Annals. Food Science and Technology
2015

INFLUENCE OF EDIBLE OILS, TIME, TEMPERATURE, MOLECULAR GROUPS ON MOLECULAR IODINE ABSORBANCE IN VISIBLE REGION

Rahul **Gangidi**[1], Sukumar **Debnath**[1]
[1]CSIR-Central Food Technological Research Institute (CFTRI), Traditional Food and Sensory Science department, Mysore, Karnataka [570020], India.
Email: debnath@cftri.res.in

Abstract
Conjugated -linoleic-acid (CLA), is well known for its health benefits. The CLA can be produced in edible-oils directly and less-tediously by photo-isomerization, in the presence of photo-sensitizing molecular-iodine. However, the influence of edible oil type, temperature, time and chemical-groups present in edible-oils, on molecular-iodine that influences CLA production, was not explored. The objectives were to study the effects of edible-oil type, time, temperature and molecular groups, in the absence of light, on free molecular-iodine absorbance in visible region.
Molecular-iodine was added to edible-oils (0.125%). Molecular-iodine (0.25%) was added to groundnut-oil and then was either stored for 0,1,2,3 days or the molecular-iodine (0.0125%) was subjected to thermal treatments at 25, 50, 75, 100^{o}C. The molecular-iodine (0.125%) was added to 20% solvents containing chemical-groups. Molecular iodine 520nm-absorbance (maxima) was collected for the samples. Anova and Tukey-HSD pairwise-mean-comparisons were performed ($p<0.05$).
Molecular-iodine (unbound) 520nm-absorbance was highest in groundnut-oil and palm-oil and was less in sunflower-oil. There was significant-reduction ($p<0.05$) in molecular-iodine 520nm-absorbance from day 1 when compared to day 0. Temperatures of 50^{o}C , 75^{o}C and 100^{o}C treatment reduced molecular-iodine absorbance, when compared with absorbance at 25^{o}C. A free C=O group, followed by O-H group, reduced molecular-iodine 520nm-absorbance, the most. The C-O-C, (C=O)-OR, (C=O)-OH groups also reduced the molecular 520nm-absorbance, when compared to the control (CH).
Free molecular-iodine could be available at higher extent in groundnut-oil and palm-oil when compared with sunflower-oil, at less than one day storage duration, at approximately 25^{o}C; and in the absence of hydroxyl-molecular-groups.

Keywords: Conjugated linoleic acid, visible spectroscopy, food lipids, photoisomerization, photosensitizer

Submitted: 27.08.2015 Reviewed: 27.10.2015 Accepted: 18.11.2015

INTRODUCTION

Conjugated linoleic acid (CLA) is an isomer of octadecadienoic acid (linoleic acid), with a conjugated double bond system; and with all possible combination of positional isomers (starting at carbon 7, 8, 9, 10, or 11), and geometrical isomers (*cis-trans, trans-cis, cis-cis* and *trans-trans*) (Ha Y. L. et al, 1987). The CLA has positive health effects, such as antimutagen (Pariza M. W. et al, 1979), anticarcinogen (Ip C. et al, 1991), decreasing atherosclerosis (Nicolosi R. J. et al, 1997; Lee K. N et al, 1994), decreasing body fat and increasing lean body mass (Chin S. F. et al, 1994; Park Y. et al, 1997) and protecting against immune induced muscle wasting (Miller C. C. et al, 1994; Cook M. E. et al, 1993). Photoisomerized CLA enriched soy oil were found to decrease serum cholesterol and serum LDL cholesterol, lowered lipid content, and decreased liver weight in obese rats (Gilbert W. et al, 2011). The CLA enriched photoisomerized soy oil contained 25% total CLA; and 70% and 25% of the total CLA were from *trans, trans* isomers and *cis, trans* isomers, respectively (Gilbert W. et al, 2011).
Main sources of CLA are dairy and meat fats, however the current intake from them is one-tenth of the needed 3 grams per day, minimum value extrapolated from animal studies, for beneficial effects (Ip C et al, 1994; Ma D. W. L. et al, 1999). Existing dietary sources may not be able to provide needed CLA. Diversifying the sources of CLA, including from CLA enriched edible oils, could increase CLA consumption. The CLA could be produced in edible oils by photoisomerization process, simply and in large quantities. The

CLA enriched edible oils were utilized for frying oils (Jain V. P. et al, 2007), for preparation of margarine (Shah U. et al, 2014) and has other possible applications in foods and for food additive. In photoisomerization, molecular iodine (I_2; a photosensitizer) absorbs light (hv) and then cleaves into iodine radicals ($I^{\cdot}$). The cleaved iodine radical abstracts hydrogen radical ($H^{\cdot}$), from the C=C double bond of lipid molecule (LH), to produce a lipid free radical ($L^{\cdot}$) (Julliard M. et al, 1987; Yettella R. R. et al, 2011). The double bond is rearranged to a more resonance stabilized form, such that conjugated isomer (LH_c) of the fatty acid is produced. In contrast, current commercial methods required initial linoleic acid free fatty acid preparation, and then alkali isomerization at high temperatures, to form CLA (Berdeaux O. et al, 1998). The CLA fatty acids were extracted and transformed into CLA enriched triacylglycerides by interesterification (Lee J. H. et al, 2003); and this process is complex.

The CLA formation by photoisomerization was influenced by edible oil type (Gammill W. et al, 2010) (soybean oil, sunflower oil, flaxseed oil, corn oil), solvent concentration (Chintareddy V. R. et al, 2012), iodine concentration (Jain V. et al, 2006; Jain V. P. et al, 2008a), time (Jain V. et al, 2006; Jain V. P. et al, 2008a), temperature (Jain V. P. et al, 2008a); tocopherols, free fatty acid content, carotenoids, peroxide value, phospholipids (Tokle T. et al, 2009), degree of refining (Jain V. P. et al, 2008b) and other factors. Some of these factors, could have have affected hydrogen abstraction from lipids by iodine, or reacted reversibly or irreversibly with iodine, thus minimizing the availability of free molecular iodine. The CLA formation was affected by trace polar components and unsaponifiable matter (Gammill W. et al, 2010; Tokle T. et al, 2009; Jain V. P. et al, 2008b). Effects of edible oils, refining (Jain V. P. et al, 2008b) and minor intrinsic and extrinsic components (Tokle T. et al, 2009), time and temperature on CLA formation were studied, however, the effects of edible oils, time, temperature and molecular groups present in edible oils on molecular iodine (essential photosensitizer in CLA formation by photo-isomerization) were not studied.

