
CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

IRRADIATION INDUCED CHANGES IN THE *TRANS* FATTY ACID CONTENT AND PHYSICOCHEMICAL PROPERTIES OF SELECTED OILS

Prashasti Tripathi✉, O.P.Chauhan

[1]*Centre of Food Technology, University of Allahabad, Allahabad*
[2]*Defence Food Research Laboratory, Mysore.*
✉*prashasti.tripathi@gmail.com,*

https://doi.org/10.34302/crpjfst/2022.14.2.8

Article history:
Received:
1 May 2021
Accepted:
15 April 2022

Keywords:
Trans Fat;
Free Fatty Acid;
Anisidine Value;
Conjugated Diene;
Oxidation.

ABSTRACT

Among the existing technologies for food preservation, irradiation of food is recognized as a safe and effective method for a range of specific applications. Although through studies it was found that irradiation may bring biochemical changes in the food commodity. One such potential processing hazard formed due to irradiation is the generation of trans fatty acids. The current study was done to observe the effect of irradiation on trans fatty acid and other physicochemical changes in oils. In the present study, potato fingers were deep-fried separately in 3 different oils primarily consumed in India viz. soybean oil, mustard oil, and palm oil. The fried samples were then irradiated at 2,4 and 6 KGy doses and the effect of different doses of irradiation on the physicochemical properties with special reference to trans fat formation in all the selected oils were studied. Trans fat isomers observed in this study are Palmitelaidic acid (PA) C16:0, Elaidic acid (EA) C18:1t9, Vaccenic acid (VA) (C18:1t11), Linoleic acid (LA) C18:2, and Linolenic acid (LLA) C18:3, other physiochemical properties analyzed are peroxide value, anisidine value, TBA value, free fatty acid, iodine value, and total polar compound. It was observed that irradiation of lipids may lead to the formation of free radicals which may affect its properties. The changes occurring due to irradiation are found to be proportional to the linoleic and linolenic acid content of the oil. Irradiation also found to induce changes in the oxidative parameters of oils tested like peroxide value , anisidine value etc.

1.Introduction

Irradiation is a technology that improves the safety and extends the shelf life of foods by reducing or eliminating microorganisms. Ionizing radiation uses the high energy of gamma rays or accelerated electrons, thereby ionizing molecules. The use of this treatment on food could extend shelf life and protect the host against pathogenic bacteria. Currently, irradiation is permitted by USDA, FSIS, and U.S. FDA at doses up to 4.5 kGy for treating refrigerated, uncooked meat and meat by-products (FSIS 1999). On the other hand, irradiation treatment brings about some biochemical changes that could affect the nutritional adequacy of food (Giroux and Lacroix, 1998). FDA has permitted the irradiation of a number of foods like beef, pork, spices, eggshell, sprouts and fresh fruits and vegetables etc. Although through studies it was found that irradiation may bring biochemical changes in the food commodity. One such potential processing hazard formed due to irradiation is the generation of *trans* fatty acids. *Trans* fatty acids (TFA) are the geometrical isomers of unsaturated fatty acids with at least one non-conjugated, carbon-carbon double bond in the *trans* configuration rather than the more common *cis* configuration (Codex, 1985; EFSA, 2004; Kodali, 2005). Consumption of *trans* fatty

acid in diet may lead to various health issues like cardiovascular disease, obesity, coronary heart diseases, prostrate cancer, breast cancer, etc. It may also affect the cell membrane and auto immune system and can cause damage to brain cells (Zhu *et al.*, 2019). Hence it is required to minimize the intake of *trans* fatty acids, which are not indispensable to humans. Other than *trans* fat formation irradiation may also induce changes in the physiochemical properties of fat generating free radical or causing oxidation. Hence to obtain a comparative study on the extend of degradation in different oils; palm, soybean and mustard/rapeseed oil were selected which together account for 75% of the total edible oil demand, with respective shares of 46%, 16% and 14%.respectively. The effect of irradiation on the physiochemical properties of selected oils was discussed with reference to their fatty acid chain length and the degree of unsaturation.

2.Materials and Methods

Irradiation treatments were given to oil samples for dose of 2, 4, and 6 KGy and the processed samples were then analysed for *trans* fatty acid, peroxide value, anisidine value, TBA value, free fatty acid, iodine value and total polar compound.

*Irradiation of oils were done at DFRL, Mysore. *Trans* fat and other quality parameters were analyzed at CFT, University of Allahabad.

2.1. Reagents and standards

All chemicals, solvents and reagents employed were of analytical grade and purchased from Merck (India). The internal standard (IS) pentadecanoic acid (C15:0) and the individual of five Fatty acids [FA] and Fatty Acid Methyl Esters [FAME] standards: Palmitelaidic acid (PA) C16:0, Elaidic acid (EA) C18:1t9, Vaccenic acid (VA) (C18:1t11), Linoleic acid isomer mix (LA) C18:2, and Linolenic acid isomer mix (LLA) C18:3, were purchased from Sigma-Aldrich (INDIA) (purity; ≥99.99% (GC). The esterifying catalyst Boron Trifloride and solvent heptane were also purchased from Sigma (Sigma–Aldrich, India).

