10t12c were performed using PerkinElmer AutoSystem XL gas chromatograph with the aid of TC Nav software. A 120 m long capillary column with an internal diameter of 0.25 mm and 0.25 µm thickness, coated with 70% cyanopropyl polysilphenylene-siloxane was used as the stationary phase. A temperature program with an initial temperature of 170 0C with 2 min equilibration time and then a temperature gradient of 0.5 0C /min to 180 0C with 60 min holding time was applied for each sample. The total run time for the FAME was 82 min. The peak identification was carried out using the standard FAMEs and the reported literature.

RESULTS AND DISCUSSION

Infrared Spectroscopy

Some of the IR band assignments for CLA are shown in the Table 1 [12].

Table 1: Some of the IR band assignments of glycerides containing CLAs

Frequency (/cm)	Functional group and mode of vibration
3025	= CH trans stretching
3004	= CH cis stretching
2953	CH (CH3) asymmetric stretch
2924	CH (-CH2-) asymmetric stretch
2854	CH (-CH2-) symmetric stretch
1746	-C=O ester Fermi resonance
1653	-C=C- cis stretching
1465	-CH (-CH2-, CH3) bending
1377	-CH (CH3) symmetric bending
1238	-C-O, -CH2- stretching, bending
1161	-C-O, -CH2- stretching, bending
1118, 1097	-C-O stretching
967	=CH trans bending
944, 984	Conjugated =CH bending for all c, t and t, c isomers
988	Conjugated =CH bending for all t, t isomers

The Infrared spectra of the UVA-irradiated samples of 9c11t linoleic acid and 10t12c-Octadecadienoic acid methyl ester are shown in Figures 1 and 2 respectively.

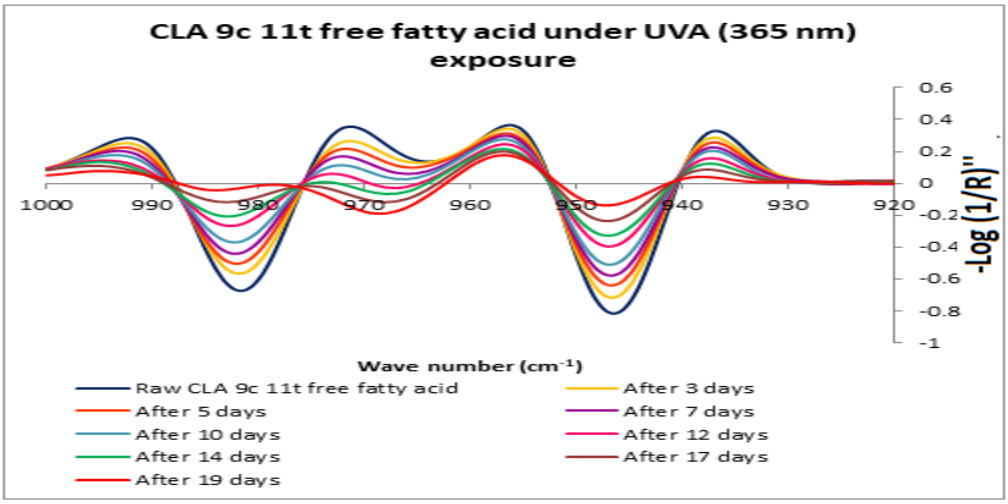


Figure 1: Second derivative profile of the infrared spectra of pure and UVA irradiated CLA 9c 11t free fatty acid in the region of 1000-920/cm