Molecular iodine may be affected by molecular groups present in edible oil's triacylglycerides, such as ester, carboxylic group, carbon-carbon double bond and carbonyl group. Inferring from earlier studies, molecular iodine also may be affected, by the molecular groups of extrinsic minor compounds, such as hydroxyl groups in moisture and peroxides; and by the molecular groups of intrinsic minor compounds, such as ether groups in waxes, hydroxyl groups in tocopherols, tocotrienols and phytosterols and others (Gammill W. et al, 2010; Jain V. et al, 2006; Tokle T. et al, 2009; Jain V. P. et al, 2008b). Molecular iodine absorbance maxima is at 520nm (Julliard M. et al., 1987). The current study explored the effects (Gammill W. et al, 2010; Jain V. et al, 2006; Jain V. P. et al, 2008b) of molecular groups influencing the molecular iodine visible light absorbance, particularly at 520nm. Molecular groups present in edible oils are ester (R-(C=O)-R), carboxylic acid -(C=O)-OH, ketone -(C=O)-, ether, -C-O-C-, hydroxyl group (-OH), and these are also present in ethyl acetate, acetic acid, acetone, diethyl ether, isopropyl alcohol, respectively. Variable number carbon-carbon non-conjugated double bonds are present in palm oil, groundnut oil and sunflower oil. In addition, role of longer hydrocarbon chains (C18) in vegetable oils when compared to short chain hydrocarbon in hexanes (C6) was studied. The effect of number of double bonds (or iodine number) on molecular-iodine visible light absorbance was also studied. Objectives of this study were to examine the influence of A) edible oils, B) time, C) temperature, and D) molecular groups; on molecular iodine absorbance in visible region.

MATERIALS AND METHODS

MATERIALS: Refined, bleached and deodorized edible oils (palm oil, groundnut oil, sunflower oil) were procured from local grocery store (Mysore, India). Granular resublimed (molecular) iodine was procured

(Nice Chemicals, Kochi, India). Acetone (Merck Speciality chemicals, Mumbai, India), diethyl ether (SD Fine Chem, Mumbai, India), isopropyl alcohol (E. Merck India Limited, Mumbai, India), ethyl acetate, acetic acid, hexanes (SD Fine Chem, Mumbai, India) were of analytical grade.

METHODS: A 25g Kg^{-1} molecular iodine was dissolved in ethyl acetate. This concentration was utilized, for preparing the subsequent iodine containing samples. A fresh iodine concentrate sample was prepared, after every 48 hours. Molecular iodine is more soluble in ethyl acetate than in non-polar solvents, such as heptane (Hildebrand J. H. et al, 1950).

Effect of edible oils on molecular iodine absorbance in visible region: A 1.25g Kg^{-1} of molecular iodine was added to either 100g Kg^{-1} groundnut oil, 100g Kg^{-1} sunflower oil, or 100g Kg^{-1} palm oil; and was dissolved in hexanes, in test tubes. Triplicate samples were prepared. A 3.75mL of the sample was placed in a quartz cuvette. Visible spectrum was collected between 415 and 580nm, with fast scan speed, 1nm slit width, 1cm path length, with UV 1800 Spectrometer (Shimadzu, Kyoto, Japan). Spectra were collected within 5 hours of sample preparation, and samples were stored at room temperature, before spectral data was collected. The data was processed with UV probe 2.42 software (Shimadzu, Kyoto, Japan). A blank spectra of samples without iodine were also collected in triplicate; and the blank spectra were averaged, and correspondingly, subtracted from the spectra of the iodine containing samples.

Effect of storage duration on molecular iodine absorbance in visible region: A 12.5g Kg^{-1} iodine in 500g Kg^{-1} groundnut oil and was stored for 0, 1, 2, 3 days at room temperature (approximately 25^{o}C). The samples were prepared in triplicate. Just before visible spectra were collected, the stored sample was further diluted with hexanes for final concentration of 2.5g Kg^{-1} iodine in sample. The blank samples without iodine were also prepared in triplicate. The blank spectra of samples without iodine were also collected; and the blank spectra were averaged, and correspondingly, subtracted from the spectra of the iodine containing samples. Spectra were collected and processed, for described previously in the "Effect of edible oils on molecular iodine absorbance in visible region", experimental section.

Effect of temperature on molecular iodine absorbance in visible region: A 1.25g Kg^{-1} iodine containing sample was prepared in groundnut oil. The samples were subjected to thermal treatment at 50$\pm$5^{o}C, 75$\pm$5^{o}C and 100$\pm$5^{o}C for 37$\pm$1.5min in an oil bath. Temperatures were selected based on possible sample temperatures during photoisomerization (Jain V. P. et al, 2008a). The samples were prepared in triplicate. The samples were further diluted to 0.125g Kg^{-1} iodine in hexanes, for spectral data collection. The samples at 25^{o}C were utilized for control. Triplicate samples for each thermal treatment, without iodine were also prepared for blanks. A blank spectra of samples without iodine were also collected; and the blank spectra were averaged, and correspondingly, subtracted from the spectra of the iodine containing samples. Spectra were collected and processed, for described previously in the "Effect of edible oils on molecular iodine absorbance in visible region", experimental section.

Effect of molecular groups on molecular iodine absorbance in visible region: A 1.25g Kg^{-1} iodine containing sample in 200g Kg^{-1} of either ethyl acetate, acetone, acetic acid, ethyl ether, isopropyl alcohol or hexanes (control) were prepared in hexanes. Blank samples without iodine were prepared. Triplicate samples were prepared for treatments and blanks. A blank spectra of samples without iodine were also collected; and the blank spectra were averaged, and correspondingly, subtracted from the spectra of the iodine containing samples. Spectra were collected and processed, for described previously in the "Effect of edible oils on molecular iodine absorbance in visible region", experimental section.