2.2. Irradiation:

Irradiation was conducted using the Co-60 gamma-radiation source and silver dichromate, which was used as a dosimeter, at the Defence Food Research Laboratory, Mysore. The applied radiation doses were 2, 4, and 6 kGy by Gamma rays in γ-chamber 5000 at the dose rate of 3.568 KGy/hr. In order to study the effect of Irradiation on *trans* fat formation 100 ml oil (soybean, mustard and palm oil) was packed and sealed in an HDPE envelop separately and it was then placed inside the irradiation chamber where they were exposed to irradiation at 2, 4 and 6 KGy at ambient temperature.(Yilmaz, 2007)

2.3. Sample preparation

Approximately 0.2 g of oil sample was *trans*ferred to the flask, 10 ml of 1.0 N methanolic NaOH was added and a known concentration of Internal Standard was added to the flask, which was then refluxed for 10 min. About 5 ml of 14 % methanolic boron trifloride (BF3/MeOH) was added and refluxed for an additional 2 min. About 5 ml n-heptane was added to the flask through condenser and then allowed to cool. Organic layer was then separated with centrifugation after adding 10 ml concentrated NaCl solution. About 1.0 ml of the top layer was *trans*ferred into a 10-ml stoppered glass tube using a *trans*fer pipette, and then the sample was diluted to the mark (10 ml) with n-heptane (AOAC , 2001)

2.4. Gas Chromatograph analysis of FAME

FAMEs were analyzed using a GC Clarus 500 Chromatograph (Perkin Elmer, India) equipped with a fused silica capillary column SP-2560 (with column length- 30 m and internal diameter 320 µm) and flame ionization detector [FID]. High-purity nitrogen (99.999 %) was used as the carrier gas with a set flow rate of 1 ml/min and hydrogen and zero air was used as

fuel gas with flow rates 45 ml/min and 450 ml/min respectively. The oven temperature program was as follows: 4 min at 130 ºC, increased/ramped by 2.5 ºC/min up to 240 ºC, and then further ramped at the rate of 5.0 ºC up to 260 ºC held for 20 min. The injector and detector temperatures were 220 and 280 ºC, respectively. The injection volume was 2 µl in split less mode (Modified AOAC, 2001).

For Determination of Peroxide value of oil/fat IS: 548-Part 1, 1964 method was used, while for Free fatty acid [FFA] analysis of oil /fat method used was IS: 548-Part 1, 1964.

2.5. Methods for determination of quality parameters of oil / fat

2.5.1. Determination of Free fatty acid (FFA) of oil /fat :

For analysis of FFA 5 gm sample was taken in a conical flask and to the flask 50 ml of 95% ethyl alcohol (neutralized by adding 0.1N NaOH drop wise) was added. Oil was dissolved in the ethanol and kept on hot plate for boiling. Sample was boiled till all the oil droplets get completely dissolved. 2-3 drops of phenolphthalein indicator was added and the sample was then titrated with 0.1N NaOH (while still hot) till a faint pink color appear in the sample that should last for at least 15 sec in the sample (IS: 548-Part 1a, 1964)

Calculation:

Free fatty acid % = 28.5× V×N /W (1)

Where, V= Volume in ml of standard sodium hydroxide required for titration

N= Normality of standard sodium hydroxide used in titration

W= Weight in ‘g’ of the sample taken for estimation.

2.5.2. Determination of Total polar compound in oil/fat:

2.5g of clean and dry sample was weighed in 50 ml volumetric and 20 ml ether solvent was added to it (87:13). The solution was warmed to dissolve the fat/oil completely and the volume was make up with ether solution. 20 ml of this solution was poured at the top of the glass column slowly with the help of a pipette. The drain was collected in clean and dry conical (pre-weighted). The non-polar components were eluted from the column by draining 150 ml ether solvent slowly. The flow should be slow that it takes 1 hour to elute the solvent completely. After the solvent is eluted completely, the flask was kept in hot air oven to evaporate the solvent. The flask was cooled and the weight of flask was measured (IUPAC, 1992).

*To prepare ether solution for elution, petroleum ether and diethyl ether was mixed in ratio 87:13.

Calculation:

TPC = 1- (weight of flask after evaporation of solvent- initial weight of flask) (2)

2.5.3. Determination of Peroxide value of oil/fat :

Approximately 0.2 ml of sample was taken in a boiling tube. To the sample 9.9 ml of chloroform/methanol (7:3 v/v) was added and swirled to dissolve the sample. Then 50 µl of 10 mM xylenol orange was added and mixed. 50 µl of Fe (II) Chloride solution was added and kept for 5 mins. Absorption was determined at 560 nm wavelength with U.V-Vis spectrophotometer. Standard curve was plotted using standard Fe (III) chloride instead of oil at 0, 1 and 2 ml concentration (IS: 548-Part 1b, 1964)

Calculation:

P.V. = [(A1- A2)×inverse of slope× 55.84 × 2 / weight of sample (3)

Where A1= sample absorbance

A2= blank absorbance

* Xylenol orange solution- 0.019 gm of xylenol orange was dissolved in 25 ml of water.

*Ferrous (Fe II) chloride solution: dissolve 0.5g ferrous sulphate ($FeSO_4.7H_2O$) was dissolved in

50 ml water and 0.4 g barium chloride ($BaCl_2.2H_2O$) in 50 ml water. The 2 solutions were mixed and add 2 ml of 10M HCl was added to it. Now filter out $BaSO_4$ and store the solution in brown bottle.

*Ferric (Fe III) chloride solution (to be used as a standard of 10µg/ml): 0.5g ferric chloride was dissolved in 50ml of 10 M HCl and 2 ml of 30% hydrogen peroxide solution was added to it. The solution is boiled for 5 minutes to remove excess of H_2O_2. Cooled and diluted to 500 ml with distilled water. 1 ml of the prepared reagent was diluted to 100 ml with chloroform/methanol (7:3 v/v) solution.

