Antioxidant properties of orange and lemon peels and their efficacy in preventing lipid peroxidation in stored oils

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ABSTRACT
The role of natural antioxidant compounds in preserving the frying stability of oils is increasingly recognized. Orange (Citrus x sinensis) and lemon peels (Citrus x aurantiifolia) are rich sources of antioxidant components. The objective of the current study was to determine antioxidant properties of orange and lemon peels and evaluate their efficacy in preventing lipid peroxidation in heated and stored unrefined sunflower (Helianthus annuus) and groundnut oils (Arachis hypogaea). Peel powder was added at 0.5 and 1.0% level. The oils were subjected to thermal treatment, stored and analyzed on 0, 10, 20, and 30 days for the determination of free fatty acid (FFA) and peroxide value (PV). The obtained results showed that orange peel had higher total polyphenolic, tannins, β-carotene and total carotene contents. The highest DPPH radical scavenging activity was observed in pure methanolic extract (70%). FFA ranged from 0.03-0.1 and 0.04-0.1% in sunflower oil heated at 60 ºC with 0.5 and 1.0 g peel powder, respectively. FFA was in the range of 0.35-0.45% for groundnut oil samples heated at 60 ºC with 0.5 g peel powder, whereas PV ranged between 3.65 and 8.4 meq/kg. The reduction in lipid peroxidation during storage of heat treated peel powder incorporated samples was remarkable for those heated at 60 ºC and stored for 10 days. Lemon peels were also rich in total phenols (716 mg/100 g) and flavonoids (168 mg/100 g) and showed considerable antioxidant activity. At the end of the storage duration, both thermally treated groundnut and sunflower oils showed lesser FFA over the ranges 0.12-0.15% and 0.38-0.52%, respectively and PV ranging from 8.96 to 11.5 meq/kg and from 7.9 to 10.9 meq/kg, respectively than control oils (FFA-0.18-0.9% and PV-12.2-21.2 meq/kg). This study showed that orange and lemon peels may be used as natural antioxidant agents to prevent lipid peroxidation.

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1. Introduction
There is a continued demand by the consumers to lower or eliminate chemical additives in food samples. This has resulted in search for alternatives, particularly those that could be taken from natural plant origin. Scientific investigations have shown that the EOs and extracts from various plant materials can act as food preservatives by preventing the proliferation of microorganisms along with acting as natural antioxidants since they possess a wide variety of bioactive compounds (Mohammadhosseini et al., 2016; Mohammadhosseini, 2017; Mohammadhosseini et al., 2017; Mohammadhosseini et al., 2019). Antioxidants can scavenge free radicals or other reactive oxygen species (ROS) that attack a variety of compounds in living cells and are of great help to strengthen defensive barriers in humans. Other activities susceptible to oxidation include enzyme inhibition and metal binding (Mohammadhosseini et al., 2016). Thus, plant materials with health promoting properties should be encouraged to be used by food processing sector for improving the oxidative stability of their product along with minimizing risks occurring through the use of synthetic additives (Frezza et al., 2017; Erenler et al., 2018).

In fact, vegetable oils have a tendency to be oxidized during use and storage which alter the texture of oil to a plastic like consistency besides becoming rancid...
Oils that are more unsaturated are readily prone to oxidation than those with less saturation tendency (Parker et al., 2003; Mahuya et al., 2008). As the degree of unsaturation increases, the rate of formation and the amount of primary oxidation compounds that are accumulated at the end of the induction period tends to increase (Martin-Polvillo et al., 2004). These changes are known to be accelerated in the presence of oxygen and metal ions. As an alternative option to prevent such adverse changes, nowadays antioxidants are added to both liquid and solid fats in order to enhance the oxidative stability of the oils (Guillen and Cabo, 2002). Antioxidants are used as food additives to prevent food from being deteriorated. These are added to food products such as oil, bread, cookies, biscuits and dairy products to enhance their shelf life via preventing lipid peroxidation and protecting from oxidative damage. Several experimental investigations have documented the positive effect of antioxidants on the stability of vegetable oils. An important class of antioxidants consists of the phenolic compounds, butyl hydroxy anisole (BHA), butyl hydroxy toluene (BHT), propyl gallate, and tert-butyl hydroquinone (TBHQ). These are widely used in vegetable oils recommended for domestic and industrial processes. However, there is a rising awareness regarding possible adverse effects of application of synthetic antioxidants for food usage. Thus, consumers are demanding for better, safer and health promoting natural antioxidants. Vegetable oils in their natural form are also known to possess constituents which could function as natural antioxidants. Amongst them, the important natural antioxidants are ascorbic acids, tocopherols, carotenoids, chlorogenic acids and flavanols (Ullah et al., 2003).

