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Effect of heating on oxidative stability of palm and rice bran mixture oils

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Abstract: The oxidative stability of Palm oil (PO) is studied and compared with the mixture of rice bran oil (RBO) at different ratios using rheological, Fourier transform infrared (FTIR) spectroscopy and *in-vitro* analysis. The mixtures are subjected to repeated five cycles of heating to frying temperature and the changes of viscosity at every time of heating are observed. The structural changes in the chemical composition of the oil on heating are analysed using FTIR. The increase in the percentage of transmission are observed at 2854 cm^{-1} (CH_2 stretching) and decrease in the formation of carbonyl compounds(aldehyde, ketone, ester etc.,) are observed at 1100 to 1700cm^{-1}. The free radicals formed in the oils on oxidation are studied by the % of inhibition of peroxides with the concentration of oils using ABTS and DPPH radical scavenging assay. It has been observed the % of inhibition decreases with increase in the concentration of RBO with PO. The study gives a result about the mixture of PO with RBO to use in snacks industry with oxidative stability, increase nutritive value and highly economical.

Keywords*:* Rheology, RBO, PO, FTIR, ABTS and DPPH.

Introduction

India accounts for 10% of the world's edible oil consumption of that 80% of the total oilseeds grown in India. The total availability of vegetable oils is about 7.5MT, which is not adequate to meet the domestic demand. The processing industry and the solvent extractors suffer from pervasive sickness because of insufficient and high-cost of raw material and uncompetitive processing makes oils and oil mills and affects export prospects, which are further eroded by invasion of low priced imported oils. According to PFA (Prevention of Food Adulteration Act) 4th Amendment Rules 1992, blending of any two vegetable oils (wherein the component oil used in the admixture is not less than 20%) has been permitted so as to increase the overall availability of oils of consumers' choice. Hence blending of vegetable oils as per the combinations presented in order is used to ensure optimal health benefits. "Blended edible vegetable oil" means an admixture of any two edible vegetable oils of the quality and the proportion. It is beneficial to consume a mix of oils to maintain a balance between the fatty acids, antioxidants, minerals and vitamins. As using a combination of two oils may not be a practical thing to do but today a number of blended oils are available in the market that are the best buys and are suitable for frying.

Viscosity is one of the most important parameters required to establish the superiority and permanence of food system in food industry [1, 2]. Viscosity measurement and control has a great importance in food industry and acute knowledge of viscosity is necessary for various food industrial processes to find the quality of them [1, 3, 4]. The resistance occurs between one parts of the fluid that move relative to another one is called viscosity.

Hence viscosity is closely correlated with the unsaturated and the chain length of the fatty acids that constitute the triglycerides [5-7].

Over the past 20 years in the field of food research, mid- FTIR spectroscopy is used as effective analytical tool facilitating particularly the studies of lipids [8 -10]. Moreover the spectrum shows the difference in the transmission band, varying intensity for the vibrations of functional group in the composition of oil.

Rice bran oil contains unsaturated fatty acids 47% of it is monounsaturated (oleic acid), 30% of polyunsaturated (linoleic acid) and 21% of saturated fatty acids. The oil contains antioxidant oryzanol that may offer some health benefits to prevent coronary heart diseases; phytosterols, compounds believed to help lower cholesterol absorption; and relatively high amounts of vitamin E [11]. Palm oil contains fatty acids like saturated palmitic acid 44.3%, monounsaturated oleic acid 38.7% and polyunsaturated linoleic acid 10.5%, antioxidants vitamin E especially tocotrienols, β- Carotene, Vitamin K and magnesium [12]. Palm oil which is a popular vegetable oil in India is relatively cheaper is blended with high potent antioxidants rice bran oil which is cost-effective compared to other type of mixture. Blending of these oils may be able to increase quality and stability, including heat stability: primary and secondary oxidation and resist the formation of polymers and polar compounds.

In the present work, the variations of the viscosities of the mixture of edible oils (palm oil and rice bran oil) at different ratios are studied. The structural change in the mixture of oils after heating it to frying temperature is studied using FTIR analysis. The formation of peroxides in the heated mixtures of palm oil and rice bran oil is studied using ABTS and DPPH radical scavenging assay.