All the test tubes containing samples were

covered tightly with aluminium foils on the top to minimize evaporation losses; and also wrapped with aluminium foil to prevent excessive light induced changes. All measurements were in weight/weight basis. All the solvents utilized were analytical grade. Anova, Tukey HSD all pairwise mean comparisons at 95% significance level ($p<0.05$) and simple linear regression were performed with statistical software (SYSTAT software, San Jose, California, USA).

3.RESULTS AND DISCUSSION

Effect of edible oils on molecular iodine absorbance in visible region: The molecular iodine absorbances at 520nm in palm oil, groundnut oil and sunflower oil were 0.79, 0.71 and 0.46, respectively (Table 1). Iodine visible absorbance in palm oil and groundnut oil were not significantly different ($p>0.05$); however sunflower oil absorbance was significantly less than ($p<0.05$) palm oil and groundnut oil (Table 1). This could be due to high degree of unsaturation in sunflower oil. The iodine values of palm oil, groundnut oil and sunflower oil were 50-55, 86-107 and 118-141 (Codex standard for named vegetable oils, CODEX STAN 210, 1999), respectively; and approximate calculated ratio of polyunsaturated fatty acids (linoleic acid) to monounsaturated fatty acids (oleic acid) are 0.27, 0.53 and 2.29, respectively (Codex standard for named vegetable oils, CODEX STAN 210, 1999). Linoleic acid (predominantly *cis* 9, *cis* 12 C18:2) is present in edible oils at following proportions: 750-832g Kg^{-1} in safflower oil, 480-590g Kg^{-1} in soy oil, 483-740g Kg^{-1} in sunflower oil, 120-430g Kg^{-1} in groundnut oil, 90-120g Kg^{-1} in palm oil, 100-240gKg^{-1} in mustard oil, 150-300g Kg^{-1} in rapeseed oil (low erucic acid), 369-479g Kg^{-1} in sesame oil, 35-210g Kg^{-1} in olive oil, 10-25g Kg^{-1} in coconut oil, 20g Kg^{-1} in butter, 290-410g Kg^{-1} in rice bran oil, 467-582g Kg^{-1} in cotton seed oil, 560g Kg^{-1} in wheat germ oil, 340-656g Kg^{-1} in corn oil (Codex standard for named vegetable oils, CODEX STAN 210, 1999).

Table 1: Absorbance (520nm) of molecular iodine dissolved in palm oil, groundnut oil and sunflower oil.

Edible Oil	**Absorbance at 520nm (n=3); Average $\pm$ standard deviation**
Palm Oil	0.790 ± 0.060^{a}
Groundnut Oil	0.710 ± 0.098^{a}
Sunflower Oil	0.460 ± 0.077^{b}

* Molecular iodine (1.25g Kg^{-1}) dissolved in 48.75g Kg^{-1} ethyl acetate, 100g Kg^{-1} edible oil, and 850g Kg^{-1} hexanes. All concentrations were in w/w basis. Absorbances of blanks (without iodine) were subtracted from corresponding sample absorbances. All edibles oils utilized were refined. Letters with different superscript were significantly different at 95% significance level ($p<0.05$).

The lower absorbance in sunflower oil could also be due to higher wax content and unsaponifiable matter content. Wax content in sunflower oil is 0.1-0.4g Kg^{-1} (Davidson H. F. et al, 1996), and wax content was not reported for groundnut oil and palm oil, suggesting negligible amounts. Sunflower oil produced negligible amounts of CLA when compared to soy oil, by photoisomerization, despite having similar fatty acid profile, probably due to higher wax content (Gammill W. et al, 2010). Unsaponifiable matter content in groundnut oil, palm oil, and sunflower oil are 5.1g Kg^{-1} (Basiron Y., 1996), 4g Kg^{-1} (Young C., 1996), 15g Kg^{-1} (Davidson H. F. et al, 1996), respectively. The other constituents of unsaponifiable matter, include carotenoids, phytosterols, tocopherols, tocotrienols and other colouring matter. Total phytosterol contents for palm oil, groundnut oil and sunflower oil were 0.3-0.7g Kg^{-1}, 0.9-2.9g Kg^{-1}, 2.4-5g Kg^{-1}, respectively; and total tocopherol and tocotrienol contents were 0.15-1.5g Kg^{-1}, 0.17-1.3g Kg^{-1} and 0.44-1.55g Kg^{-1}, respectively (Codex standard for named vegetable oils, CODEX STAN 210, 1999). Carotenoid content in palm oils could be from 0.5-2g Kg^{-1} (Young C., 1996).

Groundnut oil with linoleic acid content could be more suitable for CLA formation with photoisomerization. However, CLA is yet to be produced in many linoleic acid containing edible oils, including groundnut oil. Currently,

CLA was produced in laboratory scale in soy oil (Gammill W. et al, 2010; Chintareddy V. R. et al., 2012; Jain V. et al; 2006; Jain V. P. et al 2008; Tokle T. et al., 2009; Jain V. P. et al 2008; Gangidi R. R. et al, 2004), corn oil (Gammill W. et al, 2010; Chintareddy V. R. et al, 2012), safflower oil and flax seed oil (Gammill W. et al, 2010). The other linoleic acid containing edible oils that could be possible sources of CLA, include groundnut oil, cottonseed oil, sesame oil, mustard oil, rapeseed oil, niger seed oil, palm oil, rice bran oil, olive oil, wheat germ oil, and other edible oils (Codex standard for named vegetable oils, CODEX STAN 210, 1999).

The observed colour of iodine containing palm oil, groundnut oil and sunflower oil samples were red, red and yellow, respectively. The iodine's maximum absorbance in palm oil, groundnut oil and sunflower oil were at 504, 505 and 504nm, respectively; and their corresponding maxima values were 0.8312, 0.7447 and 0.4822, respectively (Figure 1).