2.5.4. Determination of Anisidine Value of oil/fat:

Approximately 0.5 gm oil sample was weighed in 25 ml volumetric and volume made up with iso-octane. Then it was mixed thoroughly to dissolve the sample. Absorbance measured at 350nm wavelength against pure iso-octane. After measuring the absorbance 1 ml of anisidine reagent (0.25% w/v) was added to the same sample solution. Tube was then stoppered and kept in dark for 10 min. Absorbance again measured at 350 nm wavelength against pure iso-octane as reference (IS: 548-Part 1b, 1964)

* To prepare anisidine reagent dissolve 0.25gm of para-anisidine was dissolved in 100 ml acetic acid (AR grade)

Calculation:

A.V. = 37.5× [A1- A2) / W. (4)

Where ,

A1 =Absorbance of fat/oil with anisidine

A2= Absorbance of fat/oil without anisidine]

W = Weight of sample

2.5.5. Determination of Conjugated diene value of oil/fat :

Hydroperoxides from PUFAs form conjugated dienes that can be measured quantitatively by spectrophotometric UV measurement at wavelength 232 nm. To measure conjugated diene value, 0.5 gm of sample (melted and filtered to remove suspended impurity) was weighed and then diluted to 50 with iso-octane in volumetric flask. Sample was dissolved completely with shaking and then absorbance was taken at 232-262-268-274 nm wavelengths with pure iso-octane as reference/blank. The absorption value 'indicated by D' read on the spectrophotometer and with K indicated as the specific absorption value (IS: 548-Part 1b, 1964)

Calculation:

The K value is obtained from the equation:

$K = D / C \times S$ (5)

Where, C= solution concentration ing/L (10g/L)

S= cuvette thickness in centimetre.

ΔK value is determined as follows,

ΔK = K268- (K262 + K274 / 2)

* The conjugated diene value is based on the detected absorbance and is expressed as µmol hydroperoxides /g sample.

2.5.6. Determination of Iodine Value of oil /fat

Approximately 0.2 gm of dried and filtered oil sample was weighed into 250 ml stopper conical flask. In the flask 25 ml of carbon tetrachloride was added and agitated to dissolve sample. Further 25 ml of wij's iodine solution was added. The flask was then immediately stoppered, swirled to mix and kept in dark for 1 hr. After 1 hr conical was taken out and 15 ml of 10% potassium iodide solution was added to it. Immediately 100 ml of CO_2 free water (boiled and cooled) was added and the sample was then titrated with 0.1 N standard sodium thiosulphate solution using starch indicator (AOAC 993.20).

Calculation:

I.V. = 12.69 (Blank T.V.– Sample T.V.) × N of $Na_2S_2O_3$ / Weight of sample. (6)

2.6. Statistical analysis:

All values were shown as the means and standard deviations for three replicates. The statistical analyses were performed using the ANOVA function of IBM SPSS Statistics 20.0 for Windows software. Statistical significance was determined as $p<0.05$ using Duncan's multiple range test. Coefficient of variance was calculated for free fatty acid, peroxide value, total polar compound, anisidine value, iodine value and conjugated diene value. All the graphs were plotted on Sigma plot 10.0 software.

3.Results and discussion

3.1.Effect of Irradiation on TFA content in selected oils

Irradiation of samples was done at the Defence Food Research Laboratory, Mysore. The applied radiation doses were 2, 4, and 6 kGy by Gamma rays in γ-chamber 5000 at the dose rate of 3.568 KGy/hr. In order to study the effect of Irradiation on *trans* fat formation soybean, mustard and palm oil and were processed at 2, 4 and 6 KGy at ambient temperature. **Table 1 and 2** shows the content of all *trans* isomers in soybean, mustard and palm oil, respectively, irradiated at doses between 0 and 6 KGy **(Figure 1).** In the present study, the amount of total fatty acids was affected by irradiation, mainly because of the reduction in unsaturated fatty acids (*cis*-UFA). Slight but statistically significant changes ($P<0.05$) of the main polyunsaturated *trans* fatty acids such as C18:2-9c,12c, C18:1-9n were observed in processed samples for each of the 3 oils selected, irradiated at 4 KGy compared with the control sample, at a dose of 2 KGy, no significant change was observed in *trans* fatty acid content. Hence it can be concluded that irradiation induced the formation of TFAs in food when the absorbed dose exceeded 4 KGy. Among the three oils selected for study, maximum increase was observed in soybean oil followed by palm oil and then mustard oil (GC chromatogram of palm oil before and after irradiation treatment is shown in **Figure 2**). Brito et.al (2002) and Yilmaz (2007) also reported that gamma-irradiation leads to an increase in the amount of *trans* fatty acid in food, particularly elaidic acid (C18:1-9t). However in the present study trans isomerization is found more in linolenic acid. Although irradiation caused the increase of *trans* fatty acids, the total amounts of all *trans* isomers were no more than 2%, which is the *trans* fat limit in some countries like Denmark.

Table 1. Effect of irradiation on TFA content in different oils

	Soybean oil	**Mustard oil**	**Palm oil**
Fresh oil	0.63 ± 0.09^{a}	0.88 ± 0.11^{a}	0.93 ± 0.12^{a}
2 KGy	0.67 ± 0.12^{a}	0.91 ± 0.13^{a}	0.94 ± 0.14^{a}
4 KGy	0.78 ± 0.16^{ab}	0.96 ± 0.13^{ab}	0.99 ± 0.13^{b}
6 KGy	0.89 ± 0.11^{b}	1.03 ± 0.14^{bc}	1.07 ± 0.11^{bc}
F-VALUE	2.809	0.847	0.979

*Values with different superscript in the same column differ significantly ($p\leq0.05$)

*All data are expressed as mean±s.d. (n=3)

Table 2. Changes in *trans* fatty acid composition of selected fats/oils during irradiation

