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Antioxidant Stability of Edible Oil Using Rheological Behaviour and *in vitro* Analysis

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In food industry, rheological behaviour and thermal degradation are the important parameters required to determine the quality and stability of food system. In this paper, the rheological behaviour and thermal degradation of sunflower oil and palm oil, is investigated. The Newtonian behaviour of the oils are studied from the kinematic viscosity of unused edible oils (sunflower oil, palm oil, groundnut oil, coconut oil, olive oil, sesame oil) using redwood viscometer at temperatures from 30 to 90 ºC. Increase in viscosity of used (heated to frying condition, 210 ºC) edible oils like sunflower oil, palm oil are studied in the same variation of temperature shows the non-Newtonian behavoiur of the oils. This oxidation process can be prevented or retarded by the addition of synthetic or natural antioxidants. The antioxidant activities in oils on heating to smoke point are analyzed using *in vitro* studies. The free radical scavenging activity of the samples using ABTSH and DPPH is measured and the antioxidant activity of sunflower oil is much effective than palm oil.

Key Words: Rheology, Thermal degradation, Redwood viscometer.

INTRODUCTION

Rheological measurement is much useful behavioural and predictive information for product consistency and quality[1]. Temperature is also important parameter and frequently appears in rheological equation[2], in the study of flow of liquids. Edible oils represent one of the primary constituents in the formulation and manufacture of products by food industry[3]. Viscosity simply means the resistance of one part of the fluid to move relative to another one. Therefore, viscosity is closely correlated with the structural parameters of the fluid particles. The oil viscosity has a direct relationship with some chemical characteristics of the liquids, such as the degree of unsaturation and the chain length of the fatty acids that constitute the triglycerides[4]. Viscosity slightly decreases with increased degree of unsaturation and rapidly

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increases with polymerization, which led to the development of the idea to use edible oils as bio-diesel fuel[5]. When the oil is heated to the frying condition the unsaturation decreases and it becomes saturated due to oxidation. Edible oils are complex mixtures of many triglycerides with different chain lengths[6].

Atmospheric oxidation is the most important cause of detoriation in fats[7]. Oxidation is accelerated by means of exposure to heat, light, amount of oxygen available and humidity. The intense frying of oils causes an oxidizing thermal degradation with the formation of decomposition, such as aldehydes, ketones, free fatty acids and hydroxy compounds that in high levels can be harmful to human health. When oxygen travels through the fat, it is absorbed by the fat and reacts mainly in the double links, thus the polyunsaturated components of fats oxidize much faster than unsaturated ones[8,9].

A paradox in metabolism is that, majority of complex life requires oxygen for its existence but oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species[10]. Consequently, organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids. In general, antioxidant systems either prevent these reactive species from being formed or remove them before they can damage vital components of the cell[11]. Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytoplasm and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation[11]. These compounds may be synthesized in the body or obtained from the diet[12].

In the present study the viscosity of some edible oils (sunflower oil, palm oil, coconut oil, olive oil, sesame oil and groundnut oil) are measured for heating and cooling in the temperature range of 30 to 90 °C. Thermal degradation of the edible oil is studied from viscosities of sunflower oil, palm oil used in frying condition and the saturation of the triglycerides is studied from repeated cycles of heating and cooling. The antioxidant effect in used and unused palm oil and sunflower oil in *in vitro* studies has been evaluated.

EXPERIMENTAL

Edible oils like coconut oil, groundnut oil, olive oil, palm oil, sesame oil and sunflower oil are purchased in local commerce.

DPPH$^{\bullet}$ radical scavenging assay: This method is based on the reduction of a methanol solution of DPPH$^{\bullet}$ in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H[13]. This transformation results in a change in colour from purple to yellow, which was measured spectrophotometrically by the disappearance of the purple colour at 517 nm. Chloroform solutions of oil (25, 50, 75,100 µg/mL of chloroform) are added to 2 mL of a methanol solution of DPPH$^{\bullet}$ free radical or methanol alone (control)[14,15]. The reaction mixture is shaken

by cyclomixer and then kept in the dark for 0.5 h under ambient conditions. The absorbance is measured at 517 nm and the antioxidant capacity is expressed as percentage inhibition, calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{A_{(cont)} - A_{(test)}}{A_{(cont)}} \times 100 \quad (1)$$

where, $A_{(cont)}$ is the absorbance of the control and $A_{(test)}$ the absorbance of the sample at 517 nm. IC_{50} is the antioxidant concentration that inhibits the $DPPH^{\bullet}$ reaction by 50 % under the experimental conditions. This was calculated by plotting percentage inhibition against different concentrations of oil. Low IC_{50} values indicate high radical-scavenging activity of cation. All analyses were run in triplicate and averaged.