Materials and Methods

Edible oils palm oil and rice bran oil are purchased in local commerce. Rice bran oil and palm oil blended to the ratios 3:1 (RPO1), 1:1 (RPO2) and 1:3 (RPO3) to form 100ml. The oil is heated to 190°C to study the effect of mixture of oils on heating for the duration (0.5, 1, 1.5, 2 and 2.5hrs.). Oils are periodically removed from heating and cooled to room temperature after each heating desired time of heating

Viscosity measurement

Kinematic viscosity (ν) of the binary mixture is measured using Redwood viscometer which is based on the principle of laminar flow through capillary tube. Redwood time t in second is measured in the collection of 50cc of the sample. The kinematic viscosity is calculated from the following relation:

$(\nu) = (A^* t - B/t) \times 10^{-4}\ m^2/s$ …… (1)

A & B are constants; When $t > 34$ $A = 0.26$ & $B = 172$.

FTIR method

Perkins Elmer FTIR spectrometer with deuterated triglycin sulphate (DTGS) as a detector, resolution level of 4 cm^{-1} and the number of scans being 256 is used to take the spectra. The instrument has a scan speed of 0.20cm/s. Perkins Elmer data acquisition and processing software spectrum for windows has been used. The oil samples are collected before and after undergoing five cycles of heating to frying condition. 2 ml of liquid sample is poured over the KBr pellets (0.2mm thickness) using a capillary tube and the pellet is placed in the path of the sample beam. The spectra are recorded for three oil mixtures 3:1(RPO1), 1:1 (RPO2) and 1:3 (RPO3) from 4000 to 450cm^{-1}.

In-vitro analysis

ABTS radical scavenging activity

The ABTS scavenging test is used to determine the antioxidant activity (by estimating peroxide formation) of both hydrophilic and hydrophobic compounds. The assay measures ABTS radical cation formation induced by metmyoglobin and hydrogen peroxide. The formation of the coloured ABTS radical is suppressed by antioxidants by electron donation radical scavenging and inhibit. The quantity of antioxidant in the test sample is inversely proportional to the ABTS radical development.

ABTS is generated by mixing 2.5 ml of 7 mM ABTS with 14.7 mM ammonium per sulphate and stored in the dark at room temperature for 16 hours. The solution is diluted with water to achieve an absorbance of 0.7±0.05 O.D and is used as control. The radical-scavenging activity is assessed by mixing 2 ml of ABTS solution with different concentrations of sample (oil dissolved in CCl_4) 25, 50, 75, 100 µg /ml. The experiment is carried out according to an improved method as described by *Re et al.* 1999 [13] with slight modification. After 30 min, the percentage inhibition at 734 nm was calculated for each concentration relative to blank absorbance[7].

$$ABTS + H_2O_2 \longrightarrow ABTS^* + H_2O \quad \text{---------- (2)}$$

The reaction between ABTS and ammonium per sulphate directly generates the blue green ABTS chromophore, which can be reduced by an antioxidant, thereby resulting in a loss of absorbance at 734 *nm*. The antioxidant capacity is expressed as percentage inhibition, calculated using the following formula:

$$\text{Inhibition (\%)} = [(A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}}] \times 100 \quad \text{---------- (3)}$$

where A_{control} is the absorbance of the control reaction and A_{sample} is the absorbance of the sample. The peroxide level is determined by the reading the absorbance using UV- Spectrophotometer (Perkin Elmer, Spectrum I, Massachusetts, USA). IC_{50} is calculated by plotting percentage inhibition against different concentrations of oil. IC_{50} values denote the concentration of sample required to scavenge 50% of ABTS free radicals. Low IC_{50} values indicate high radical – scavenging activity. The experiment has been performed in triplicate, was recorded as mean ± SD.

DPPH (2, 2'-diphenyl-1 - picrylhydrazyl) free radical scavenging assay

DPPH method is also used to study the scavenging activity of antioxidants in oils. It is seemed to be endowed with good antioxidant properties. This method is based on the reduction of a methanol solution of DPPH in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H (Rubalya *et al*., 2009a). The samples are prepared by adding carbon tetrachloride solutions to oil (25, 50, 75,100 µg/ml of oil) and adding to 2 ml of a methanol added to $DPPH^{free}$ radical. The reaction mixture is shaken by cyclo-mixer and then kept in the dark for 30 min under ambient conditions.