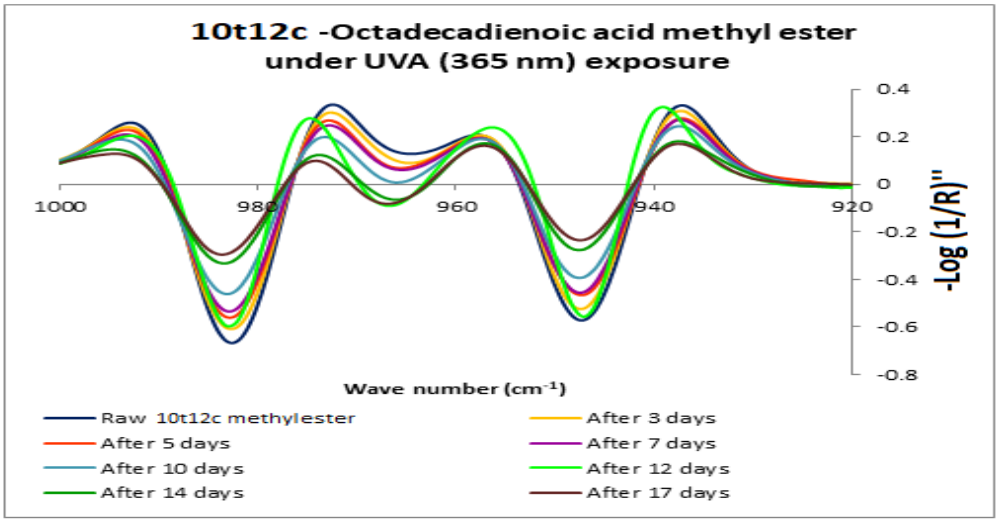


Figure 2: Second derivative profile of the infrared spectra of pure and UVA irradiated 10t12c-Octadecadienoic acid methyl ester in the region of 1000-920/cm

CLAs having cis-trans and trans-cis configurations give rise to two characteristic absorptions at around 944/cm and 984/cm due to the conjugated =CH bending. The peak at around 967/cm arises due to the methylene interrupted =CH trans bending; and therefore indicates the presence of methylene interrupted trans, cis or cis, trans or trans, trans LAs or mixture of these isomers [12,13]. The slight blue shift in the peak at around 984/cm distinctly also indicates that the UVA irradiated sample is a mixture of trans, cis or cis, trans or trans, trans CLAs with a high percentage of trans, trans isomers. The intensity of the peak at around 967/cm has increased slightly with irradiation time. This implies that the methylene interrupted trans character of the double bonds increases with UVA irradiation [14]. Meanwhile, the decrease in the intensity of the peaks around 944/cm and 984/cm illustrates a decrease in the conjugated cis, trans or trans, cis isomers [15].

Gas Chromatography

Methylated ester of CLA 9c 11t free fatty acid: The gas chromatograms of the FAME of the UVA irradiated 9c11t linoleic acid are shown in Figure 3. The most significant peaks in the chromatograms are the peaks arising from 9c11t, trans, trans CLAs and 18:1(c) configurations. In addition to these major peaks, other minor peaks of the CLA isomers are also present. The small peaks around 48 min to 51 min in the UVA irradiated samples are due to the formation of methylene interrupted 18:2 configurations. This corresponds to the increase in the intensity of the IR peak at 967/cm during prolonged irradiation of UVA. Therefore, the formation of methylene-interrupted isomers of linoleic acid is possible during UVA irradiation.

The variation of the relative concentrations of the major isomers present in the UVA irradiated samples of 9c 11t linoleic acid are graphically represented in Figure 4. These profiles illustrate that the relative percentage of 9c 11t configuration shows an increase upon UVA irradiation for about 10 days; and starts to decrease thereafter. On the other hand, the relative percentages of trans, trans CLAs and 18:1(c) configurations show a decrease upon UVA irradiation for 10 days, and then, their percentages start to increase.