$ABTSH^{\bullet}$ radical cation scavenging: The $ABTS^{\bullet}$ scavenging test is widely used to determine the antioxidant activity of both hydrophilic and lipophilic compounds. The reaction between $ABTS^{\bullet}$ and ammonium per sulfate directly generates the blue green $ABTS^{\bullet}$ chromophore, which can be reduced by an antioxidant, thereby resulting in a loss of absorbance at 734 nm. The experiment is carried out according to an improved method as described by Re *et al.*[16], with slight modification. $ABTS^{\bullet}$ 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 h. The solution is diluted with water to achieve an absorbance of 0.7 O.D at 734 nm. The radical-scavenging activity is assessed by mixing 2 mL of this $ABTS^{\bullet}$ solution with different concentrations of sample dissolved in chloroform (25, 50, 75, 100 µL). 1.0 mL of chloroform along with 2.0 mL of $ABTS^{\bullet}$ was used as control. The final absorbance is measured at 734 nm. The antioxidant capacity is expressed as percentage inhibition, calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{A_{(cont)} - A_{(test)}}{A_{(cont)}} \times 100 \quad (2)$$

where $A_{(cont)}$ is the absorbance of the control and $A_{(test)}$ the absorbance of the sample at 734 nm. IC_{50} is the antioxidant concentration that inhibits the $ABTS^{\bullet}$ reaction by 50 % under the experimental conditions. This is calculated by plotting percentage inhibition against different concentrations of oil. Low IC_{50} values indicate high radical scavenging activity. All analyses were run in triplicate and averaged.

Methods: Kinematic viscosity (ν) is defined as the ratio of absolute viscosity (η) to mass density. The Redwood viscometer (manufactured by Associated Instrument manufacturers India Private Limited, New Delhi, India) consists of an oil cup furnished with a pointer, which ensures a constant head and orifice at the center of the base of inner cylinder. The orifice is closed with a ball, which is lifted to allow the flow of oil during the experiment. A temperature controller maintains the temperature. The cylinder, which is filled up to, fixed height with liquid whose viscosity is to be determined, is heated by water bath to the desired temperature. The orifice is opened and the time required for collecting 50 mL of oil is measured.

The kinematic viscosity is calculated from the following relation:

$$(\nu) = (A^{\bullet}\ t\text{-}B/t) \times 10^{-4}\ m^2/s \quad (3)$$

A and B are constants, t = redwood time which measure the rate of flow in seconds.

when $t < 34$ A = 0.0026 and B = 1.175

and when $t > 34$ A = 0.2600 and B = 172

The copper cup in the viscometer is washed with CCl_4 after each observation. Each reading is taken from the average of three trials.

RESULTS AND DISCUSSION

Fig. 1 shows the variation of viscosity with temperature. The viscosity is calculated during the rise of temperature 30-90 °C in the step increase of 10 °C from the redwood seconds substituted in the formula given in the experimental. It is observed that the viscosity decreases with increase in temperature.