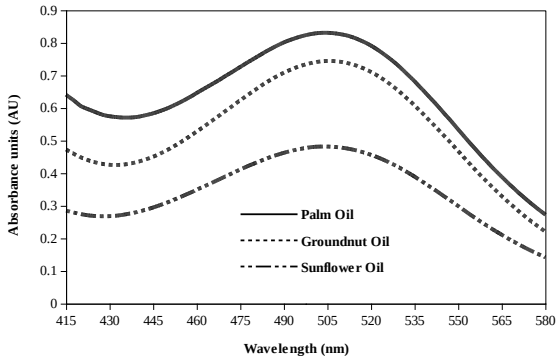


Figure 1 – Visible spectrum of (415-580nm) of molecular iodine dissolved in palm oil, ground nut oil and sunflower oil.

* Molecular iodine (1.25g/Kg) is dissolved in 48.75g/Kg ethyl acetate, 100g/Kg edible oil, an 850g/Kg hexanes. All concentrations were in w/w basis. Absorbances of blanks (without iodine) were subtracted from corresponding iodine containing sample absorbances.

The absorbance maxima blue shifted, with respect to molecular iodine absorbance in hexane at 514nm. Absorbance at 520nm in hexanes (2.449), without reactive unsaturation, long hydrocarbon chains and other unsaponifiable matter, was at least 3 times higher than maximum absorbance in groundnut oil. Average absorbance values at 520nm (n=3) for palm oil, groundnut oil, and sunflower oil blanks were 0.048 $\pm$ 0.002, 0.003 $\pm$ 0.0003, 0.005 $\pm$ 0.007, respectively; and for hexanes (without oil or iodine) was -0.003 $\pm$ 0.004 (data not shown).

In the earlier studies, it was found that 99% CLA formed, from linoleic acid, in 110g Kg^{-1} soy oil dissolved in hexanes, in less than 4 hours (Chintareddy V. R. et al, 2012). However, lower viscosity, solvent content and low concentration of edible oil could have allowed greater light penetration, allowing higher yields of CLA (Gangidi R. R. et al, 2014), when compared to photoisomerization, in highly viscous vegetable oils (1000g/1Kg) in other studies (Gammill W. et al, 2010; Jain V. et al 2006; Jain V. P. et al 2008a; Tokle T. et al 2009; JainV. P. et al 2008b; Gangidi R. R. et al., 2014). Additionally, role of containers may have been overlooked, for stainless steel vessels (Chintareddy V. R. et al, 2012) were utilized for photoisomerization in the study, and glassware for photoisomerization in other studies (Jain V. et al, 2006; Jain V. P. et al, 2008a; Tokle T. et al, 2009; Jain V. P. et al, 2008b; Gangidi R. R. et al, 2014). Further studies on molecular iodine absorbance, in visible region, for other available oils needs to be conducted. Also the effects of concentration of edible oil in solvent, and the effects of metal containers on CLA formation by photoisomerization, could be explored in future studies.

Solubility of iodine in edible oils is low and non-uniform. However, iodine readily dissolves in slightly-less non-polar solvents, such as ethyl alcohol (100g Kg^{-1}). Iodine's solubility in ethyl alcohol is 10 to 20 times higher than that of solubility in non-polar solvents, at room temperature (Hildebrand J. H et al, 1950) This could be due to dielectric constant (an indication of non-polarity) of iodine, being approximately 11, and very similar to that of dielectric constant of ethyl acetate, which is 7; and dielectric constant of edible oils is approximately 3. Ethyl acetate was selected for a solvent, for it has similar molecular groups found in edible oils; and a

25g Kg^{-1} iodine concentrtion, is lower than possible maximum solubility of iodine in ethyl acetate.

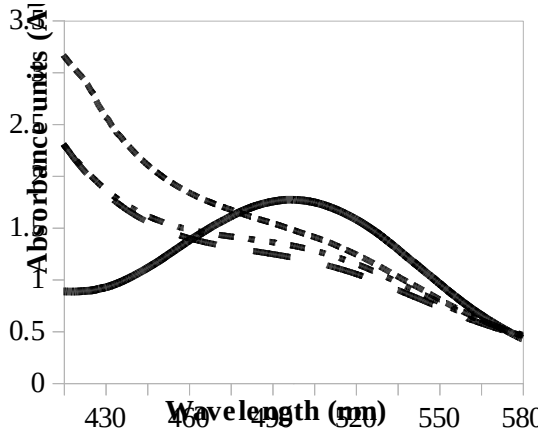
Effect of time on molecular iodine absorbance in visible region: The spectra of iodine in groundnut oil samples stored day 1, day 2 and day 3 showed varied spectral characteristics when compared to day 0 sample spectra (Figure 2). The absorbances at 520nm of day 0, day 1, day 2 and day 3 were 1.579, 1.241, 1.148 and 1.051, respectively (Table 2). The values were higher than that of earlier groundnut oils, for the iodine concentration in time based data was 2.5g Kg^{-1} (Table 2), and in earlier edible oils data (Table 1) was 1.25g Kg^{-1}. There was a significant decrease in 520nm iodine absorbance from day 0 to day 1, 2 and 3 ($p<0.05$) (Table 2). However, there was no significant difference between day 1, day 2 and day 3 absorbance, for the room temperature stored samples. This could be due to reversible or irreversible binding of the iodine to molecular groups present in groundnut oil, such as phytosterols (4g Kg^{-1}) (Codex standard for named vegetable oils, CODEX STAN 210, 1999), total tocopherols and tocotrienol contents (0.17-1.3g Kg^{-1}) and total sterols of 0.9-2.9g Kg^{-1} (Codex standard for named vegetable oils, CODEX STAN 210, 1999), with time.

Table 2: Effect of storage duration on absorbance (520nm) of molecular iodine dissolved in groundnut oil.