This transformation results is a change in colour from purple to yellow, which has been measured spectrophotometrically by using UV- Spectrophotometer (Perkin Elmer- Spectrum I, Massachusetts, USA). The disappearance of the purple colour change at 517 nm is observed.

$$DPPH^* + AH \longrightarrow DPPH + A^* \quad \text{---------- (4)}$$

The percentage of inhibition (antioxidant capacity) is computed by measuring the absorbance at 517 nm, using the following formula,s

$$\text{Inhibition (\%)} = [(A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}}] \times 100 \quad \text{----------(5)}$$

Where, A_{Control} is the absorbance of the control and A_{Sample} the absorbance of the sample at 517 nm. Antioxidant activity expressed as IC_{50} is calculated by plotting percentage inhibition against different concentration of oil. IC_{50} value denotes the concentration of oil sample required to scavenge 50% DPPH radical. All observation has been carried out in triplicate and recorded as mean ± SD.

Statistical analysis

All data on total antioxidant activity are the average of triplicate. To examine the effect of type of compound and concentration on antioxidant activity, graph pad software version 5.0 is used (r^2 =0. 0.9949, $p<0.005$, $n>9$). The data were recorded and analysed by SPSS (version 12).One-way analysis of variance is performed by ANOVA procedures. Significant differences between means were determined by Tukey's multiple range tests, p-Values <0.05 were regarded as significant and p-value<0.001 were very significant.

Results and Discussion

Changes of viscosity of oil mixtures on heating

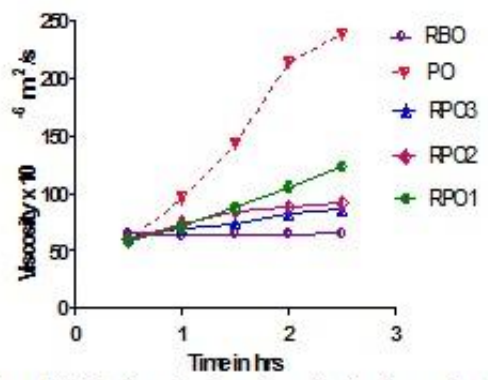

Figure 1 Variation of viscosity with the time of heating

Kinematic viscosity of the oil is calculated from the formula given in the experimental by heating the oil to the frying temperature up to 190°C for 0.5, 1.0, 1.5, 2 and 2.5 hrs. After each desired time of heating the oil was cooled to 28°C and the oil viscosity was measured. Figure 1 shows the variation of viscosity of rice bran, palm oil and its mixtures with different times of heating. It is observed that palm oil shows steep increase in viscosity on heating to 0.5 hrs. The change in viscosity of PO after five cycles of heating is 75.9%, RPO1 changes by 52%, RPO2 differs by 33% and RPO3 decreases to 26% compared to other mixtures of oils. The oil with large amount of polyunsaturated fatty acids has low viscosity [5, 14]. Increase in the quantity of RBO decreases and controls the viscosity with the cycles of heating due to the presence of strong antioxidant like oryzanol, sitosterol etc., that slow or prevent the oxidation of fatty acids and other composition of oils. Rancidity leads to the formation of primary product peroxide that leads to the formation of secondary product like carbonyl or hydroxyl groups bonded to carbon chain making flux among molecules that increases viscosity [7, 15].

The variation of viscosity show the antioxidant stability is more in the mixture of oils than palm oil hence it confirms that the degree of saturation in the composition of oils are less. This study implies that even though viscosity is a simple marker for thermal degradation, it strongly establish that instead of using palm oil the mixture of rice bran and palm oil could be used in frying low cost effect.

FTIR Analysis

Increase in viscosity is due to the changes in the structure of unsaturated fatty acids and the structural variation can be studied using FTIR analysis. Figure 2, 3, 4 shows FTIR spectra of the mixtures of heated RPO1, RPO2 and RPO3 oils after fifth cycle of heating. The band observed at 3004.68 cm^{-1} and 1654.37 cm^{-1} assigned due to the vibration of *cis*-double bond of C-H stretching in the fatty acids of oils [1, 16]. It is observed that the transmission bands at 1458, 1744 and 3472 cm^{-1} are changed due to partial thermal degradation of oil. The presence of transmission 1417 cm^{-1} is due to rocking vibrations of - CH bonds of *cis* disubstituted olefins. The broadening of transmission at 1744 cm^{-1} is owed to the production of saturated aldehydes functional groups or other secondary oxidation products of peroxides [17, 18].