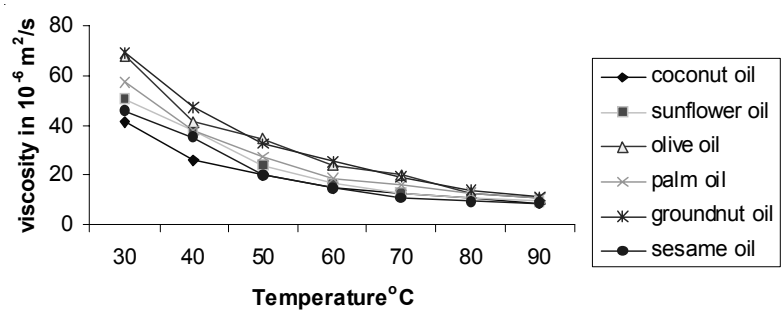

Fig. 1. Variation of viscosity with temperature

At 90 °C almost all the oils have the same viscosity. The fall in viscosity is owing to the high thermal movements among molecules that reduce intermolecular forces and makes flow among them easier by reducing the viscosity. The presence of double bonds in fatty acid that exist in *cis* configuration (oleic and linoleic) form, produces 'kinks' in the geometry of the molecules[3]. This prevents the chains coming close together to form intermolecular contacts, resulting in an increased capability of the oil to flow. The variation of viscosity of the oils is also calculated in cooling in the same range of temperature and found to be the same implying that there is no degradation in this temperature range. Viscosity is related to the concentration of polyunsaturated chains rather than monounsaturated chains because of π bonds, which makes bonding more rigid, decreasing rotation between C-C bonds[10].

Rheological behaviour of edible oil after frying condition is studied from Figs. 2 and 3. It is found that viscosity of unused oil is lesser than used oil. The viscosity of used oil is greater than unused indicates the liquid may change from Newtonian to non-Newtonian and becomes dilatants fluid. It is known that certain properties of fatty acid residues in the molecule of triglycerol have significant effects on the fluidity of the oil.

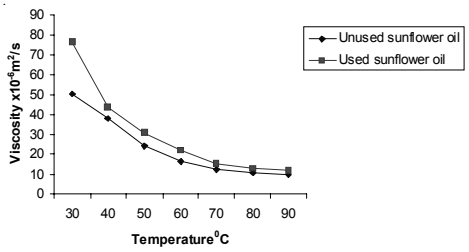

Fig. 2. Variation of viscosity with temperature of sunflower oil

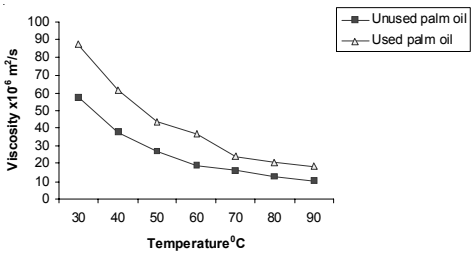

Fig. 3. Variation of viscosity with temperature of palm oil

Most of the bonds in the hydrocarbon chain of fatty acids are single bonds. This linear 'zig-zag' organization enables the chains to be lined up close to each other and intermolecular force vander Waals interaction can take place[4]. This system inhibits flow of oil, resulting in the relatively high viscosity of the oils.

The study of thermal degradation of the oil is carried out by heating the oil to the frying temperature up to 210 °C for 0.5, 1.0, 1.5, 2.0 h. After heating to desired time, viscosity is measured at 30 °C. Fig. 4 shows the increase in the viscosity with the time of heating, clearly indicates the increase in saturation. During heating *cis*-unsaturated fatty acids are partly converted to *trans*-isomer[17]. This *trans*-isomer makes the fatty acid saturated. Sunflower oil series (3) has the lowest viscosity variation compared to the palm oil, since it has 70 % of polyunsaturated fatty acids[3]. The 50 % polyunsaturated fatty acids in palm oil has higher tendency to saturate. The natural antioxidants in palm oil get evaporated leads to viscosity increases with frying time due to oxidation, isomerism and due to polymerization reaction. Oxidation reaction leads to the formation of carbonyl or hydroxyl groups bonded to carbon chain making flux among molecules that increases viscosity.

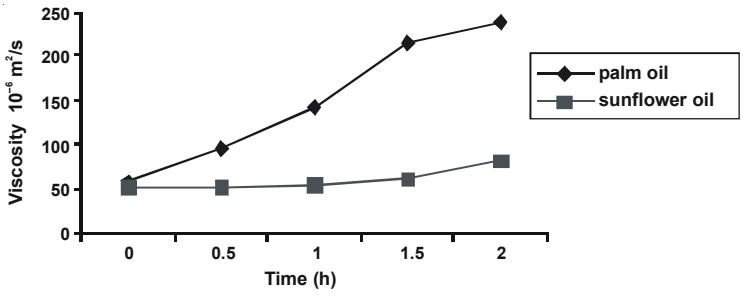

Fig. 4. Time *vs.* viscosity variation

***In vitro* analysis:** Palm oil contains saturated fatty acids like palmitic acid 44.3 %, oleic acid-38.7 % and linoleic acid-10.5 %, vitamin E especially tocotrienols, vitamin K and magnesium[17]. According to British Pharmacopoeia, sunflower oil contains high concentration of palmitic acid: 4-9 %, stearic acid: 1-7 %, oleic acid:

14-40 %, linoleic acid: 48-74 % lecithin, tocopherols, carotenoids and waxes. The antioxidant activity of the palm oil may be due to the presence of carotenoids and vitamin E. The presence of β-carotene is the reason for the yellow colour of the palm oil may also be an important factor for the free radical scavenging activity[18], gave a commentary on the antioxidant effect of β-carotene and its role in cardio protection. The antioxidant activity of the sunflower oil may also be due to the presence of vitamin E and tertiary butyl hydroquinone (TBHQ).

The percentage inhibition of ABTS/DPPH free radical by palm oil is increased along with the concentration of oil. It is well known that $ABTS^{\bullet}$ activity is closely correlated with $DPPH^{\bullet}$ ($r^2 = 0.949$, $p < 0.005$, $n > 9$) because both are responsible for the same chemical property of hydrogen or electron donation.

From Table-1, the IC_{50} value of unused palm oil is found to be higher than that of used oil by 40 % [100-{(24.03/40.05)×100}] in ABTS ($r^2 = 0.9858$, $p < 0.005$, $n > 9$) radical scavenging activity. The free radical scavenging activity of unused palm oil decreases when used oil is increased along with the concentration as shown in Figs. 5 and 6. Similarly, in DPPH radical decolourization assay, the IC_{50} value is increased by 49.6 % [100-{(24.03/40.05)×100}]. The IC_{50} value of sunflower oil is plotted against concentration as in Figs. 7 and Fig. 8. It is observed both used and unused oil increases. The IC_{50} value of unused sunflower oil given in Table-2 is found to be greater than that of used sunflower oil by 49.6 % [100-{(25.42/50.39) × 100}] in ABTS ($r^2 = 0.9961$, $p < 0.005$, $n > 9$) radical decolourization assay and 41.7 % [100-{(28.81/49.45) × 100}] in DPPH ($r^2 = 0.\ 0.9949$, $p < 0.005$, $n > 9$) radical decolourization assay. The antioxidant activity of unused palm oil is high because it has higher concentration of carotene. But it gets volatile when it is heated.

TABLE-1
ABTS AND DPPH RADICAL DECOLOURIZATION OF PALM OIL

Concentration (μg/mL)	Palm oil $ABTS^{\bullet}$		Palm oil $DPPH^{\bullet}$	
	Used	Unused	Used	Unused
25	35.5 ± 0.2	48.3 ± 1.1	19.96 ± 0.3	35.9 ± 1.3
50	58.4 ± 1.7	67.7 ± 0.3	48.40 ± 2.1	59.9 ± 0.4
75	72.9 ± 0.8	76.4 ± 0.7	66.40 ± 1.0	70.7 ± 0.9
100	94.7 ± 1.4	82.9 ± 0.1	93.40 ± 1.7	78.8 ± 0.1
IC_{50}	40.05	24.03	50.39	25.42

TABLE-2
ABTS AND DPPH RADICAL DECOLOURIZATION OF SUNFLOWER OILS

Concentration (μg/mL)	Sunflower oil $ABTS^{\bullet}$		Sunflower oil $DPPH^{\bullet}$	
	Used	Unused	Used	Unused
25	31.0 ± 0.2	53.2 ± 0.4	14.4 ± 0.5	41.95 ± 0.5
50	47.4 ± 1.1	64.5 ± 1.3	56.0 ± 1.6	56.00 ± 1.6
75	60.7 ± 0.3	72.3 ± 0.8	65.6 ± 1.0	65.60 ± 1.0
100	71.5 ± 0.4	83.9 ± 1.5	64.7 ± 0.5	80.80 ± 1.8
IC_{50}	50.39	25.42	49.45	28.81