Sample	Linoleic acid methyl ester (%)	Linolenic acid methyl ester (%)	Elaidic acid methyl ester (%)	Vaccenic acid methyl ester (%)	Palmitelaidic acid methyl ester (%)	*Trans* fat % in product
Soybean control	0.14±0.02	0.32±0.02	0.15±0.08	ND.	ND.	0.63±0.14[a]
Soybean 2 KGy	0.15±0.07	0.34±0.03	0.16±0.03	ND.	ND.	0.67±0.12[a]
Soybean 4 KGy	0.20±0.09	0.41±0.07	0.27±0.03	ND.	ND.	0.88±0.26[ab]
Soybean 6KGy	0.14±0.04	0.37±0.03	0.16±0.03	ND.	ND.	0.69±0.11[b]
Mustard control	0.13±0.06	0.39±0.05	0.33±0.05	ND.	0.03±0.01	0.88±0.13[a]
Mustard 2 KGy	0.15±0.04	0.41±0.07	0.35±0.01	ND.	0.02±0.01	0.91 ±0.16[a]
Mustard 4 KGy	0.14±0.06	0.44±0.03	0.38±0.02	ND.	0.03±0.03	0.96±0.13[ab]
Mustard 6 KGy	0.15±0.08	0.46±0.02	0.38±0.03	ND.	0.02±0.03	0.98±0.14[bc]
Palm control	0.20±0.4	0.15±0.06	0.45±0.02	ND.	0.13±0.05	0.93±0.21[a]
Palm 2 KGy	0.18±0.1	0.14±0.02	0.48±0.04	ND.	0.13±0.05	0.94±0.19[a]
Palm 4 KGy	0.19±0.2	0.15±0.01	0.49±0.03	ND.	0.15±0.05	0.99±0.17[b]
Palm 6KGy	0.19±0.2	0.16±0.03	0.52±0.05	ND.	0.16±0.05	1.04±0.11[bc]

*All data are expressed as mean±s.d. (n=3)

*Values with different superscript in the same column differ significantly ($p \leq 0.05$)

The formation of *trans* configuration has been confirmed to occur during the irradiation of *cis* fatty acids. Brito et.al (2002) observed an average increase of 80.4 % in TFA amount when they evaluated the effect of TFA content in fresh bovine meat irradiated with doses between 1–5 kGy. Brito et.al (2002) found that although, the gamma radiation has been an excellent method to conserve meat, the molecular structure of this meat were changed during irradiation. Other studies have shown that *cis–trans*-isomerization of unsaturated fatty acids occurs during gamma irradiation of barley grains (Geibler, 2003) and frankfurters (Fan, 2009). More recently, Yilmaz and Gecgel C. (2013) also found significant increases in *trans* fatty acid content of ground beef at doses of 3 kGy or above. At 5 kGy, total *trans* fatty acid content was increased from 7.0%

to 9.4%, a 34% increase. On contrary Chen (2007) found no significant effect of irradiation (0 to 3.2 kGy) on the C18:1 *trans* content of the total lipid fraction of beef semi-tenderness muscle; however, some changes occurred in the much lower concentrations of C16:1 *trans* and C18:2 *cis*, *trans* content of the neutral and polar lipid fractions. Wang *et. al* (2012) reported that irradiation led to a significant increase of total *trans* fatty acid content in ground beef and liquid egg with an absorbed dose range between 6.743 and 11.472 kGy (P\0.05). The change in C18:1-9t content was the most significant compared with other *trans* fatty acids. Irradiation of barley grains (Geibler, 2003) with a dose of 10 kGy resulted in no measurable *trans* isomers.