Duration	**Absorbance at 520nm (n=3); Average ± standard deviation**
Day 0	1.579 ± 0.214^{a}
Day 1	1.241 ± 0.107^{b}
Day 2	1.148 ± 0.018^{b}
Day 3	1.051 ± 0.023^{b}

* A 2.5g Kg^{-1} molecular iodine dissolved in 97.5g Kg^{-1} ethyl acetate, 100g Kg^{-1} groundnut oil, and 800g Kg^{-1} hexanes. All concentrations were in w/w basis. Absorbances of blanks (without iodine) were subtracted from corresponding sample absorbances. Letters with different superscript were significantly different at 95% significance level ($p<0.05$).

Figure 2 - Effect of time on visible spectrum (415-580nm) absorbance of molecular iodine dissolved in groundnut oil.



* A 2.5g/Kg molecular iodine dissolved in 97.5g/Kg ethyl acetate, 100g/Kg groundnut oil and 800g/Kg of hexanes. All concentrations were in (w/w) basis. Absorbances of blanks (without iodine) were subtracted from corresponding iodine containing sample absorbances.

Further changes after day 3 could be possible, however photoisomerization could be prohibitively expensive with the increase in number of days. The photoisomerization may further reduce the availability of molecular iodine, however it may have to be studied in future studies. In addition, the reversible and irreversible binding of the iodine to various molecular groups needs to be further explored for possibilities of reuse of extracted iodine for photoisomerization. It was found that iodine can be regenerated during photoconjugation (Julliard M. et al, 1987), for the concentration of CLA formed was much higher than the concentration of added iodine (Chintareddy V. R. et al, 2014; Jain V. et al, 2006; Jain V. P. et al, 2008a).

The molecular iodine 520nm-absorbance reduced with time, suggesting possibility of less molecular iodine availability with time; and this may also explain the gradual reduction in CLA formation with time (Jain V. et al, 2006; Jain V. P. et al, 2008a; Gangidi R. R. et al, 2014) rather than exclusively due to equilibrium reached between CLA and linoleic acid. Higher inclination of slopes of CLA formation, within one day of photoisomerization, were reported in previous literature (Jain V. et al, 2006; Jain V. P. et al, 2008a; Gangidi R. R. et al, 2004), when compared to slopes after day 1. The reduction

in free molecular iodine absorbance, from day 1 in groundnut oil, could explain the reduced slopes from day 1 or 2, during earlier photoisomerization studies (Jain V. et al, 2006; Jain V. P. et al, 2008a; Gangidi R. R. et al, 2014). By removing added-iodine (Yettella R. R. et al, 2013) and the formed trace polar compounds during photoisomerization, and then re-adding fresh iodine, there may be a possibility of higher CLA formation in edible oils. However, addition of fresh iodine to already photoisomerized oil, marginally increased the CLA content during photo-isomerization (Yettella R. R. et al, 2013). In addition, iodine and CLA were found to be proxidants. CLA enriched soy oil's stability was lower in the presence of residual iodine (Yettella R. R. et al, 2012), and for this reason lower photoisomerization times could be preferable, before the edible oil is re-processed for removal of peroxides, and bound and free iodine. Natural day light could possibly be utilized to form CLA by photoisomerization, and potentially residual iodine recovered and reused, however such a concept needs to the explored, in the future studies.

The spectral maxima of day 1 to day 3 shifts to lower wavelengths when compared to day 0. The maximum absorbance of day 0, day 1, day 2 and day 3 was at 495nm, 415nm, 415nm and 415nm, respectively; and the corresponding values were 1.765, 3.160, 2.301 and 2.302, respectively (Figure 2). Higher precision of day 3 absorbance (0.023) when compared with day 0 absorbance (0.214), could be due to lower absorbance values for day 3, when compared with absorbance values for day 0 (Table 2).

The observed colour of day 0 oil was red; and day 1, day 2 and day 3 edible oils was pale red in colour. This could probably be due to iodine reversibly or irreversibly binding to various molecular groups, present in edible oils. This suggests that the colour may not be a very good indicator, for measuring the availability of free molecular iodine content. Very long term storage studies may have to be conducted in dark, and under high intensity light, found in lamps utilized for photoisomerization studies, to determine the free iodine availability with time. The values of day 0, day 1, day 2, day 3 blanks, at 520 nm, were 0.0390 $\pm$ 0.019, 0.070 $\pm$ 0.019, 0.024 $\pm$ 0.005, 0.030 $\pm$ 0.006, respectively.

Effect of temperature on molecular iodine absorbance in visible region: For 25^{O}C, 50^{O}C, 75^{O}C and 100^{O}C treated samples, molecular-iodine absorbance maxima at 520nm, was 0.163, 0.058, 0.032 and -0.021, respectively (Table 3). The absorbance values were lower in this study, when compared to previous studies, due to lower iodine content (0.0125g Kg^{-1}) in samples that had either 0.25g Kg^{-1} (Table 2, Figure 2) or 0.125g Kg^{-1} (Table 1, Figure 1) iodine. Original groundnut oil was observed to have light yellow in colour. The observed colours for iodine containing samples treated for 100^{O}C was dark yellow, for 75^{O}C and 50^{O}C were light red and for 25^{O}C samples was red colour. The heat treatment significantly reduced ($p<0.05$) iodine molecular absorbance at 50^{O}C, 75^{O}C, 100^{O}C when compared with 25^{O}C. For 25^{O}C, 50^{O}C, 75^{O}C and 100^{O}C, the absorbance maxima was at 512nm, 515nm, 514nm and 415nm, respectively; and their respective absorbance values were 0.164, 0.059, 0.032 and -0.004, respectively (Figure 3). The negative values for 100^{O}C could be due to destruction of light absorbing chromophore components in fresh edible oil, and also due to reaction of iodine with edible oil. Higher iodine content at 25^{O}C also explains previous studies (Jain V. P. et al, 2008a) optimum CLA formation between 25^{O}C and 50^{O}C, probably due to negligible loss of free molecular iodine, and also could be due to higher rates of brownian-interaction between iodine, light and linoleic acid. Blank values of 25^{O}C, 50^{O}C, 75^{O}C and 100^{O}C were 0.018 $\pm$ 0.012, 0.013 $\pm$ 0.0002, 0.008 $\pm$ 0.005 and 0.019 $\pm$ 0.024, respectively (Data not shown).