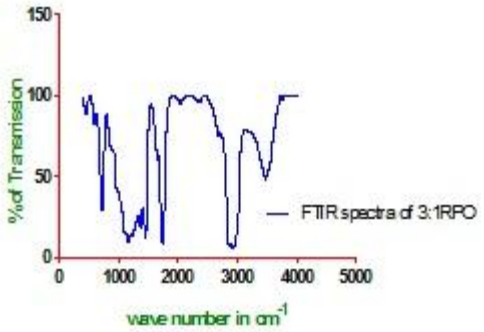

Figure 2FTIR spectrum of mixture of 3:1 RPO1

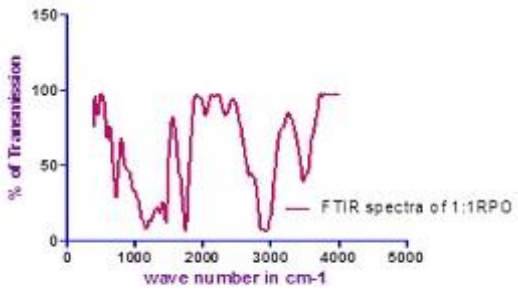

Figure 3 FTIR spectrum of mixture of 1:1 RPO2

Figure 5 shows the variation of unsaturated compounds (CH_2) in the mixtures of rice bran and palm oils. The observed bands at 2924.44 and 2856.95 cm^{-1} is due to stretching vibration of CH_2 groups in the fatty acids [17]. It is observed that the depth and breadth of the valley increases due to addition of more percentage of rice bran oil with palm oil in the mixtures.

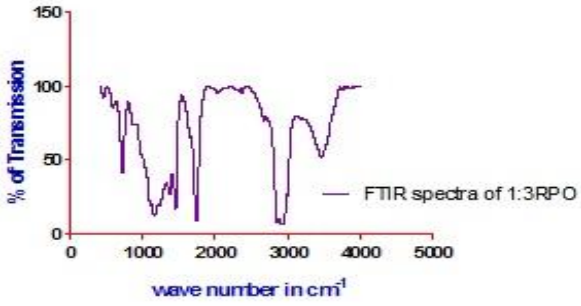

Figure 4 FTIR spectrum of mixture of 1:3 RPO3

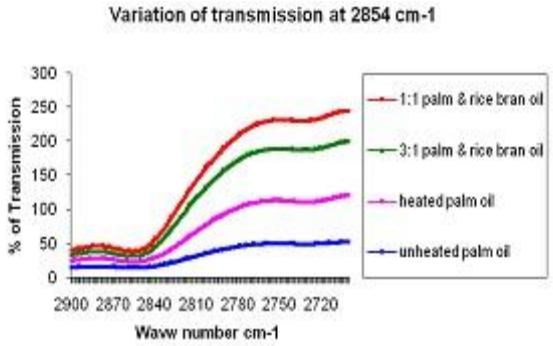

Figure 5 Variation of unsaturation composition at 2856.95 cm^{-1}

Figure 6 shows the absorbance in the region 1000-1744cm^{-1} result in the increase in the formation of carbonyl compounds like aldehyde; ester and ketone are formed from initial peroxide compounds [9, 12]. The presence of transmission 1417 cm^{-1} may be due to rocking vibrations of -CH bonds of *cis* disubstituted olefins. Also the broadening of transmission at 1744 cm^{-1} is owed to the production of saturated aldehydes functional groups or other secondary oxidation products of peroxides. The band observed at 1374.28 and 1162.11 cm^{-1} is due to stretching of the C-O ester group that arises when water molecule is eliminated due to the chemical reactions between fatty acids and glycerol during degradation [17]. Figure 7 illustrate the changes in the percentage of transmission due to CH_2 rocking vibrations of *cis*- disubstituted olefins at 722cm^{-1} [1, 19]. The graph illustrates the zero *trans* shortening of palm oil on addition rice bran oil.

From the above experiential bands of the mixture of oils, it exemplify the presence of unsaturated fatty acid in the oil has been augmented and the RBO/PO mixture might be used for frying with less adverse effect.