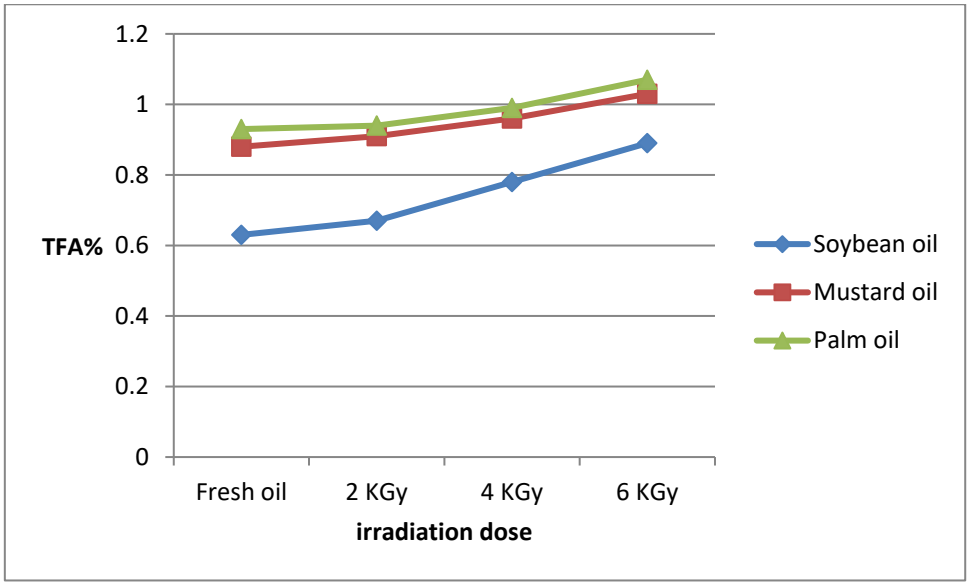


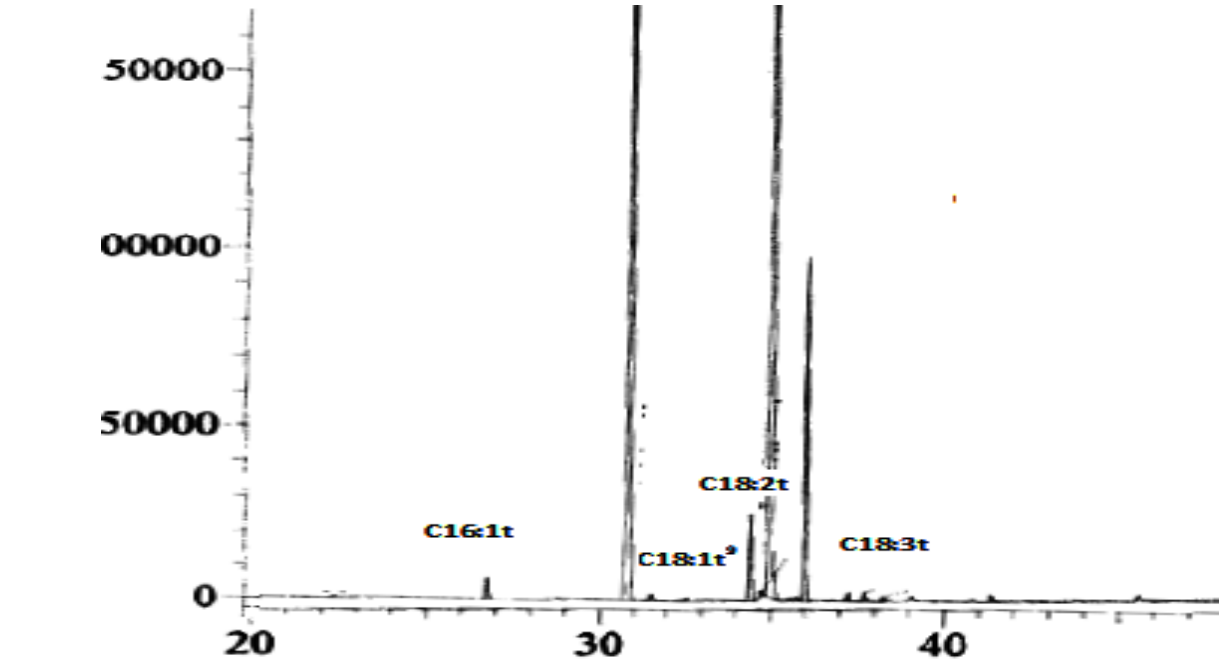
Figure 1. *Trans* fatty acid content in selected oils at different irradiation dose

Food irradiation is a promising technology that has been commercially established because it can effectively extend food shelf-life, control pathogenic bacteria, and delay the ripening or maturation of certain fruits or vegetables (Prakash 2016, Kume *et. al*, 2009). WHO, IAEA, and FAO consider food irradiated with doses of up to 10 kGy safe for consumption (Joint, 1981). The high energy of ionizing radiation may cause chemical modifications in the lipid fraction, which results in radical formation. The *cis*/*trans* isomerization of carbon double bonds has been reported to occur in vivo by a free radical attack against the cell membrane (Ferreri et.al.2004).

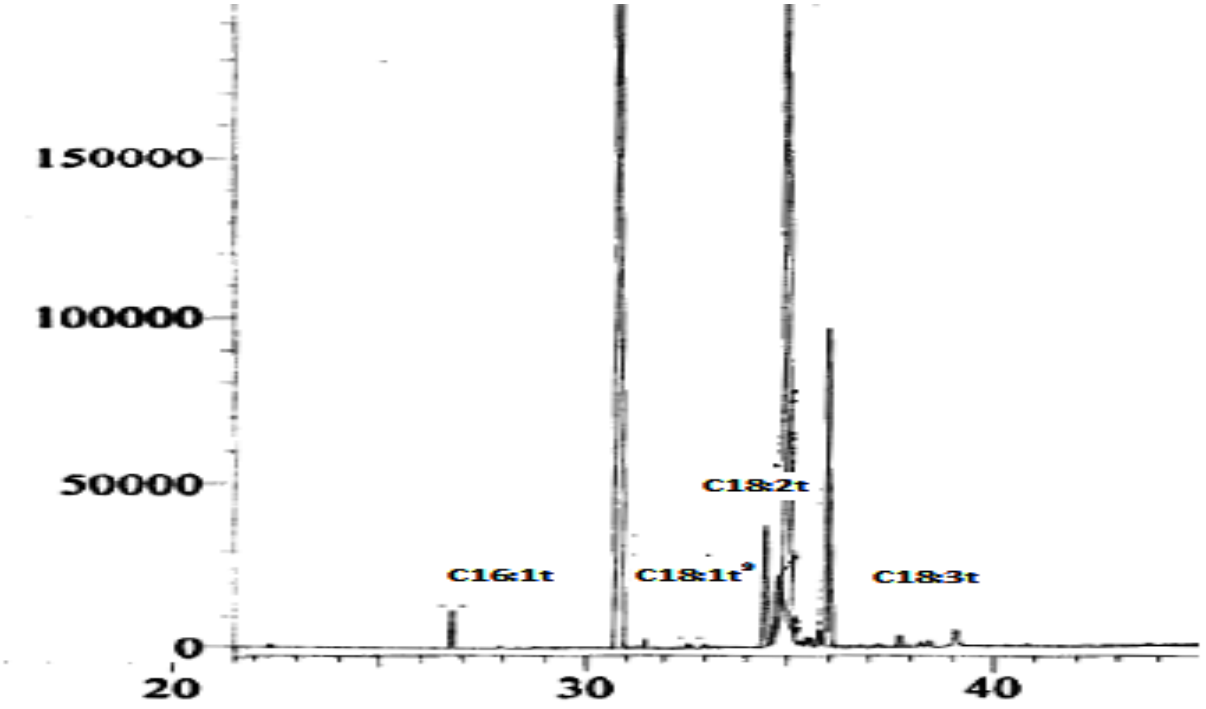
The formation mechanism of TFA due to irradiation was quite different from that during hydrogenation. A radical-catalyzed isomerization of carbon–carbon double bonds in unsaturated fatty acids is reasonable for this case. XH was converted into X via hydrogen abstraction because of gamma-irradiation. The isomerization process occurred between the radical adduct X and a double bond of the fatty acid, and then *trans* isomers were formed via a β-elimination of X (Chatgilialoglu 2002). XH can take the form of compounds such as RSH or RSSH in food, as established in previous studies (Zambonin et.al.2006).