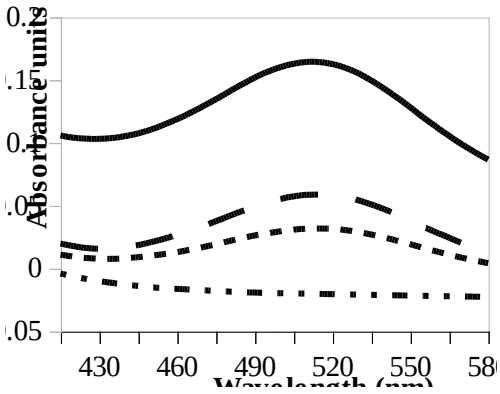


Figure 3 - Effect of temperature on absorbance (415-580nm) of molecular iodine dissolved in groundnut oil.
* A 1.25g/Kg iodine dissolved in 48.5g/Kg ethyl acetate and 950g/Kg ground nut oil. The samples were treated at 25°C, 50°C, 75°C and 100°C for 37±1.5 minutes. All concentrations were in w/w basis. The treated samples (100g/Kg) were dissolved in 900g/Kg hexanes prior to spectral data collection. Absorbances of blanks (without iodine) were subtracted from corresponding iodine containing sample absorbances.

Table 3: Effect of temperature on absorbance (520nm) of molecular iodine dissolved in groundnut oil.

Temperature	**Absorbance at 520nm (n = 3); Average ± standard deviation**
25°C	0.163 ± 0.065[a]
50°C	0.058 ± 0.009[b]
75°C	0.032 ± 0.002[b]
100°C	-0.021 ± 0.012[b]

*A 1.25g Kg^{-1} iodine dissolved in 48.75g Kg^{-1} ethyl acetate, and 950g Kg^{-1} groundnut oil. The samples were treated at 25°C, 50°C, 75°C and 100°C for 37±1.5minutes. All concentrations were in w/w basis. The treated samples (100g Kg^{-1}) were dissolved in 900g Kg^{-1} hexanes, prior to spectral data collection. Absorbances of blanks (without iodine) were subtracted from corresponding sample absorbances. Letters with different superscript were significantly different at 95% significance level ($p<0.05$).

Effect of iodine content on molecular iodine absorbance in visible region: The 0.25g Kg^{-1} (Table 2), 0.125g Kg^{-1} (Table 1) and 0.0125g Kg^{-1} (Table 3) iodine in 95-100g Kg^{-1} groundnut oil samples in hexanes, showed absorbance of 1.579 ± 0.214, 0.710 ± 0.098 and 0.163 ± 0.065 respectively, at 520nm. Considering effects due to solvent composition to be minimal, there was a linear increase in absorbance ($p<0.05$; R^2 adjusted = 0.95; slope>0 at $p<0.05$), with increase in iodine concentration. This data further shows decrease in absorbance, in sunflower oil (Table 1), when compared to absorbance in groundnut oil; day 2 and day 3 samples when compared to day 0 samples (Table 2); 75°C and 100°C treated samples when compared to 25°C (Table 3), were due to decrease in free molecular iodine. Loss of molecular iodine could be due to many factors, importantly, the reaction with C=C double bonds. It was thought that free molecular iodine reacts with double bonds (Rossel J. B., 1986), and in addition, unsaponifiable matter could also have reduced availability of free molecular iodine content. There may be a possibility of measuring iodine content by visible absorbance, for freshly prepared iodine containing edible oils. At least 1.25g Kg^{-1} of iodine in groundnut oil dissolved in hexanes, could be detected ($p<0.05$) with visible spectroscopy, with further possibilities of even lower iodine content measurement. However, visible absorbance methods, for stored samples may not be feasible, for similar iodine concentration, variable absorbances were found with time (Table 2 and Figure 2).

Effect of molecular groups on molecular iodine absorbance in visible region: Acetone's carbonyl (C=O) and isopropyl alcohol's hydroxyl (OH) group were most reactive, and decreased absorbance of molecular iodine, at 520nm (Figure 4). However, carbonyl group of acetic acid and ethyl acetate were less degradative than carbonyl group of acetone; and hydroxyl group of acetic acid was less degradative, suggesting triacylglycerides and free fatty acids in edible oils, are least likely to affect molecular iodine concentration. However, hydroxyl group present in moisture, lipid peroxides (hydroperoxides), tocopherols and phytosterols could most likely affect the molecular iodine concentration, and so, likely affect molecular iodine's availability for CLA formation.

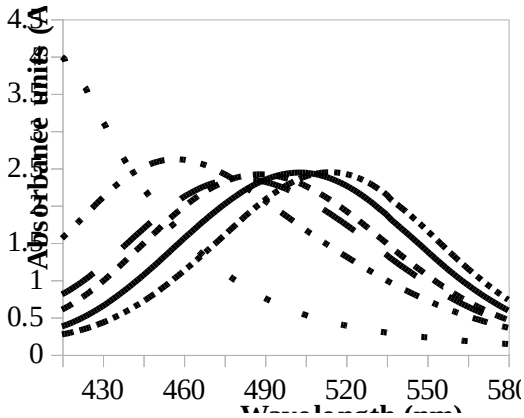


Figure 4 - Effect of molecular groups in acetone (C=O), isopropyl alcohol (O-H), ethyl ether (C-O-C), ethyl acetate (COOR), acetic acid (COOH) and hexanes (CH) on molecular iodine absorbance in visible region (415-580nm).
* A 1.25g/Kg molecular iodine was dissolved in 48.75g/Kg ethyl acetate, 200g/kg sample solvent and 750g/Kg hexanes. Absorbances of blanks (without iodine) were subtracted from corresponding iodine containing sample absorbances.