Benavente et al. (2000) demonstrated that the shelf life of fats and oils could be extended via utilization of natural antioxidants from olive leaf. In another study, fried potato chips were shown to retain optimum sensory quality characteristics when treated with the antioxidant component from methanolic extract of peanut hulls on storage (Rehman, 2003).

Fruit and vegetable processing in India generates substantial quantities of waste. The by-products of fruits are considered as an abundant source of antioxidant polyphenols (Kammerer et al., 2014). The peels and pomace are regarded as the potential source of sugars, minerals and organic acids, dietary fibers and phenolics which are known to have a wide range of actions including antioxidants, antimutagenic, cardio preventive, antibacterial and antiviral activities (Moure et al., 2001). Thus, utilization of waste as a source of polyphenols and antioxidants could be noted to have considerable economic benefit to food processors.

There has been an increased interest in natural antioxidants in recent years for their vital role in preventing the autooxidation of fats, oils and fat containing food products. In a study by Singh and Immanuel (2014), the peels of pomegranate, lemon and orange were used as proper sources of natural antioxidants. The results indicated that among the three extracts, pomegranate exhibited a high percentage of antioxidant activity and phenolic content of 92.7% and 249.41 mg/g in comparison to lemon and orange peel extract. Furthermore, maximum total phenolic content was found in lemon extract (0.9 mg/g). Paneer samples prepared by addition of natural antioxidant extracts from these peels were subjected to sensory studies which showed that the extracts at the level of 2% were acceptable and shown to have the greater ability to prevent peroxide formation. The ability to prevent peroxide formation in paneer sample was in the order of pomegranate peel> lemon peel> orange peel.

The extraction of phenolic compounds from citrus peels have gained interest as a source of natural antioxidants and antimicrobial agents in food stuffs (Muhammad, 2010). These compounds have high antioxidant activity and have been documented to exert antimicrobial effects against various food borne pathogens (Hayat et al., 2010; Espina et al., 2011; Delgado-Adámez et al., 2012a, b; El-Seedi et al., 2012) as they possess high contents of terpenoids, tannins, quinones, phenolic acids and polyphenols (Calvo et al., 2006; Lee and Lee, 2010).

Antioxidant substances in citrus waste (e.g. peels) have been proposed to kill microbial flora in soil and increase the acidity of the medium (Sharma et al., 2017). The citrus waste is also widely used as an additive to afford protection against oxidative degradation of foods (Kumaran and Karunakaran, 2006). This property is attributed to the ability of phenolic compounds which help to scavenge free radicals, break radical chain reactions exerting chelating action on metals (Nayak et al., 2015). Fruit peels are known to possess naturally biologically active compounds viz natural antioxidants, that could be used as cheap sources of functional ingredients as well as food additives (Marín et al., 2002; Puupponen-Pimiä et al., 2002; Hayat et al., 2009; Galanakis, 2012; Norah et al., 2012). The present investigation was planned with an objective of exploring the antioxidant properties of orange and lemon peel powders and evaluating their efficacy as inhibitors of oxidative processes in stored oils.

2. Experimental

2.1. Materials

The oils needed for the study, namely sunflower (Helianthus annuus) oil (Phalada Agro Research Foundations Pvt. Ltd. Mysore, Karnataka, India) and groundnut (Arachis hypogaea) oil (Dharani Farm co-operative Ltd. Mysore, Karnataka, India) manufactured by the process of cold press and free of any additives were obtained from the local organic shop. Fresh lemon (Citrus limon) and oranges (Citrus sinensis) were purchased from the local market and subjected
to further processing steps as required for the study. Chemicals used for the study comprising L-ascorbic acid, β-carotene, and 2,2’-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma (Sigma-Aldrich, USA) Chemical Co, and all other chemical compounds were obtained from E-Merck, Mumbai or Qualigens Fine Chemicals, Mumbai, India.