In-vitro Analysis

Figure 8 illustrates the percentage of inhibition using ABTS* for the mixture of oil RPO1 (r^2=0.991, p<0.005, n>9) is 57.06%. The percentage of inhibition varies sharply showing effective radical scavenging activity and reaches saturation at 100µ g/ml. There is a significant difference (p< 0.01) in scavenging activity. The effect of antioxidant activity of the mixture RPO2 (r^2=0.980, p<0.005, n>9) is illustrated in Figure 9 and is found to be 56.18%. Figure 10 shows a steep variation of percentage of inhibition in the mixture of oil RPO3 (r^2=0.944, p<0.005, n>9) at the concentration 50 µg/ml and reaches saturation at 75µ g/ml. The IC_{50} Value of this mixture of oils is 48.73%. Comparing the radical scavenging activity in the three mixtures of oils it is observed that the IC_{50} values decrease in the order; RPO1 > RPO2 > RPO3.

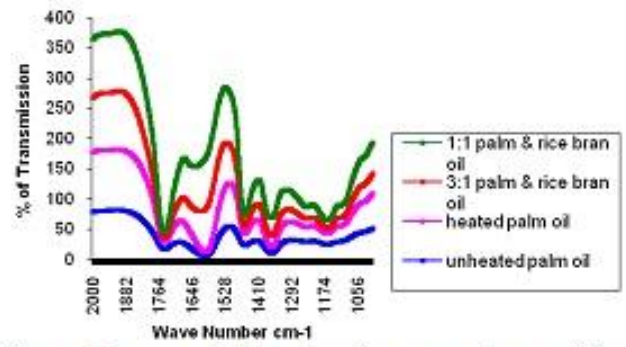

Figure 6 Decrease in the carbonyl compound composition at 1000 and 1744 cm^{-1}

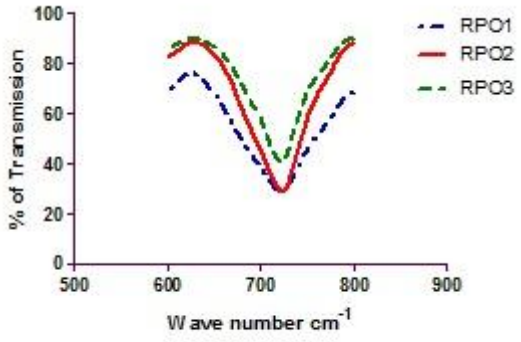

Figure 7 Variation of unsaturation composition at 722 cm^{-1}

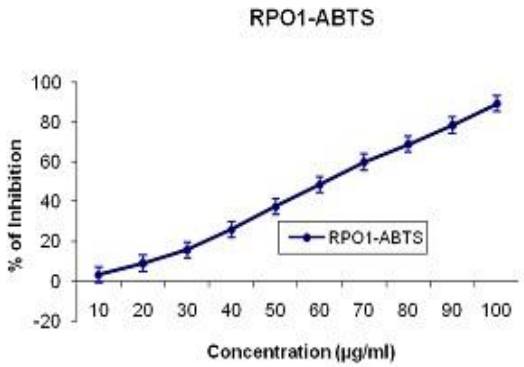

Figure 8 Variation of % of Inhibition using ABTS assay with the concentartion of the mixture RPO1

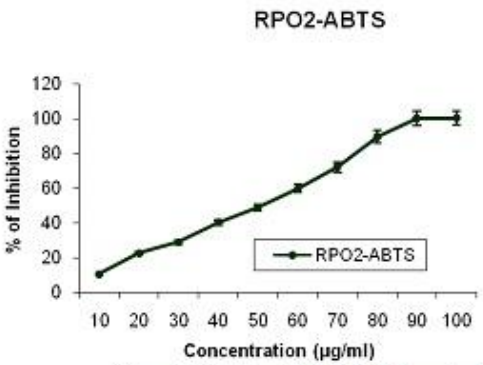

Figure 9 Variation of % of Inhibition using ABTS assay with the concetration of the mixture RPO2

Fgure 11 illustrates a steep variation in the inhibition percentage with concentration up to100 µg/ml. The IC_{50} value of RPO1 (r^2=0.9889, p<0.005, n>9) is 71.86%. The percentage of inhibition of the mixture RPO2 (r^2 =0.976, p<0.005, n>9) as in Figure 12 increases sharply up to100 µg/ml and its IC_{50} value is 60.65%. Figure 13 predicts the scavenging activity of the mixture of oils RPO3 (r^2 =0.991, p<0.005, n>9) reaches saturation at 50 µg/ml. Hence the IC_{50} value is comparably low 58.97%. Comparing all the three mixtures of oils it is found that the antioxidant activity decrease in the order; RPO1 > RPO2 > RPO3.