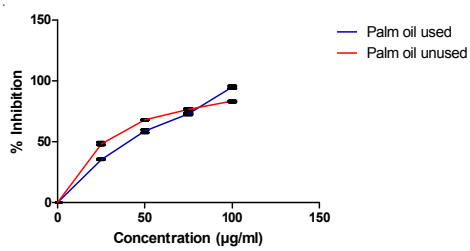

Fig. 5. ABTS radical decolourization of used and unused palm oil

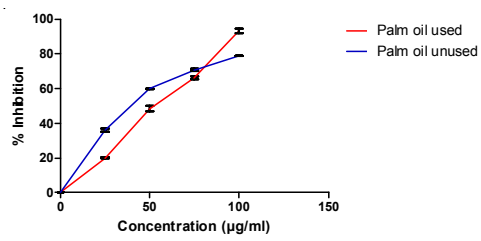

Fig. 6. DPPH radical decolourization of used and unused palm

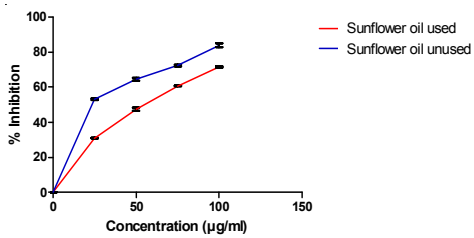

Fig. 7. ABTS radical decolourization of used and unused sunflower oil

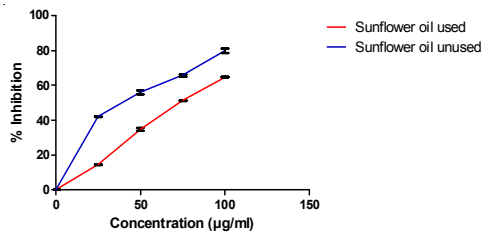

Fig. 8. DPPH radical decolourization of used and unused sunflower oil

The sunflower oil has higher IC_{50} value like used palm oil. But on heating, the loss of antioxidant activity in ABTS$^{\bullet}$ radical decolourization assay is observed as lower in sunflower oil when compared to the palm oil. But no such difference has been observed in the DPPH$^{\bullet}$ radical decolourization assay[19,20].

Statistical analysis: Statistical analysis is made using software SPSS version 12. Table-3 shows the data analysis studied for the calculated viscosity (dependent) with rise in temperature (independent). It was found that the power function to be the best curve fit with minimum error if the r sq value approaches 1. It can be noted that r sq value ranges from 0.976 to 0.998. The most important part of the result is F-ratio and the associated significance value. Larger the F-ratio larger would be the chance[21]. Therefore one can conclude that present regression model result is significantly better prediction of accuracy.

TABLE-3
REGRESSION COEFFICIENT OF THE SAMPLE

Name of the oil	R square value	F value	Standard error
Coconut oil	0.99482	960.03	0.04240
Sunflower oil	0.99814	2684.10	0.02700
Olive oil	0.97787	220.96	0.10000
Palm oil	0.99771	2718.37	0.03201
Groundnut oil	0.99279	678.58	0.06000
Sesame oil	0.97629	20585.00	0.10000
Sunflower oil used	0.99808	2595.00	0.04100
Palm oil used	0.98954	473.00	0.08000

Conclusion

Kinematic viscosity is measured for edible oils at temperatures range from 30-90 °C indicates the non-degradation of the oil used. The viscosity of used oil is found to be greater than unused oil which shows the thermal degradation. The oil becomes dark, high viscous with unpleasant smell. Significant variation in sunflower oil and palm oil decreases the nutritional value of oil as a consequence of which digestion capability of humans is affected. Under this condition the viscosity of oils increases. The determination of unsaturation in oils increases the oxidation of lipoproteins that lead to atherosclerosis, hypertension, coronary artery disease stroke, *etc*. In metabolic activity saturated fatty acids is converted into diacylglycerol which alter colonic epithelial cells that leads to colon cancer. The antioxidant in the oils breaks oxidation by adding hydrogen atom to free radicals. The antioxidant activity of both palm oil and sunflower oil is found to be lost during heating. The percentage of lose in antioxidant activity of palm oil during heating, is more than that of not heated is owing to the presence of high percentage of carotene. Sunflower oil in ABTS and DPPH decolourization assay are almost the same. From the above results the antioxidant activity of sunflower oil is much effective than palm oil.

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