2.2. Methods

The study design consisted of sequential steps including i) preparation of peel powders from orange and lime, ii) analysis of antioxidant components and activity of peel powders, iii) addition of peel powders to unrefined groundnut and sunflower oils, iv) subjecting the oils to thermal treatment, v) storage of oils and evaluation of lipid peroxidation by free fatty acids and vi) peroxide value analysis.

2.2.1. Preparation of peel powders

The peels from orange and lemons were separated and dried in a hot air oven at 60 °C for 8 h. The dried peels were ground into a fine powder using a mixer and sieved in a 60 µ mesh. The powders were then transferred to air tight containers and stored at 4 °C until further use.

2.2.2. Thermal treatment of oils

Unrefined groundnut and sunflower oils were used as the substrate for oxidation studies. Accurately, weighed quantities of peel powders (0.5% and 1%, respectively) were added to a 20 mL portion of the oil. The glassware containing peel powder and oil samples were wrapped with aluminum foil to prevent photo-oxidation. The wrapped tubes were then subjected to heat treatment at temperatures of 60 °C, 80 °C and 100 °C, respectively for 4 h in a temperature controlled incubator. They were then stored under darkness at 27 °C until the completion of predetermined time interval of storage. Two sets of control samples, namely (i) without heat treatment and with and without the addition of peel powders and (ii) with heat treatment and without the addition of peel powders were also maintained under similar storage conditions for further comparison. The oil samples of each treatment were withdrawn on 0, 10, 20 and 30 days for analyzing the FFA and PV contents.

2.2.3. Antioxidant properties of peel powders

Peel powders were analyzed for their total phenolics, flavonoids, tannins, total carotenoids and β-carotene contents. Antioxidant activity was determined using the corresponding assays and the extraction media used were composed of water, methanol, ethanol and 80% methanol.

2.2.3.1. Estimation of antioxidant components

Total phenolic content of peel powders was measured using the Folin-Ciocalteau assay and a phenolic acid (tannic acid) to set up a calibration curve following the method of Matthaus (2002). Results were expressed as tannic acid equivalent (TAE)/100 g of sample. The total flavonoid content was determined using the Dowd method (Arvouet-Grand et al., 1994) and expressed as rutin equivalent (mg of RE/g of extract). Colorimetric estimation of tannins was based on the measurement of blue color formed by the reduction of phosphotungstomolybdic acid by tannin like compounds in alkaline solution. Total tannin content was expressed as mg TAE/100 g of sample (Ranganna, 1986). Samples were extracted in acetone and transferred to petroleum ether phase for carotenoids estimation. Total carotene was read colorimetrically using petroleum ether for baseline correction. β-Carotene was separated by column chromatography and read colorimetrically (Ranganna, 1986).

2.2.3.2. Determination of antioxidant activity

Total antioxidant activity was determined by phosphomolybdenum method and expressed as the number of equivalents of ascorbic acid (Prieto et al., 1999). Radical scavenging activity of the samples was determined according to Shimada et al. (1992) method, which is based on the scavenging of the DPPH (1,1’-diphenyl-2-picrylhydrazyl) radical. Reducing power was measured by the formation of Perl’s Prussian blue at 700 nm which is based on the reduction of Fe³⁺/ ferricyanide complex to the ferrous form by antioxidants (Oyaizu, 1986). The Fe²⁺ formed was monitored by measuring the formation trend of Perl’s Prussian blue.

2.2.4. Peroxide Value (PV) and Free Fatty Acid value (FFA)

Oil was analyzed for FFA and PV initially and on every 10th day up to 30 days of storage. FFA (as % oleic acid) was estimated using an alkali titration method (AOCS, 2000). PV (meq kg/oil) was measured by titration with sodium thiosulfate solution (0.1 M) using starch as an indicator (AOCS, 2000).

2.3. Statistical analysis

The results were compiled to obtain mean and SD values. The data were also analyzed using t-test to determine the level of significance between the treatments using the statistical software excel stat version 16.0.