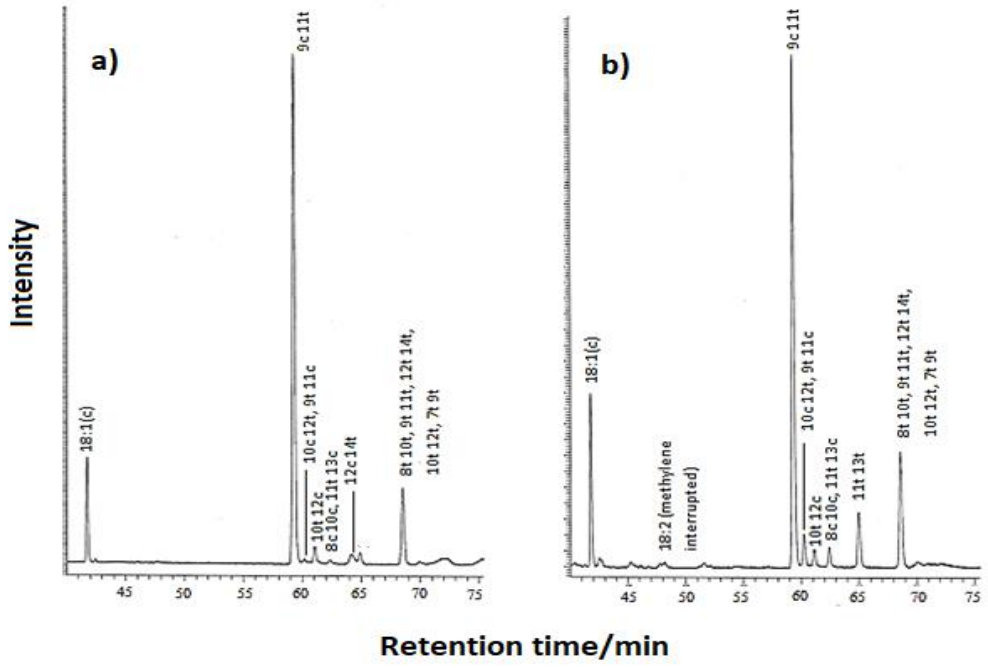
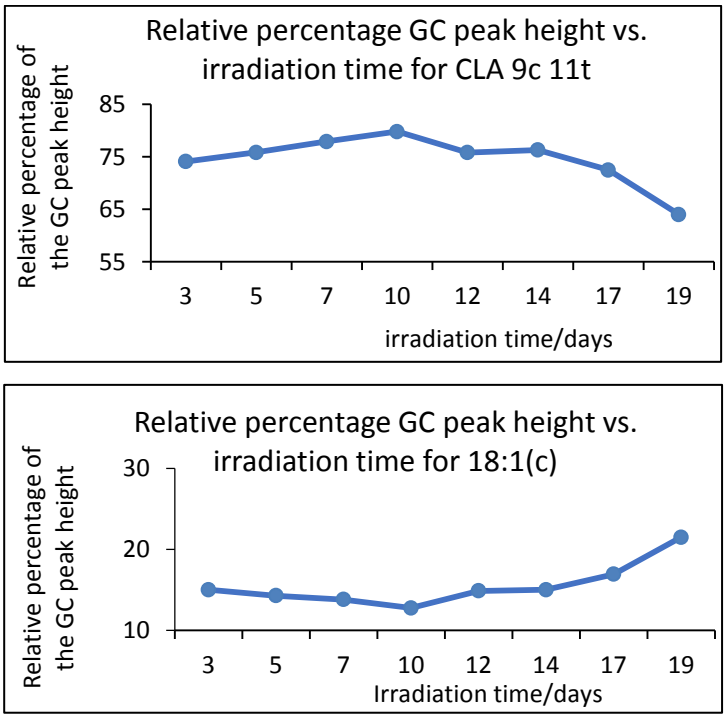


Figure 3: a) The gas chromatogram of FAME of the UVA irradiated CLA 9c 11t free fatty acid after 3 days b) The gas chromatogram of FAME of the UVA irradiated CLA 9c 11t free fatty acid after 19 days



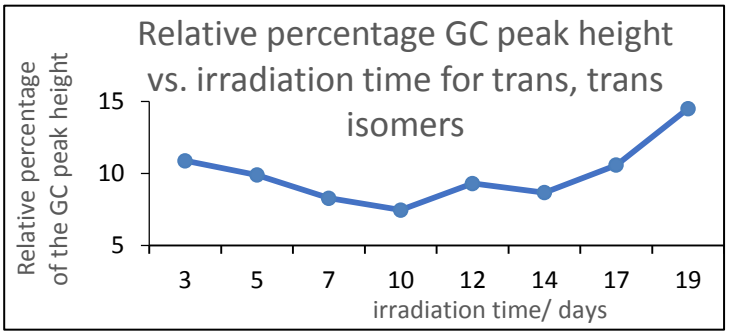


Figure 4: The graphs of relative percentage GC peak height vs. irradiation time for 18:1 (c), CLA 9c 11t and other trans, trans isomers

10t 12c-Octadecadienoic acid methyl ester: The gas chromatograms of the FAME of the UVA irradiated 10t12c linoleic acid are shown in Figure 5. The peaks arising due to 10t12c, 18:1 (c), 9c11t configurations are present in the chromatogram of the raw 10t12c conjugated linoleic acid methyl ester. However, upon UVA irradiation, other peaks such as 18:1(t), methylene interrupted 18:2 and other isomers of CLA have appeared. The small peak around 50 min in the UVA irradiated samples are due to the formation of methylene interrupted 18:2. This further supports the increase in the intensity of the IR peak at 967/cm during prolonged irradiation of UVA. Therefore, the formation of methylene-interrupted isomers of linoleic acid is possible during UVA irradiation. The appearance of peaks corresponding to trans, trans CLA positional isomers upon UVA irradiation; which were not present in the raw 10t12c linoleic acid sample prior to irradiation suggests that there is a possibility of formation of trans, trans CLA isomers during prolonged UVA irradiation.