Minami (2012) studied the effect of gamma radiation on soybean and soybean oil. Irradiation at 10 to 80 KGy under anaerobic conditions did not markedly changes the fatty acid composition. While 10 KGy radiation does not affect the fatty acid composition even under aerobic condition while 40 KGy irradiation considerably altered the fatty acid composition under aerobic condition. Moreover 40 KGy irradiation produces significant amount of *trans* fatty acid under aerobic condition, but not under

anaerobic conditions. Irradiating soybean oil induced lipid per-oxidation and reduced the radical scavenging activity under the anaerobic condition.



Palm oil- Fresh



Palm oil- After Irradiation

Figure 2. GC chromatogram of Palm oil before and after irradiation (6 KGy) treatment

3.2.Free fatty acid (FFA)

Free fatty acid is the relative measure of rancidity as FFA is normally formed during the decomposition of oil glycerides due to the action of moisture, temperature and/or lipolytic enzyme lipase. Free fatty acids increase the thermal oxidation of oils, and their unsaturation rather than chain length led to significant effects on thermo-oxidative degeneration. The effect of Irradiation on free fatty acid value is given in **Table 3** and a very slight significant change was observed in the FFA content of oils with maximum increase for soybean oil (with co-efficient of variance 0.0888).

Table 3. Effect of Irradiation on free fatty acid value of selected oils

Processing	SOYBEAN OIL	MUSTARD OIL	PALM OIL
Fresh sample	0.18±0.06	0.35±0.21	0.29±0.12
2 KGy	0.19±0.05	0.3±0.11	0.32±0.08
4 KGy	0.19±0.09	0.34±0.17	0.3±0.09
6 KGy	0.22±0.14	0.36±0.15	0.35±0.05
C.V.	0.088823	0.077925	0.083992

*Free fatty acid expressed as % of oleic acid.

*All data are expressed as mean±s.d. (n=3)

3.3. Total polar compound

Total polar compound of the fresh oil samples selected for the study are 0.11±0.03, 0.08±0.04 and 0.11±0.02 (TPC expressed as % by weight) for soybean oil, mustard oil and palm oil respectively. The effect of Irradiation on total polar compound (TPC) is given in **Table 4.** Most significant increase in TPC was observed for mustard oil followed by palm oil and then soybean oil indicating more degradation of triglycerides in mustard oil.

Table 4. Effect of Irradiation on TPC value of selected oils

Processing	SOYBEAN OIL	MUSTARD OIL	PALM OIL
Fresh sample	0.11±0.03	0.08±0.03	0.11±0.05
2 KGy	0.12±0.02	0.1±0.02	0.12±0.06
4 KGy	0.12±0.05	0.11±0.05	0.14±0.06
6 KGy	0.10±0.02	0.1±0.02	0.12±0.04
C.V.	0.085105	0.129057	0.102719

* TPC expressed as % by weight

*All data are expressed as mean±s.d. (n=3)

3.4. Peroxide value

The peroxide value is defined as the amount of peroxide oxygen per 1 kilogram of fat or oil. It gives a measure of the extent to which an oil sample has undergone primary oxidation, Peroxide value of the fresh oil samples selected for the study are 0.8±0.05 mEq/Kg, 0.92±0.11 mEq/Kg and 0.83±0.08 mEq/Kg for soybean oil, mustard oil and palm oil, respectively. A very significant change was observed in peroxide value of oils after irradiation treatment **(Table 5).** Maximum peroxides were generated in soybean oil followed by mustard oil and palm oil. The result observed in this study is found to be in agreement to other findings[15], showing the oxidation of lipids resulting in formation of peroxides. These peroxides may further undergo secondary oxidation thus forming conjugated dienes.

Table 5. Effect of Irradiation on Peroxide value of selected oils

Processing	**SOYBEAN OIL**	**MUSTARD OIL**	**PALM OIL**
Fresh sample	0.81±0.17	0.92±0.21	0.80±0.18
2 KGy	0.83±0.14	0.94±0.18	0.81±0.16
4 KGy	0.88±0.13	0.97±0.22	0.84±0.14
6 KGy	0.96±0.11	0.99±0.28	0.82±0.09
C.V.	0.061415	0.033422	0.020638

*Peroxide value expressed as mEq/Kg of oil

*All data are expressed as mean±s.d. (n=3)

Table 6. Effect of Irradiation on Anisidine value of selected oils

Processing	**SOYBEAN OIL**	**MUSTARD OIL**	**PALM OIL**
Fresh sample	2.13±0.65	7.68±1.05	4.83±0.97
2 KGy	2.43±0.43	7.65±0.99	4.97±0.05
4 KGy	2.51±0.19	7.78±1.32	5.07±0.65
6 KGy	2.64±0.43	7.97±1.08	5.21±0.82
C.V.	0.089141	0.030284	0.011828

*All data are expressed as mean±s.d. (n=3)

3.5. Anisidine value

p-Anisidine reacts with secondary oxidation products formed by combination of free radicals with O2 generating hydroperoxides such as aldehydes and ketones in fats and oils to form products that absorb at 350 nm wavelength of light. It is particularly good at detecting unsaturated aldehydes, which are the ones that are most likely to generate unacceptable flavors. Different processing treatments lead to a variety of chemical reactions which can be categorized as hydrolysis, oxidation, and polymerization of the triacylglycerol molecule. The decomposition products formed by these processes may be volatile or nonvolatile and undergo further degradation. Hydrogenated soybean oil with 0.1% linolenic acid had more hydrolytic degradation, but lower p-anisidine values and

polymer formation, than the soybean oil with 2.3% linolenic acid (Tompkin, 2004). **Table 6** showing the effect of irradiation on anisidine value indicates most significant increase in soybean oil after processing. The mustard oil is having more complex fatty acids and palm oil have comparatively lesser unsaturated fatty acids hence among the three oils soybean oil is found to undergo oxidative changes significantly more compared to other two oils.