3. Results and Discussion

3.1. Antioxidant components of orange and lemon
peel powders

The results of the study have been shown in Tables 1-3 and Figs 1-2. Data pertaining to the antioxidant components of orange and lemon peel powders have been represented in Table 1, as well. As seen, the tannin content of orange peel was significantly higher (31.3 mg/100 g) than lemon peel (26.6 mg/100 g). Tannins, as water soluble polyphenols, are present in many plant foods. These are considered nutritionally undesirable because they precipitate proteins, inhibit digestive enzymes and affect the utilization of vitamins and minerals. Tannins in food plants serve as a natural defense mechanism against microbial infections and are found in a wide variety of foods such as millets, barley, dry beans, peas, apple, banana, black berries, cranberries, dates and grapes (Chung et al., 1998). Okwu and Emenike (2006) analyzed the phytonutrients in fruits and found the tannin content of sweet orange and tangerine to be higher (0.04 mg/100 g) followed by grape content (0.03 mg/100 g). The presence of tannins could be responsible for the bitter principle and sour taste of some citrus species.

Approximately, 115 different carotenoids have been reported in citrus fruits. The color of orange and its peel is due to carotenoid content. Pink grape fruit contains the highest amount of β-carotene. Other citrus fruits also contain high levels of carotenoids such as lutein, zeaxanthin and β-cryptoxanthin (Mangels et al., 1993). The present study indicated the presence of higher amounts of β-carotene in orange peel (645 µg/100 g sample) in comparison to lemon peel which had only 188 µg/100 g of these natural pigment. In addition, remarkably higher total contents of carotene were present in orange peel compared to those found in the lemon peel (28,789 µg vs. 2,445 µg/100 g).

On the other hand, the highest phenolic concentration was found in the water extracts of orange peel (1873 mg/100 g). For 80% methanol, pure methanol and ethanol, they were in the order of 1703, 1603 and 1153 mg/100 g, respectively. For lemon peel, the samples extracted with water showed higher phenolic content (716 mg/100 g) compared with other media (range, 373-687 mg/100 g). Polyphenols constitute a complex group of substances, namely coumarins, xanthines, stilbenes, flavonoids, lignans and tannins (Saura-Calixto and Bravo, 1995). These are suggested to have promising anticancer property and found to be present in highest concentration in citrus peel which is mainly composed of ferulic, sinapic, coumaric and caffeic acid as well as hesperidin and naringin. Gorinstein et al. (2001) in a comparative analysis of biochemical characteristics of citrus fruits reported highest total phenolic content in peels of lemons (190 mg/100 g) followed by oranges (179 mg/100 g) and grape fruit (155 mg/100 g).

In the present study, the total phenolic content of both orange and lemon peel powders were shown to be much higher than the previously reported

Table 1
Total polyphenolic, flavonoid and antioxidant activity of lemon and orange peel powders.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Tannin content (mg/100g)</th>
<th>Total Carotene (µg/100g)</th>
<th>β-Carotene (µg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange peel</td>
<td>31.3±6.65</td>
<td>28497±292</td>
<td>648±3.5</td>
</tr>
<tr>
<td>Lemon peel</td>
<td>26.6±0.02**</td>
<td>2416±28.3***</td>
<td>186±1.5**</td>
</tr>
</tbody>
</table>

Antioxidant properties: Extraction in different media

<table>
<thead>
<tr>
<th>Extraction media</th>
<th>Tannin content (mg/100g)</th>
<th>Total phenolic content (mg/100g)</th>
<th>Flavonoid content (mg/100g)</th>
<th>Total antioxidant activity (µmol ascorbic acid/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange peel</td>
<td>1873±57.0</td>
<td>1703±33.0</td>
<td>1603±4.00</td>
<td>1153±28.0</td>
</tr>
<tr>
<td>Lemon peel</td>
<td>716±14.0**</td>
<td>687±28.0**</td>
<td>493±0.17</td>
<td>373±10.0**</td>
</tr>
</tbody>
</table>

Flavonoid content (mg/100g)

| Orange peel        | 145±0.04                 | 165±0.03                       | 181±0.02                  | 78.5±0.71                                    |
| Lemon peel         | 168.5±0.30**             | 220±0.10                      | 60.6±0.88                 | 78.0±0.53**                                  |

Statistically significant difference between orange and lemon peel powder application of Student’s ‘T’ test. *significant differences; ns: no significant difference