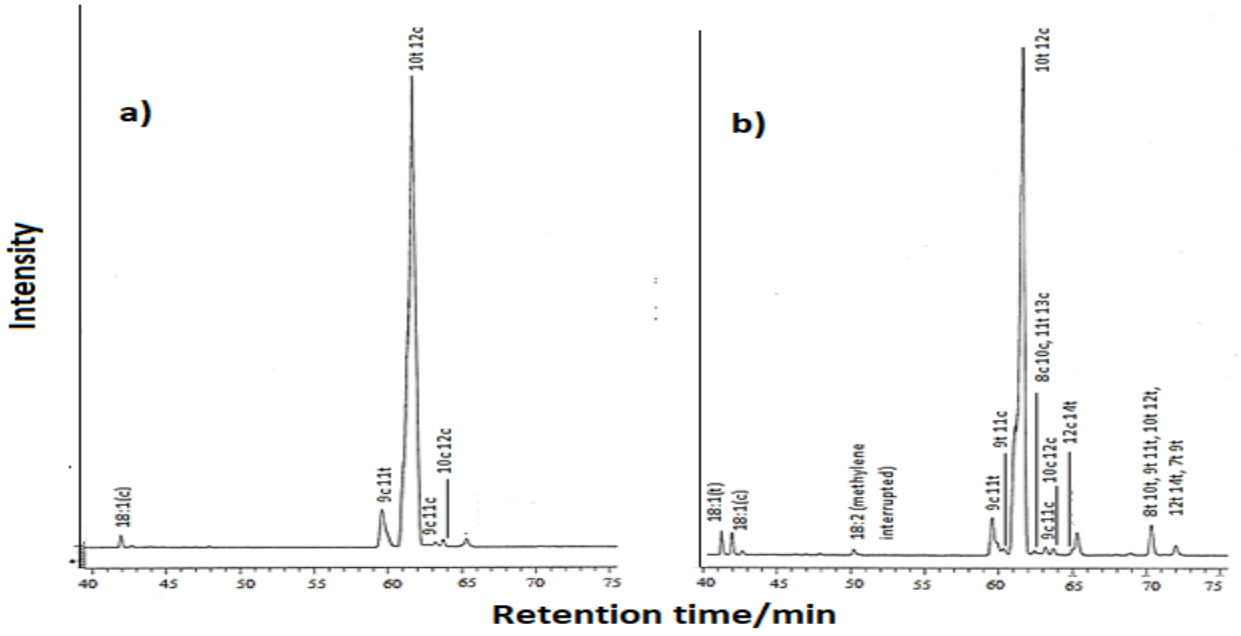


Figure 5: a) The gas chromatogram of raw 10t 12c-Octadecadienoic acid methyl ester b) 10t12c-Octadecadienoic acid methyl ester after 17 days

The variation of the relative percentages of the major isomers present in the UVA irradiated samples of 10t12c linoleic acid are graphically represented in Figure 6. These profiles indicate that the relative percentages of 18:1(c),

18:1(t) and trans, trans CLA configurations show an increase upon UVA irradiation. Meanwhile, the relative percentages of 10t12c shows a decrease upon UVA irradiation.