3.6. Iodine value

The Iodine value (IV) of an oil/fat is the number of grams of iodine absorbed by 100 g of the oil/fat, when determined by using wiij's solution. The iodine value is a measure of the amount of unsaturation (number of double bonds) in fat/oil. During heat treatment, a progressive decrease in unsaturation was observed in all oils by measurement of IV. This decrease can be attributed to the destruction of double bonds by oxidation, s*cis*sion, and polymerization (Cowan, 1954; Cuesta, 1993). It was followed that the more saturated the oil is the lesser is the degradation of oil into secondary metabolites**.**

As shown, the highest significant ($p < 0.05$) change in the IV was shown by the mustard oil and soybean oil, thus indicating that the highest decrease in double bonds occurred due to oxidative rancidity in the proposed media **(Table 7).** This observation could be due to the presence of a high amount of PUFAs in oil.The greater the degree of unsaturation (or high IV), the more rapid the oil tends to be oxidized, particularly during deep-fat frying. Abbas (2016), reported that iodine value (IV) for microwave heated palm oil, sunflower oil and blend of palm-sunflower oil gradually decreased with increasing heating times. The reduction in IV was highest in sunflower oil (5.73) and lowest in palm-sunflower oil blend (4.11).

Table 7. Effect of Irradiation on Iodine value of selected oils

Processing	**SOYBEAN OIL**	**MUSTARD OIL**	**PALM OIL**
Fresh sample	126±4.29	102±3.09	48±2.07
2 KGy	125±4.17	101±3.69	48±1.78
4 KGy	125±3.98	101±2.74	47±1.43
6 KGy	125±4.21	100±2.18	47±1.84
C.V.	0.006532	0.009456	0.012155

*All data are expressed as mean±s.d. (n=3)

3.7. Conjugated diene

The ultraviolet spectrophotometric analysis of oil indicates the degree of oxidation, being its value expressed as specific extinction co-efficients. The K_{232} value is indicative of carbonyl compounds present in oil. The maximum permitted value of K_{232} in edible oil is 2.50 (Malheiro 2009). A significantly negative co-relation was found for irradiation treatments for increase in K_{232} value.

Absorbitivity of fresh oil sample for K-232 value are 0.21±0.10, 0.24±0.08, and 0.17±0.05 for soybean oil, mustard oil, palm oil respectively. If the increase in conjugated diene was observed for all the processing treatment, it was found that coefficient of variation for diene value during irradiation treatment ranged from 0.0688 to 0.0404 only with no significant change **(Table 8).**

Table 8. Effect of Irradiation on Diene value of selected oils

Processing	SOYBEAN OIL	MUSTARD OIL	PALM OIL
Fresh sample	0.21±0.07	0.24±0.05	0.16±0.05
2 KGy	0.21±0.04	0.26±0.02	0.18±0.06
4 KGy	0.23±0.09	0.26±0.09	0.20±0.02
6 KGy	0.25±0.05	0.27±0.06	0.21±0.10
C.V.	0.068823	0.053422	0.040421

*All data are expressed as mean±s.d. (n=3)

Gecgel (2013), studied Changes in some physicochemical properties and fatty acid composition of irradiated meatballs irradiated using a ^{60}Co irradiation source (with the dose of 1, 3, 5 and 7 kGy). The physicochemical results showed total acidity, peroxide and thiobarbituric acid (TBA) values increased significantly as a result of irradiation doses. The fatty acid profile in meatball samples also changed with irradiation. Gecgel (2013) also reported an increase in t*rans* fatty acids (C16:1*trans*, C18:1*trans*, C18:2*trans*, C18:3*trans*) with increasing irradiation doses.

4.Conclusions:

Slight but statistically significant changes ($P<0.05$) of the main polyunsaturated *trans* fatty acids such as C18:2-9c,12c, C18:1-9n were observed in the samples for each of the 3 oils selected with the irradiation dose of 4 kGy compared with the control sample, at a dose of 2 KGy, no significant change was observed in *trans* fatty acid content. Hence it can be concluded that irradiation induced the formation of TFAs in food when the absorbed dose exceeded 4 kGy. Among the three oils selected for study, maximum difference was observed in soybean oil followed by palm oil and then mustard oil. Oxidative changes for peroxide and anisidine value was most significant in soybean oil, however no significant change was observed for iodine value and conjugated diene value after irradiation in all the selected samples.

5.References:

Abbas, A. M. ,Mesran, M., Hadi Bin, R. Lapit, N., Hidayu, O. (2016), Effect of microwave heating with different exposure times on the degradation of corn oil, *International food research journal.*, 23 (2), 842-848.

AOAC, Official Method 969.33. (1990), "AOAC Fatty Acid in Oils and Fats Preparation of Methyl Ester Boron Trifluoride Method," 15th Edition, AOAC International, Washington DC, 400– 405.

AOAC, Official method 993.20. (1998), Ca 5a-40, Free Fatty Acids. *Official Methods and Recommended Practices of the American Oil Chemists' Society.* (5th ed). Champaign, IL.