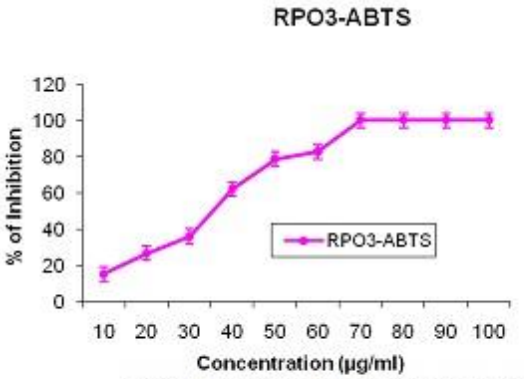

Figure 10 Variation of % of Inhibition using ABTS assay with the concetration of the mixture RPO3

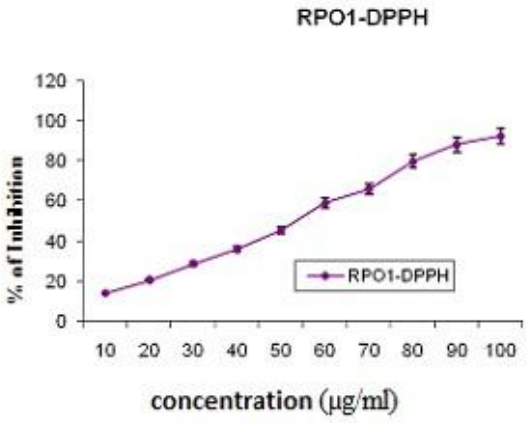

Figure 11 Variation of % of Inhibition using DPPH assay with the concentration of the mixture RPO1

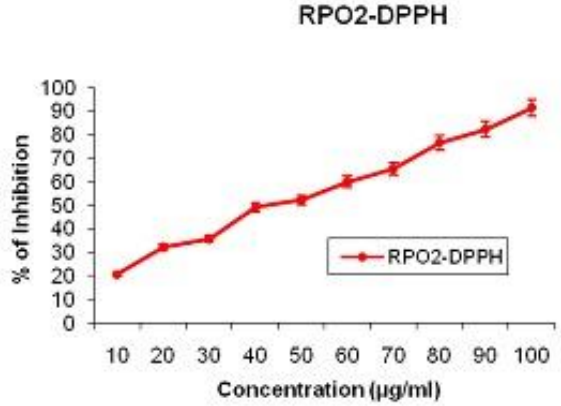

Figure 12 Variation of % of Inhibition using DPPH assay with the concetration of the mixture RPO2

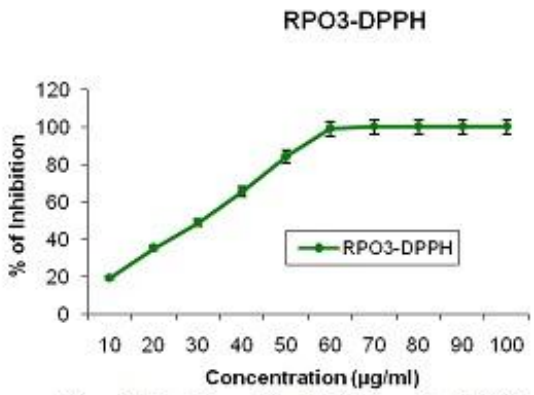

Figure 13 Variation of % of Inhibition using DPPH assay with the concetration of the mixture RPO3

Conclusions

Kinematic viscosities of the palm and rice bran oil mixture are studied that indicates the escalation of unsaturated compounds in the mixtures. The unsaturated compounds in oils is studied using FTIR technique

from the valley that arises due to rotation and vibration of molecular functions (chemical group) the oxidative deterioration are evaluated. The structural transform and increase in *cis* - transform of mixture of oils heated to frying condition is also studied. The ABTS and DPPH radical scavenging assay proves the formation of peroxides are less in the mixtures. The study make public that the percentage of conversion of unsaturated fatty acid into saturated fatty acid in the mixture of oils is less than palm oil and suitable for frying with oxidative stability, economical and less adverse effect.