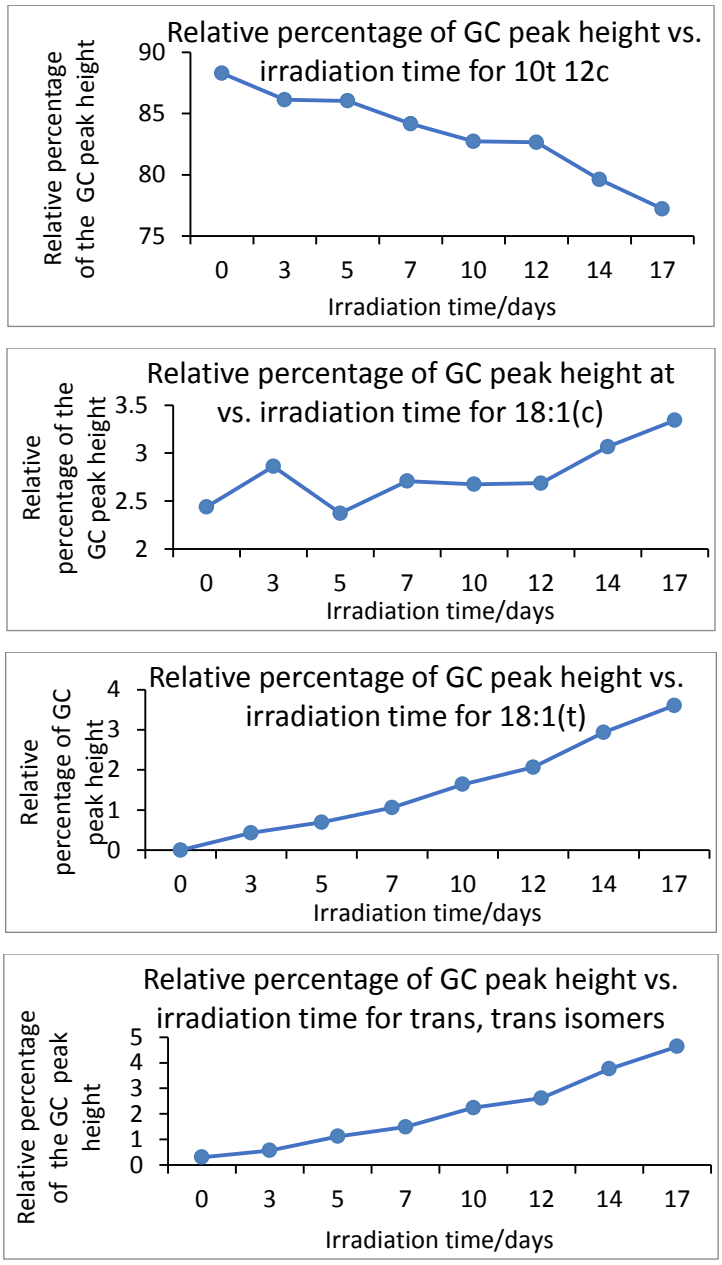


Figure 6: The graphs of relative percentage GC peak height vs. irradiation time for 18:1 (t), 18:1 (c), CLA 10t12c and CLA trans, trans isomers

CONCLUSION

The initial changes taking place under the UV irradiation of 9c11t CLA fatty acid shows that the content of 9c11t increases slightly during UV irradiation. However, the results from the IR and GC analyses confirm that the biologically important CLA isomer 9c11t and the isomer 10t12c decrease in concentrations when exposed to prolonged UV radiation. These isomers isomerise into other configurations of CLA and form other new compounds such as 18:1c and 18:1t.

The final mixture of the UV irradiated samples contain certain isomers of CLA with a higher percentage of trans, trans CLA isomers. The formation of 18:1 isomers was not expected because this involves addition of hydrogen atoms.

The mechanistic aspects of the formation of specific CLA isomers, 18:2 and 18:1 isomers during UV irradiation are under investigation and will be reported in the future.

REFERENCES

[1] Linoleic acid: Structure, Properties, Metabolism, Food Sources. http://www.tuscany-diet.net/lipids/list-of fatty-acids/linoleic/, **2018.**
[2] Essential Fatty Acids. http://www.pcrm.org/health/health-topics/essential-fatty-acids, **2018.**
[3] Good Food Sources for 18:2 Linoleic Acid. http://healthyeating.sfgate.com/good-food-sources-182-linoleic-acid-11013.html
[4] PR O'Quinn; JL Nelssen; RD Goodband; MD Tokach. *Anim Health Res Rev*. **2000**, 1, 35.
[5] D Kritchevsky. *Nutr Bull*. **2000**, 25, 25.
[6] MA Belury. *Annu Rev Nutr*. **2002**, 22, 505.
[7] GS Kelly. *Altern Med Rev*. **2001**, 6, 367.
[8] RC Khanal. *Asian-Aust J Anim Sci.* **2004**, 17, 1315.
[9] RR Yetella; B Henbest; A Proctor. *J Am Oil Chem Soc*. **2013**, 90, 863.
[10] JTJ Reaney; ND Westcott. *Inform*. **2002**, 13,802.
[11] Ultraviolet Radiation. https://hps.org/hpspublications/articles/uv.html ,**2018**.
[12] AA Christy. *Chem Phys Lipids*. **2009**, 161, 86.
[13] AA Christy. *Lipids*. **2009**, 44, 1105.
[14] AA Christy; RF Rifla. *Advances in Natural and Applied Sciences*. **2016**, 10, 19.
[15] AA Christy; SL Arachchi. *Advances in Natural and Applied Sciences.* **2016**, 10, 168.