AOAC, Official Method 996.06. (2001), "AOAC Fat (Total, Saturated and Unsaturated) in Foods, Hydrolytic Extraction Gas Chromatographic Method," 18th Edition, AOAC In- ternational, Arlington.

Brito, M. S., Villavicencio, A., , Mancini-Filhoj. (2002), Effects of irradiation on *trans* fatty acids formation in ground beef. *Radiation Physics and Chemistry*, 63, 337– 340.

Chatgilialoglu, C., Altieri A., Fischer, H. (2002), The kinetics of thiyl radical-induced reactions of monounsaturated fatty acids esters, *Journal of American chemical* society, 124(43), 12816-12823.

Chen, Y. J., Zhou, G. H., Zhu, X. D., Xu, X. L.,

Tang, X. Y., Gao, F. (2007), Effect of low dose gamma irradiation on beef quality and fatty acid composition of beef intramuscular lipid. *Meat Science*, 75(4), 23–31.

Codex Alimentarius. (1985). Guidelines on nutrition labelling, CAC/GL 2-1985. www.codexalimentarius.org/input/download/standards/34/CXG_002e.pdf [accessed 06.08.16].

Cowan, J. C. (1954), Polymerization, copolymerization, and isomerization. *Journal of American Oil Chemist Society.*, 31, 529-535.

Cuesta, C., Muniz, F. J. S., Garrido Polonio, C., Varela, S. L., Arroyo, R. (1993), Thermoxidative and hydrolytic changes in sunflower oil used in fryings with a fast turnover of fresh oil, *Journal of American oil chemists society,* 70(11), 1069-1073

European Food Safety Authority (2004). Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a Request from the Commission Related to the Presence of *Trans* Fatty Acids in Foods and the Effect on Human Health of the Consumption of *Trans* Fatty Acids. *The EFSA Journal,* 81, 1-49.

Fan, X., Kays, S.E., (2009), Formation of *trans* fatty acids in ground beef and frankfurters due to irradiation, *Journal of food science*, 74(2), C79-C84.

Ferreri, C., Samadi, A., Sassatelli, F., Landi, L., Chatgilialoglu, C. (2004), Regioselective cis-*trans* isomerization of arachidonic double bonds by thiyl radicals: The influence of phospholipid supramolecular organization, *Journal of American oil chemists society,* 126(4), 1063-1072.

FSIS USDA (1999), Rulemaking/Federal Register docket no. 97-076F, irradiation of meat food products

Gecgel, U. (2013), Changes in some physic-chemical properties and fatty acid composition of irradiated meatballs during storage, *Journal of food science and technology,* 50(3), 505-513.

Geibler, C., Brede, O., Reinhardt, J. (2003,) *Cis–trans*-Isomerization of unsaturated fatty acids during gamma-irradiation of barley grains. *Radiation Physics and Chemistry*, 67, 105–113.

Giroux, M., Lacroix, M. (1998), Nutritional adequacy for irradiated meat- a review, *Food research international*, 31(4), 257-264.

IS (2010b). Indian standards (IS) 548 (1964 reaffirmed 2010) - methods of sampling and tests for oils and fats, Part 1,sampling, physical and chemical tests, determination of peroxide value in fats and oils, 63-65.

IS (2010a). Indian standards (IS) 548 (1964 reaffirmed 2010) - methods of sampling and tests for oils and fats, Part 1,Sampling, physical and chemical tests, determination of acid value in fats and oils, 2, 9-31.

IUPAC (1992), *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th. Ed., C. Paquot and Hautfenne (Ed.). *International Union of Pure and Applied Chemistry*, Blackwell Scientific Publications Inc, Oxford

Joint, F. (1981) Wholesomeness of irradiated food: report of a Joint FAO/IAEA/WHO Expert Committee (meeting held in Geneva from 27 October to 3 November 1980). World Health Organization

Kodali, D.R. (2005). *Trans* Fats – Chemistry, Occurrence, Functional Need in Foods and Potential Solutions. In: Kodali, D.R. and List, G.R. (eds.) *Trans* Fats Alternatives, AOCS Press, Champaign Illinois, United States, 1-25.

Kume, T., Furuta, M., Todoriki, S., Uenoyama, N., Kobayashi, Y. (2009), Status of food irradiation in the world. *Radiation Physics and Chemistry*, 78, 222–226.

Malheiro, R., Oliveira, I., Boas, M. B., Pereira, J.A. (2009), Effect of microwave heating with different exposure times on physical and chemical parameters of olive oil, *Food and Chemical Toxicology,* 47, 92–97..

Minami, I., Nakamura, Y., Todoriki, S., Murata, Y. (2012), Effect of γ-radiation on the fatty

acid composition of soybean and soybean oil, *Journal of Bioscience, biotechnology and biochemistry,* 76(5), 900-905.

Prakash, A. (2016), Particular applications of food irradiation fresh produce, *Radiation Physics and Chemistry*, 129, 50-52.

Yilmaz, I., Gecgel, U.,(2007), Effects of gamma radiation on *trans* fatty acid composition in ground beef, *Food Control*, 18(6), 635-638.

Zambonin, L., Ferreri, C., Cabrini, L., Prata, C., Chatgilialoglu, C., Landi, L. (2006), Occurance of *trans* fatty acids in rat fed a *trans*-free diet: A free radical mediated formation, *Free radical biology and medicine*, 40(9), 1549-1556.

Zhu, K., Jia, H., Zhao, S., Xia, T., Guo, X., Wang, T., Zhu, L. (2019), Formation of environmentally persistent free radicals on microplastics under light irradiation, *Environmental science and technology*, 53.