Acknowledgement

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References

1. Adolfo Valdes F and Ana Garcia B., A study of the evolution of the physicochemical and structural characteristics of olive and sunflower oil after heating at frying temperatures, Food chem., 2006, 98, 214-219.
2. Rubalya Valantina S and Neelamegam P., Study of rheological behaviour and thermal degradation in vegetable oils on heating, Asian J. of Chem., 2012a.24, 1975-1978.
3. Abramovic H and Klofutar C., The temperature dependence of dynamic viscosity for some vegetable oil, Acta Chem. Slov, 1998, 45, 69-77.
4. Fountain C W, Jennings J, Mckie C, Oakman P and Fetterolf M L., Viscosity of common seed and vegetable oils, J. of Chem. Edu., 1997, 74, 224-227.
5. Rubalya Valantina S and Neelamegam P., Antioxidant potential in vegetable oil. Res. J. Chem. Environ., 2012b, 16, 87- 94.
6. Moya Moreno M C M, Menndoza Olivares D, Amezquita F J, Peris V and Bosch Reig F., Study of the formation of carbonyl compounds in edible oils and fats by H-NMR and FTIR, J. Mol. Str., 1999a, 482-483.
7. Rubalya Valantina S, Neelamegam P and Gayathri K., Estimating antioxidant stability in sunflower and rice bran oil on heating using in-vitro analysis, Biomed. 2009, 29, 31-35.
8. Arnold R G and Hartung T E, Infrared spectroscopy determination of the degree of unsaturation of fats and oils, J. Food Sci., 1971, 36, 166-168.
9. Jag Mohan, Organic Spectroscopy Principles and Applications**.** 2nd ed. Narosa Publishing House. New Delhi. 2001, 29-95.
10. Moya Moreno M C M, Menndoza Olivares D, Amezquita F J, Gimeno Adelantado J V and Bosch Reig F., Analytical evaluation of polyunsaturated fatty acids degradation during thermal oxidation of edible oils by FTIR. J. Talanta 1999b, 50, 267-275.
11. Fereidoon Shahidi, Bailey's Industrial oil and Fat Products, 6th ed. Wiley- Inter science Publication, New York. 2005,603-675.
12. Tang T S., Fatty acid composition of edible oils in the Malaysian market with special reference to trans-fatty acid, J. of oil Palm res., 2002, 14, 1-8.
13. Re R, Pellegrini N, Protegente A, Pannala A, Yang M and Rice Evans C., Antioxidant activity applying improved ABTS radical cation decoloursation assay, Free Rad. Biol. Med., 1999, 26, 1231-1237.
14. Fasina O O, Hallman H, Craig M and Schmidt Clements C., Predicting temperature dependence viscosity of vegetable oils from fatty acid composition, JAOCS. 2006, 83, 899-903.
15. Santos J C O, Santos I M G and Souza A G., Effect of heating and cooling on rheological parameters of edible vegetable oils, J. Food Eng**.** 2005, 67, 401-405.
16. Ismail A A, Van de Voort F R, Emo G and Sedman J., Rapid quantitative determination of free fatty acid in fats and oils by FTIR spectroscopy. JAOCS, 1993. 70, 335-341.
17. Hamed S F and Mousa Allam A., Application of FTIR spectroscopy in the determination of antioxidant efficiency in sunflower oil, J. App. Sci. Res**.,** 2006, 2, 27-33.
18. Rubalya Valantina S, Arockia Sahayaraj P and Angelin Prema A., Antioxidant stability in palm and rice bran oil using simple parameters, Rasayan J. Chem., 2010, 3, 44-50.
19. Vlachos N Y, Skopelitis M, Psaroudaki V, Konstantinidou, Chatzilazarou, and Tegou A E., Application of Fourier transform-infrared spectroscopy to edible oils, Anal. Chem. Acta**.,** 2006, 573–574, 459-465.

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