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STABILIZATION OF EDIBLE OIL BY ONION PEEL AND RATAN JOT EXTRACT ON STORAGE TO PREVENT HUMAN HEALTH HAZARD

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ABSTRACT

Edible oil can be oxidatively stabilized using natural phenolic extracts. The oxidation of oil eliminates health benefits of edible oil. They produces reactive species in it that cause many human health hazard. The phenols ring structure defines its antioxidant activity in oil medium. Onion peel and ratan jot have been reported to contain the phenols of different nature (or structure). This study aims to delay oxidation in edible oil on storage by adding aqueous methanolic extract of onion peel and ratan jot in it. Total phenolic content (TPC) as determined by Folic-Ciocalteu method in onion peel was found to be 48.02 and 33.64 gm of gallic acid per kg in ratan jot extract. Antioxidant capacity (AC) as determined by DPPH radical scavenging activity method was found to be 87 % in onion peel extract and 55 % in ratan jot extract. The extracts were added in three different concentrations (300, 600 and 900 mg/kg) to preheated edible (sunflower) oil. Treated and untreated (control) oil samples were then stored in dark at ambient temperature (approx. 36^0C) for eighty days. The samples were then analyzed periodically with an interval of ten days for lipid oxidation parameters, peroxide value (PV), free fatty

acid (FFA) and para anisidine value (P-AV). Both extracts showed dose dependent antioxidant activity and were effective in preventing formation of hazardous compound in edible oil. However, Onion peel extract due to stabilized the edible oil againts detrimental spoilage for longer period than the ratan jot extract at all the concentrations.

KEYWORDS: *Human Health, Plant Phenols, Edible oil, Oxidation, Total Phenolic Content, Antioxidant Capacity.*

1. INTRODUCTION

Edible (vegetable) oil undergoes an oxidative degradation process during processing and storage. The oxidation of oil not only eliminates health benefits of edible oil but also produces reactive species that may initiate cáncer.[1] Scientists have explored number of methods to maximize the stability of edible oil. For utility of vegetables oil with its optimal nutritive value many efforts have been done to stabilize them. These efforts include partial hydrogenation[2]; blending linolenic and linoleic acid rich oils with more stable oleic or saturated fatty acid rich oils[3,4] and the use of synthetic and natural antioxidants.[5,6] However, hydrogenation is not safe as trans fatty acids are produced during the process which are reported to be involved in carcinogenesis and cardiac problems.[7] Blending of oil may decrease the health benefits associated with the consumption of polyunsaturated fatty acids. The health concerns have been raised on the addition synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ) in edible oil.[8] Natural antioxidants are the safer alternatives then their synthetic counterparts. Therefore, there is a growing interest for use of natural antioxidants to stabilize the edible oils.[9] Phenols are one of the most important groups of natural antioxidants.[10] They occur only in plants and are known to protect easily-oxidizable constituents of food from oxidation. Phenols act as antioxidants through their radical scavenging in which they break the free radical chain reaction by donating hydrogen atom.[11] The resulting phenoxy radical may be reduced to its original compound by enzymatic or nonenzymatic reactions.[12] Another possible antioxidant mechanism is via metal chelation and restriction of the accessibility of the metal ion for participation in Fenton-type reactions.[13]

The plant waste as a source of phenol antioxidants has been investigated during last five decades. For example pomegranate peels and seed oils have been reported to contain a substantial amount of polyphenols such as quercitin and kaempferol, flavond diglycoside and

ellagic acid etc.[14, 15,16] Gallic and chlorogenic acids have been identified as the predominant phenolic copmounds in potato peel and sugar beet pulp.[17] However, very few researches have been done on the utilization of plant material by characterization of the phenolic compounds on basis of their reducing capacity and their antioxidant capacities to control lipid oxidation.[18,19]

Simic et al. (2007) suggested that multiple OH substitution and conjugation are important for the free radical scavenging activity and reduction potentials of phenols. They reported that quercitin, rutin (flavonoids) and caffeic acid (phenolic acid) posses low oxidation potential and have high antioxidant properties.[20] However, this study was performed by using pure phenolic compounds were used in a linolenic acid model.