The acetone, isopropyl alcohol, diethyl ether, ethyl acetate, acetic acid and hexanes absorbances at 520nm were 0.396, 1.316, 1.741, 1.939, 2.265 and 2.417, respectively (Table 4). Iodine in hexanes showed characterstic maxima at approximately 514nm, and had the highest absorbance of all the solvents. The hexanes had minimal of reactive molecular groups, and possibly greater likelyhood of CLA formation, if edible oil were dissolved in hexanes (Figure 4). Iodine absorbance maxima was at 520nm (Julliard M. et al, 1987). In previous published studies, higher formation of CLA was found, when edible oil (110g Kg^{-1}) in hexanes was photoisomerized in the presence of iodine (Chintareddy V. R. et al, 2012), when compared with edible oils (1000g Kg^{-1}) were utilized directly (without solvents) (Jain V. et al, 2006; Jain V. P. et al, 2008a; Tokle T. et al, 2009; Jain V. P. et al 2008b; Gangidi R. R. et al, 2004). In addition, n-hexane, an isomer of hexane, is an approved solvent for edible oil solvent extraction, and maximum allowed residual limit is 0.005g Kg^{-1} (Food Safety and Standards (Food Products Standards and Food Additives) Regulations, Food Safety and Standards Authority of India, 2011), in refined edible oils. n-Hexane may be used for a solvent in CLA formation by photoisomerization. Other solvents are not permitted in edible oil extraction from oil seeds.

Table 4: Effect of molecular groups on molecular iodine absorbance (520nm).

Sample solvent (molecular groups)	**Absorbance at 520nm (n=3); Average ± standard deviation**
Acetone (-C=O)	0.396 ± 0.046^{a}
Isopropyl alcohol (-C-OH)	1.316 ± 0.017^{b}
Diethylether (-C-O-C-)	1.741 ± 0.017^{c}
Ethyl Acetate (-COOR)	1.939 ± 0.001^{d}
Acetic acid (-COOH)	2.265 ± 0.012^{e}
Hexanes (-CH)	2.417 ± 0.011^{f}

* A 1.25g Kg^{-1} molecular iodine dissolved in 48.75g Kg^{-1} ethyl acetate, 200g Kg^{-1} sample solvent, and 750g Kg^{-1} hexanes. Absorbances of blanks (without iodine) were subtracted from corresponding sample absorbances. Letters with different superscript were significantly different at 95% significance level ($p<0.05$).

Molecular weight of acetone, acetic acid, ethyl acetate, diethyl ether, isopropyl alcohol and hexanes were 58, 60, 88, 74, 60, 86, respectively; and their densities 0.79, 1.048, 0.902, 0.714, 0.785, 0.659, respectively; and molal concentration (g Kg^{-1}) in samples were is 3.44, 3.33, 2.27, 2.70, 3.33 and 2.32, respectively, suggesting similar number of molecules for a unit weight for all the solvents. The molal concentration in 200g Kg^{-1} sample was highest for acetone, and lowest for ethyl acetate.

The observed colour in glass test tubes for molecular iodine (0.125%) dissolved in 20% solvents of acetone, isopropyl alcohol, diethyl ether, ethyl acetate, acetic acid and hexanes were dark yellow, yellow, yellowish-red, orange-red, light-red and light-red, respectively. Iodine maximum absorbance in acetone and isopropyl alcohol was marginally shifted to lower wavelengths. Ethyl acetate, acetone, acetic acid, diethyl ether, isopropyl alcohol and hexane had maxima at 488nm,

473, 503, 481, 456 and 514nm, respectively; and average (n=3) absorbance at their respective maxima were 2.488, 1.156, 2.439, 2.356, 2.618 and 2.443, respectively (Figure 4). Blank values at 520nm for acetic acid, acetone, isopropyl alcohol, diethyl ether and ethyl acetate were 0.010 $\pm$ 0.006, 0.008 $\pm$ 0.005, 0.0002 $\pm$ 0.003, -0.002 $\pm$ 0.002, 0.004 $\pm$ 0.002, respectively (Data not shown).

CONCLUSIONS

Free molecular iodine was available at a higher extent in groundnut oil and palm oil when compared with sunflower oil, at less than or equal to day 1 storage duration, at 25^{O}C, and in the absence of hydroxyl molecular groups. Further studies could be conducted on long term effects of time and temperature, on iodine absorbance under dark and photo-isomerization conditions, and in other available edible oils.

Acknowledgements

First author is very grateful for Research Associateship from Council of Scientific and Industrial Research (CSIR), Human Resource Development Group (HRDG), New Delhi, India. We are thankful to Director, CSIR Central Food Technological Research Institute (CFTRI, Mysore, India) for his encouragement and support.

REFERENCES

1. Ha YL, Grimm NK and Pariza MW, *Anticarcinogens from fried ground meat: heat-altered derivatives of linoleic acid*, Carcinogenesis, 1987, **8**: 1881–1887.
2. Pariza MW, Ashoor SH, Chu FS and Lund DB, *Effects of temperature and time on mutagen formation in pan fried hamburger,* Cancer Lett, 1979, **7**: 63-69.
3. Ip C, Chin SF, Scimeca JA and Pariza MW, *Mammary cancer prevention by conjugated dienoic derivative of linoleic acid*, Cancer Res, 1991, **51**: 6118-6124.
4. Nicolosi RJ, Rogers EJ, Kritchvesky D, Scimeca JA and Huth PJ, *Dietary conjugated linoleic acid reduces plasma lipoprotein and early aortic atheroscelorosis in hypercholesterolemic hamsters*, Artery, 1997, **22**: 266-277.
5. Lee KN, Kritchevsky D and Pariza MW, *Conjugated linoleic acid and atherosclerosis in rabbits*, Atherosclerosis, 1994, **108**: 19–25.
6. Chin SF, Storkson JM, Albright KJ, Cook ME and Pariza MW, *Conjugated linoleic acid is a growth factor for rats as shown by enhanced weight gain and improved feed efficiency*, J Nutr, 1994, **124**: 2344-2349.
7. Park Y, Albright KJ, Liu W, Storkson JM, Cook ME and Pariza MW, *Effects of conjugated linoleic acid on the body composition in mice*, Lipids, 1997, **32**: 853-858.
8. Miller CC, Park Y, Pariza MW and Cook ME, *Feeding conjugated linoleic acid to animal partially overcomes catabolic responses due to endotoxin injection*, Biochem Biophys Res Commun, 1994, **198**: 1107-1112.
9. Cook ME, Miller CC, Park Y and Pariza MW, *Immune modulation by altered nutrient metabolism: Nutritional control of immune induced growth depression*, Poult Sci, 1993, **72**: 1301-1305.
10. Gilbert W, Gadang V, Proctor A, Jain V and Devareddy L, *Trans-trans conjugated linoleic acid enriched soybean oil reduces fatty liver and lowers serum cholesterol in obese zucker rats*, Lipids, 2011, **46**: 961-968.
11. Ip C, Singh M, Thompson HJ and Scimeca JA, *Conjugated linoleic acid suppresses mammary carcinogenesis and proliferation activity of the mammary gland in the rat*, Cancer Res, 1994, **54**: 1212–1215.
12. Ma DWL, Wierzbicki AA, Field CJ and Clandinin MT, *Conjugated linoleic acid in canadian dairy and meat products*, J Agric Food Chem, 1999, **47**: 1956–1960.
13. Jain VP and Proctor A, *Production of conjugated linoleic acid-rich potato chips*, J Food Sci, 2007, **72**: 75-78.
14. Shah U, Patel AR, Walle DV, Rajarethinem PS, Proctor A and Dewettinick K, *CLA-rich soy oil margarine production and characterization*, J Am Oil Chem Soc, 2014, **91**: 309-316.
15. Julliard M, Luciani A, Chevalier JL, Cecchi G and Ucciani E, *Photosensitized*