Fig. 1. Free radical scavenging activity of lemon and orange peel extracted in various media.
Citrus flavonoids have potent antioxidant activity that were absent in orange fiber. However, in the present study, the flavonoid content of eight different varieties of citrus peel had a negligible amount of 21.6 mg/g on dry basis. The position and degree of hydroxylation is of primary importance in determining the antioxidant activity of flavonoids (Shahidi et al., 1992). Citrus flavonoids have been extensively investigated because of their health promoting properties (Middleton and Kandaswami, 1994). In the present investigation, the flavonoid content of orange peel powder extracted in water, 80% methanol, pure methanol and ethanol were 145, 165, 181 and 78.5 mg/100 g, respectively. The corresponding values for lemon peel were also found to be 168.54, 220, 606 and 78.0 mg/100 g. Wang et al. (2007) analyzed the flavonoid content of eight different varieties of citrus fruits grown in Taiwan and found that seven varieties had less than 20 mg flavonoid per gram. The lemon peel had a negligible amount of 21.6 mg/g on dry basis. However, in the present study, the flavonoid content of lemon peel powder was much higher than those reported previously. The total antioxidant activity of both peels was highest in water media followed by 80% methanol, methanol and ethanol. Measured as μmol of ascorbic acid/g in water extract, the values were 79,480 and 68,750 for orange and lemon peel, respectively.

Table 2
Effect of storage on free fatty acids and peroxide value of thermally treated sunflower oil.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Free Fatty Acids (% oleic acid)</th>
<th>Peroxide Value (Meq/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days 0 10 20 30 0 10 20 30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unheated oil samples</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.08 ± 0.01 0.12 ± 0.01</td>
<td>6.21 ± 0.01 7.01 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Orange peel (0.5%)</td>
<td>5.00 ± 0.00 6.00 ± 0.00</td>
</tr>
<tr>
<td>Orange peel (1%)</td>
<td>0.02 ± 0.01</td>
<td>3.81 ± 0.03 4.56 ± 0.03</td>
</tr>
<tr>
<td>Lemon peel (0.5%)</td>
<td>0.06 ± 0.01</td>
<td>5.62 ± 0.03 6.96 ± 0.03</td>
</tr>
<tr>
<td>Lemon peel (1%)</td>
<td>0.10 ± 0.01</td>
<td>4.82 ± 0.03 6.30 ± 0.03</td>
</tr>
<tr>
<td>Oil treated at 60°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.06 ± 0.01 0.15 ± 0.01</td>
<td>5.16 ± 0.01 6.44 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Orange peel (0.5%)</td>
<td>0.05 ± 0.01 0.06 ± 0.01</td>
</tr>
<tr>
<td>Orange peel (1%)</td>
<td>0.04 ± 0.01</td>
<td>3.65 ± 0.01 6.13 ± 0.01</td>
</tr>
<tr>
<td>Lemon peel (0.5%)</td>
<td>0.06 ± 0.01</td>
<td>4.64 ± 0.01 7.09 ± 0.01</td>
</tr>
<tr>
<td>Lemon peel (1%)</td>
<td>0.02 ± 0.01</td>
<td>5.15 ± 0.01 7.16 ± 0.01</td>
</tr>
<tr>
<td>Oil treated at 80°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.06 ± 0.01 0.15 ± 0.01</td>
<td>5.16 ± 0.01 6.44 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Orange peel (0.5%)</td>
<td>0.05 ± 0.01 0.06 ± 0.01</td>
</tr>
<tr>
<td>Orange peel (1%)</td>
<td>0.04 ± 0.01</td>
<td>3.65 ± 0.01 6.13 ± 0.01</td>
</tr>
<tr>
<td>Lemon peel (0.5%)</td>
<td>0.06 ± 0.01</td>
<td>4.64 ± 0.01 7.09 ± 0.01</td>
</tr>
<tr>
<td>Lemon peel (1%)</td>
<td>0.02 ± 0.01</td>
<td>5.15 ± 0.01 7.16 ± 0.01</td>
</tr>
</tbody>
</table>

*Statistically significant difference in comparison to control.