Deep fried/baked foods consumption among people getting increased day by day. This may cause heatlh hazard due to the production of oxidation product in edible oil using for making deep fried/baked food. Thus such edible oils which are resistant against oxidation becomes a need of today's society. The available edible oils in markets either can not fulfil this requirement or they use synthetic antioxidant to attain oxidative-resistant oils. The purpose of the present research was to formulate oxidative resistant edible oil in the most safest manner. For this plant waste consisting structurally different phenols, have been taken to act as antioxidants in edible oil. The onion peel and ratan jot extract were selected to reduce of oxidation PUFA and MUFA in edible oil i.e. sunflower. Onion peel is a beneficial source of flavonoids i.e. quercitin.[21,22] While significant amount of phenolic acids i.e. hydroxybenzoic and hydroxycinnamic acid are identified in ratan jot.[23] Flavonoids due to multiple OH substitution and conjugation in their structure are found to have low oxidation potential and thus high free radical scavenging capacity than phenolic acid.[20] Total phenolics by Folin Method and radical scavenging activity by DPPH method was performed to correlate the difference in antioxidant efficacies of the phenols found in two extract with their structures. Refined, bleached and deodorized (RBD) sunflower oil was used to evaluate antioxidant efficacy of onion peel and ratan jot extract under accelerated storage because of its higher content of polyunsaturated fatty acids, the stabilization effect is more pronounced in chosen edible oil i.e. sunflower oil.[24]

2. MATERIALS AND METHODS

2.1. Materials

Refined, bleached and deodorized (RBD) sunflower oil was provided by a local oil manufacturer (Hamza vegetable oil refinery & ghee Mills (Pvt.) Ltd, Karachi). All the chemicals and reagent used were of analytical reagent grade and were purchased from Fisher Scientific, Surrey and Merck, Hertfordshire. Onions and ratan jot were purchased from a local market (Empress market, Karachi)

2.2. Extraction

The extracts of onion peel and ratan jot were obtained by a method[25] with some modification. This method utilizes the ultrasonic shaking, which increases the yield of the extract. Briefly, washed, dried and ground (80 mesh) samples of onion peel and ratan jot were introduced into the 80% aqueous methanol (methanol: water, 80:20 v/v) with a ratio of 1:30 (sample: solvent) for 30 mins along with ultrasonic shaking (Dawe instrument Ltd. U.S.A)**.** The extracts were separated from the residues by filtering through Whatman No. 1 filter paper. The combined extracts were concentrated and freed of solvent under vacuum at 45^{o}C, using rotary evaporator (Buchi, R-200, Flawil, Switzerland). The dried crude concentrated extracts were stored in a refrigerator (-4^{o}C) until used for analysis.

2.3. Total phenolic content

Total Phenolic content of the extract was estimated using Folin-Ciocalteu method as described by Gutfinger, (1981).[26] Gallic acid was used as a standard.

2.4. Evaluation of antioxidant capacity

Radical scavenging activity of the extracts was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Hossain et al. 2011.[27]

2.5. Oil enriched with extract

The extracts were added in three different concentrations (300,600, 900 ppm) to preheated sunflower oil with stirring at 50^{0}C for approximately 30 mins until uniformly mixed. For control, sunflower oil was subjected to same treatment except the addition of extract.

2.6. Storage condition

The control and extract containing samples (1 L) were placed in plastic bottle of without neck and stoppers and stored in dark at ambient temperature (36 °C) for a period of eighty days.

The level of oxidative deterioration was assessed after every 10 days by evaluating the bperoxide value (PV), para-anisidine value (P-AV) and free fatty acid (FFA).

2.7. Statistical analysis

The study was carried out in three replications. Data was analyzed using statistical package for social scientists (SPSS version 17). Analysis of variance (ANOVA) followed by Duncan multiple range test was used to distinguish the treatments at $p<0.05$. Pearson's correlation coefficient was used to establish the relations between PV, P-AV and FFA.

3. RESULT AND DISCUSSION

The onion peel and ratan jot extracts were found to contain significant amount of total phenolic content (TPC) and therefore exhibited high free radical scavenging activity. The onions peel extract is a richer source of TPC than ratan jot and in consequence it has a better free radical scavenging capacity (table 1).

Table 1: Total phenolic content and free radical scavenging activity of 80% methanolic extract of Onion peel and Ratan jot extract

	TPC (gm GAE/kg)	DPPH Inhibition (%)
Onion Peel Extract	48.02	87
Ratan Jot Extract	33.64	55.34

Both extracts are capable of inhibiting the lipid oxidation in Edible (sunflower) oil. The phenols present in onion peel in view of their structural identity has greater antioxidant capacity than ratan jot. Considerable amount of hydroperoxides were formed in edible oil during storage as shown by raise in peroxide value in the control sample which reached upto 68 meq.O_2/kg after 80 days of storage. The table 2 shows the comparative account of reduction of PV in control by the extract of onion peel and ratan jot, the data also reveals that peroxide value reducing capacity is dose dependent characteristic and onion extract is superior in controlling the oxidation of oil, perhaps because of large quantity of quercitin present in it.