conjugation of methyl 9,12-octadecadienoate, J PhotoChem, 1987, **38**: 345–355.
16. Yettella RR, Henbest B and Proctor A, *Effect of antioxidants on soy oil conjugated linoleic acid production and its oxidative stability,* J Agric Food Chem, 2011, **59**: 7377–7384.
17. Shah U, Proctor A, Lay J and Moon K, *Determination of CLA trans, trans positional isomerism in CLA-rich soy oil by GC-MS and silver ion chromatography,* J Am Oil Chem Soc, 2012 **89**: 979-985.
18. Berdeaux O, Voinot L, Angioni E, Juaneda P and Sebedio JL, *A simple method of preparation of methyl esters trans 10, cis 12 CLA and cis 9, trans 11 octadecadienoates from methyl linoleate,* J Am Oil Chem Soc, 1998, **75**: 1749-1755.
19. Lee JH, Kim MR, Kim HR, Kim LH, and Lee KT, *Characterization of lipase-catalyzed structured lipids from selected vegetable oils with conjugated linoleic acid: their oxidative stability,* J Food Sci, 2003, **68**: 1653–1658.
20. Gammill W, Proctor A and Jain VP, *A comparative study of high linoleic acid vegetable oils for the production of conjugated linoleic acid,* J Ag Food Chem, 2010, **58**: 2952-2957.
21. Chintareddy VR, Oshel RE, Doll KM, Yu Z, Wu W, Zhang G and Verkade JG, *Investigation of conjugated linoleic soy oil as drying oils and CLA sources,* J Am Oil Chem Soc, 2012, **89**: 1749-1762.
22. Jain V and Proctor A, *Photocatalytic production and processing of conjugated linoleic rich soy oil,* J Ag Food Chem, 2006, **54**: 5590-5596.
23. Jain VP and Proctor A, *Pilot scale production of conjugated linoleic acid-rich soy oil by photo-irradiation,* J Food Sci, 2008a, **73**: E183-192.
24. Tokle T, Jain V and Proctor A, *Effect of minor oil constituents on soy oil conjugated linoleic acid production,* J Ag Food Chem, 2009, **57**: 8989–8997 (2009).
25. Jain VP, Tokle T, Kelkar S and Proctor A, *Effect of degree of processing on soy oil conjugated linoleic acid yields,* J Ag Food Chem, 2008b, **56**: 8174–8178.
26. Hildebrand JH, Benesi HA and Mower LM, *Solubility of iodine in ethyl alcohol, ethyl ether, mesitylene, p-xylene, 2,2-dimethyl butane, cyclohexane and perfluor-n-heptane,* J Am Chem Soc, 1950 **72**:1017–1020.
27. Codex Standard for named Vegetable Oils, CODEX STAN **210**, 1999, pp. 1-15.
28. Davidson HF, Campbell EJ, Bell RJ and Pritchard RA, Sunflower oil, chapter 12, in Edible oil and fat products: oil and oilseeds, volume 2, Bailey's industrial oil and fat products (5th edition), edited by Hui YH, published by John Wiley & sons, Inc., New York, 1996, pp. 610.
29. Basiron Y, Palm Oil, chapter 5, in Edible oil and fat products: oil and oilseeds, volume 2, Bailey's industrial oil and fat products, 5th edition, edited by Hui YH, published by John Wiley & sons, Inc., New York, 1996, pp. 281.
30. Young C, Peanut oil, chapter 7, in Edible oil and fat products: oil and oilseeds, volume 2, Bailey's industrial oil and fat products, 5th edition, edited by Hui YH, published by John Wiley & sons, Inc., New York, 1996, pp. 383.
31. Gangidi RR and Proctor A, *Photochemical production of conjugated linoleic acid from soy oil. Lipids*, 2004, **39**: 577-582.
32. Gangidi RR and Lokesh BR, *Conjugated linoleic acid formation in edible oils by photoisomerization – A review,* J Food Sci, 2014, **79**: R781-R785.
33. Yettella RR, Henbest B and Proctor A, *Effect of UV processing treatment on soy oil conjugated linoleic acid yields and tocopherol stability,* J Am Oil Chem Soc, 2013, **90**: 863-870.
34. Yettella RR, Castrodale C and Proctor A, *Effect of added conjugated linoleic acid and iodine concentrations on conjugated linoleic acid-rich soy oil oxidative stability,* J Am Oil Chem Soc, 2012, **89**: 1939-1941.
35. Rossell JB, Classical analysis of oils and fats (chapter 1), In: Analysis of oils and fats. Edited by Hamilton RJ and Rossell JB, Published by Elsevier applied science publishers Ltd, Essex, England, 1986, pp. 23.