Table 2: Peroxide values of oil samples during accelerated storage

Days	Control	Oinion Peel Extract			Ratan Jot Extract		
		300 ppm	600 ppm	900 ppm	300 ppm	600 ppm	900 ppm
0	$7.3\pm1.15^{a\ 1}$	$6.16\pm0.288^{a\ 1}$	$6.5\pm0.50^{a\ 1}$	$6.6\pm0.529^{a\ 1}$	$6.5\pm0.50^{a\ 1}$	$7.4\pm0.793^{a\ 1}$	$7.16\pm0.763^{a\ 1}$
10	$11.6\pm0.57^{d\ 2}$	$6.33\pm0.577^{a\ 1}$	$7.33\pm0.577^{a,b1}$	$6.6\pm0.529^{a,b1}$	$9.83\pm0.288^{c\ 2}$	$7.6\pm0.577^{b\ 1}$	$7.26\pm1.101^{a,b1}$
20	$19.3\pm1.52^{e\ 3}$	$13.66\pm0.577^{c\ 2}$	$11.83\pm0.288^{b\ 2}$	$9.9\pm0.173^{a\ 2}$	$13.66\pm0.577^{c\ 3}$	$17.16\pm1.040^{d\ 2}$	$14.5\pm1.322^{c\ 2}$
30	$19.6\pm2.80^{c,d\ 3}$	$14.83\pm1.040^{a\ 2}$	$20.33\pm0.577^{d\ 3}$	$20.66\pm0.577^{d\ 3}$	$17.33\pm1.154^{b\ 4}$	$17.83\pm0.288^{b,c2}$	$17.33\pm1.154^{b2,3}$
40	$27.6\pm1.52^{f\ 4}$	$18.50\pm1.50^{a\ 3}$	$23.16\pm0.763^{d\ 4}$	$21.16\pm0.763^{c\ 3}$	$20.83\pm1.04^{b,c\ 5}$	$19.16\pm1.040^{a,b2}$	$19.16\pm0.763^{a,b3}$
50	$38.6\pm2.51^{c\ 5}$	$28.83\pm1.040^{a,b\ 6}$	$26.66\pm3.055^{a\ 3}$	$27.16\pm1.040^{a\ 4}$	$34.66\pm1.154^{b,c4}$	$33.96\pm0.057^{b,c5}$	$25.33\pm5.033^{a\ 4}$
60	$49.6\pm0.57^{e\ 6}$	$36.5\pm1.322^{b,c,d\ 7}$	$38.16\pm2.020^{d\ 4}$	$32.66\pm1.154^{a\ 5}$	$37.50\pm0.866^{c,d5}$	$35.66\pm2.081^{b,c5}$	$35.03\pm1.001^{b\ 5}$
70	$59.1\pm0.76^{e\ 7}$	$44.66\pm1.154^{c,\ d\ 8}$	$43.03\pm0.950^{b,c\ 5}$	$36.5\pm1.322^{a\ 6}$	$46.33\pm1.527^{d\ 6}$	$45.33\pm2.309^{c,d6}$	$41.03\pm1.001^{b\ 6}$
80	$68.3\pm1.52^{e\ 8}$	$51.16\pm1.040^{c\ 9}$	$49.83\pm0.288^{b,c\ 6}$	$41.66\pm2.081^{a\ 7}$	$56.33\pm2.081^{d\ 7}$	$50.83\pm1.040^{c\ 7}$	$47.16\pm1.040^{b\ 7}$

Values are mean ± SD of three measurements. Values in each row with different superscript letters present significance difference ($p<0.05$) between each types, days are fixed. Values in each row with different superscript numbers present significance difference ($p<0.05$) between days, types are fixed.

The high efficacy of onion peel extract is supposed to be due to presence of quercitin.[21,22] On the other hand ratan jot contains phenolic acids i.e. hydroxybenzoic and hydroxycinnamic acid[23] which show limited lipid oxidation inhibition in comparision to quercitin due to structural differences.[20] The 3'4'dihydroxy substitution in the B ring, coexistence of the 2,3-double bond in conjugation with the 4-keto group and the 3-hydroxy group on the C ring of quercitin molecule provides excellent electron delocalization and stabilization of the phenoxy radical.[28] While hydroxybenzoic and hydrooxycinnamic acid in ratan jot extract are the monohydroxylated and monosubstituted phenols thus provide less stabilization of phenoxy free radical.[20] Moreover, monohydroxy phenolic acids are less efficient as antioxidants than polyhydroxy phenolic acids.[29, 30]

Such free radical scavenging structural features of quercitin result in high anitioxidant capacity which prevents the oxidation in polyunsaturated fatty acid present in edible oils including sunflower oil. It has been found that many compounds, including benzoic and cinnamic acid derivatives, can behave like prooxidants depending on their concentration [31]. However, in case of our study antioxidant activity of both extracts has been observed under the concentration used in the current study.

Another measure of degree of oxidation in vegetable oils is p-anisidine value (P-AV). It measures the secondary oxidation products formed following the hydroperoxide formation. The secondary oxidation products of lipid oxidation are aldehydes, ketones and lactones which are found in most oxidized substrates. PAV measures particularly the formation of 2-alkenals.[32] Noticeable amount of 2-alkenals have been produced in control oil sample at the end of the storage (upto 9.56 absorbance unit per gram oil). Significant reduction in 2-alkenals formation has been observed in oil with onion peel and ratan jot extract (table 3). Both extracts antioxidant activity was found dose dependent. However, similar to primary oxidized product the inhibitory action of onion peel extract against secondary oxidized product is found to be greater than the rattan jot extract.

Table 3: P-anisidine values of oil samples during accelerated storage

Days	Control	Onion peel extract			Ratan jot extract		
		300 ppm	600 ppm	900 ppm	300 ppm	600 ppm	900 ppm
1	2.15±1.064^{d,1}	1.79±0.518^{cd,1}	0.98±0.110^{abc,1}	0.41±0.103^{a,1}	2.24±0.609^{d,1}	1.34±0.157^{b,c,d1}	0.80±0.129^{a,b1}
10	2.26±0.149^{c,1}	2.15±0.025^{c,1}	1.42±0.143^{b,1}	0.76±0.087^{a,12}	2.47±0.214^{c,1}	2.52±0.198$^{c\ 2}$	1.36±0.472$^{b\ 1,2}$
20	5.68±0.181^{d,2}	2.70±0.971^{b,12}	1.56±0.178^{a,1}	1.48±0.299^{a,23}	3.92±0.570^{c,2}	2.92±0.429^{b2,3}	1.72±0.011$^{a\ 1,2}$
30	6.10±0.039^{f,2}	3.27±0.246^{c,2}	2.85±0.647^{bc,2}	1.56±0.178^{a,23}	5.04±0.070^{de,3}	4.37±0.739^{d4,5}	2.28±0.377$^{b\ 2,3}$
40	6.42±0.172^{d,23}	3.40±1.265^{bc,23}	2.74±0.260^{abc,2}	1.43±0.375^{a,23}	3.94±1.032^{c,2}	3.72±0.960^{c3,4}	2.22±0.765^{a,b2,3}
50	7.34±0.401^{d,45}	4.65±0.229^{c,4}	3.26±0.467^{b,23}	1.74±0.259^{a,23}	4.69±0.237^{c,23}	4.69±0.237$^{c\ 5}$	3.02±0.7^{b3,4}
60	6.98±0.473^{d,34}	4.37±0.189^{c,34}	3.86±1.028^{bc,34}	1.62±0.375^{a,23}	4.40±0.347^{c,23}	4.35±0.332$^{c\ 45}$	3.17±0.260^{b3,4}
70	8.08±0.393^{d,5}	4.89±0.226^{c,4}	4.36±0.727^{c,4}	2.39±0.104^{a,3}	4.49±0.239^{c,23}	4.51±0.235$^{c4.5}$	3.06±0.465^{b3,4}
80	9.56±0.325^{c,6}	5.20±0.469^{b,4}	4.39±0.162^{ab,4}	3.38±1.512^{a,4}	4.32±0.166^{ab,23}	4.28±0.152$^{a,b\ 45}$	3.65±1.188$^{a\ 4}$

Values are mean ± SD of three measurements. Values in each row with different superscript letters present significance difference ($p<0.05$) between each types, days are fixed. Values in each row with different superscript numbers present significance difference ($p<0.05$) between days, types are fixed.

Similar results have been observed in case of FFA formation. FFAs are formed due to the hydrolysis of triglycerides and may get promoted by the reaction of oil with moisture [33]. FFA content increases to a greater extend with the increase in storage period for control (table 4). While significantly lesser FFAs are observed in both extract at all concentration. Thus the total oxidation in the edible oil with onion peel extract is found to be less than the edible oil with ratan jot extract due the presence of greater total phenols content and high % of free radical scavenger phenols in it.

Table 4: Free fatty acid of oil samples during accelerated storage

Days	Control	Onion peel extract			Ratan jot extarct		
		300 ppm	600 ppm	900 ppm	300 ppm	600 ppm	900 ppm
1	$0.20\pm0.000^{b,1}$	$0.10\pm0.000^{a,1}$	$0.10\pm0.000^{a,1}$	$0.10\pm0.000^{a,1}$	$0.16\pm0.057^{b,1}$	$0.10\pm0.000^{a,1}$	$0.10\pm0.000^{a,1}$
10	$0.30\pm0.000^{c,2}$	$0.13\pm0.058^{a,12}$	$0.13\pm0.058^{a,12}$	$0.10\pm0.000^{a,1}$	$0.20\pm0.000^{b,12}$	$0.13\pm0.058^{a,12}$	$0.10\pm0.000^{a,1}$
20	$0.35\pm0.050^{b,23}$	$0.13\pm0.057^{a,12}$	$0.13\pm0.057^{a,12}$	$0.13\pm0.057^{a,12}$	$0.16\pm0.058^{a,1}$	$0.13\pm0.058^{a,12}$	$0.13\pm0.058^{a,1}$
30	$0.40\pm0.000^{b,3}$	$0.16\pm0.058^{a,123}$	$0.20\pm0.000^{a,23}$	$0.16\pm0.058^{a,123}$	$0.20\pm0.000^{a,12}$	$0.16\pm0.057^{a,12}$	$0.16\pm0.057^{a,1}$
40	$0.55\pm0.050^{b,4}$	$0.16\pm0.058^{a,123}$	$0.16\pm0.058^{a,123}$	$0.16\pm0.057^{a,123}$	$0.23\pm0.058^{a,123}$	$0.16\pm0.057^{a,12}$	$0.16\pm0.058^{a,1}$
50	$0.60\pm0.002^{d,45}$	$0.20\pm0.000^{ab,23}$	$0.20\pm0.000^{ab,23}$	$0.20\pm0.000^{ab,23}$	$0.26\pm0.057^{b,234}$	$0.20\pm0.000^{ab,23}$	$0.16\pm0.057^{a,1}$
60	$0.65\pm0.052^{b,5}$	$0.23\pm0.057^{a,34}$	$0.23\pm0.056^{a,34}$	$0.23\pm0.058^{a,34}$	$0.30\pm0.001^{a,34}$	$0.26\pm0.058^{a,34}$	$0.26\pm0.058^{a,2}$
70	$0.75\pm0.047^{b,6}$	$0.30\pm0.001^{a,45}$	$0.30\pm0.001^{a,4}$	$0.30\pm0.001^{a,4}$	$0.30\pm0.001^{a,34}$	$0.30\pm0.001^{a,4}$	$0.30\pm0.001^{a,2}$
80	$0.80\pm0.003^{b,6}$	$0.33\pm0.059^{a,5}$	$0.30\pm0.001^{a,4}$	$0.30\pm0.001^{a,4}$	$0.33\pm0.058^{a,4}$	$0.30\pm0.001^{a,4}$	$0.30\pm0.001^{a,2}$

Values are mean ± SD of three measurements. Values in each row with different superscript letters present significance difference ($p<0.05$) between each types, days are fixed. Values in each row with different superscript numbers present significance difference ($p<0.05$) between days, types are fixed.

Correlation between oxidation analysis parameters of all oil samples is shown in table 5. Siqnificant relationship has been observed in case of control. Next to this are the edible oil with onion extract at all concentration and edible oil with ratan jot at 900 ppm. While least relation has been seen in ratan jot edibel oil simple at 300 and 600 ppm.

Table 5: Correlation between PV, P-Anisidine and FFA of oil samples

Corelation	Control	Onion peel extract			Ratan jot extract		
		300 ppm	600 ppm	900 ppm	300 ppm	600 ppm	900 ppm
PV & Ani	0.883	0.892	0.928	0.795	0.554	0.704	0.837
FFA &PV	0.962	0.852	0.871	0.894	0.826	0.869	0.9
Ani & FFA	0.912	0.669	0.808	0.733	0.53	0.586	0.799

CONCLUSION

One of the PUFA rich edible oil (Sunflower) is successfully stabilized by the plant waste extract that contained free radical scavenger phenols. Phenols ring structure is responsable for its antioxidant activity in oil medium. Both extracts is found effective in preventing formation of hazardous compound in edible oil. Regarding the onion peel extract found to be more reactive in oxidatively stabilizing process of the edible oil than the ratan jot because of the amount and nature of phenols present. Moreover, the antioxidant activity in both the extract depends on the concentration; i.e. more the concentration of the phenols, higher more will be the antioxidant activity

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