The position and degree of hydroxylation is of primary importance in determining the antioxidant activity of flavonoids (Shahidi et al., 1992). Citrus flavonoids have been extensively investigated because of their health promoting properties (Middleton and Kandaswami, 1994). In the present investigation, the flavonoid content of orange peel powder extracted in water, 80% methanol, pure methanol and ethanol were 145, 165, 181 and 78.5 mg/100 g, respectively. The corresponding values for lemon peel were also found to be 168.54, 220, 606 and 78.0 mg/100 g. Wang et al. (2007) analyzed the flavonoid content of eight different varieties of citrus fruits grown in Taiwan and found that seven varieties had less than 20 mg flavonoid per gram. The lemon peel had a negligible amount of 21.6 mg/g on dry basis. However, in the present study, the flavonoid content of lemon peel powder was much higher than those reported previously. The total antioxidant activity of both peels was highest in water media followed by 80% methanol, methanol and ethanol. Measured as μmol of ascorbic acid/g in water extract, the values were 79,480 and 68,750 for orange and lemon peel, respectively.
The free radical scavenging activity of orange and lemon peel powder are presented in Fig. 1. For orange peel, the highest activity was observed for pure methanolic extract and ranged between 19.7-70%. Water media also exhibited a comparatively higher range (24-65%) followed by 80% methanol which was slightly lower. The ethanolic extract, however, showed a much lower range of activity (4-51%). For lemon peel, the highest activity was recorded in 80% methanolic extract (7-25.41%) and the lowest in ethanolic media (3.97-14.3%). Water media and pure methanolic extracts were in between. In comparison, orange peel exhibited a slightly higher range of scavenging activity than that of lemon peel.

Kuljarachanan et al. (2009) conducted an investigation on antioxidant compounds in fresh and blanched lime residues during drying. Results indicated that fresh lime residues had good scavenging activity against DPPH radicals which reduced from 94 to 91% after blanching. This could be due to the loss or degradation of phenolic compounds or other DPPH radical scavenger components during blanching (Amin et al., 2006).

3.2. Reducing power assay

The reducing power of orange and lemon peel is represented in Fig. 2. As can be seen in this figure, in aqueous (water) media, the values ranged from 0.287-1.016 for orange peel and 0.179-0.36 for lemon peel. Additionally, the reducing power exhibited by 80% methanolic extract varied between 0.372-0.949 and 0.147-0.457 for orange and lemon peel powder, respectively. In pure methanolic extract, the activity level was also found to be higher for orange peel compared with lemon peel. However, the ethanolic extract was found to have the least activity.

The power of certain antioxidants is associated with their reducing power on account of the presence of reductones (Duh, 1998; Jayaprakasha et al., 2001). Jeong et al. (2004) reported that the reducing power of ethanolic or water extract of citrus peels increased significantly with heat treatment. An increase of 68.7 and 80.5% was seen in ethanolic extract on heating at 100 and 150 °C for 30 min. In water extract, the increase was 2.5 times on heating in similar conditions for 10 min.

3.3. Estimation of peroxide value and free fatty acid content

Table 2 provides information about the peroxide value (PV) and free fatty acid (FFA) content of sunflower oil subjected to various degrees of heat treatment with the incorporation of orange and lemon peel powder. For control samples without heat treatment, a gradual increase in the FFA content was evident. For samples treated with 0.5 g orange peel powder, the FFA ranged between 0.05-0.11% and was still lower (0.02-0.1%) with 1.0 g of orange peel powder. Lemon peel treated oils, however, showed a slightly higher FFA in comparison to control (0.17-0.18%) at the end of 30 days. Orange
Table 3
Effect of storage on free fatty acids and peroxide value of thermally treated groundnut oil.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Free fatty acids (% oleic acid)</th>
<th>Peroxide value (Meq/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Unheated oil samples</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>±0.03±0.06</td>
<td>±0.08±0.08</td>
</tr>
<tr>
<td>Orange peel</td>
<td>±0.07±0.04</td>
<td>±0.09±0.05</td>
</tr>
<tr>
<td>(0.5%)</td>
<td>±0.08±0.06*</td>
<td>±0.07±0.07*</td>
</tr>
<tr>
<td>Orange peel</td>
<td>±0.39±0.39</td>
<td>±0.42±0.42</td>
</tr>
<tr>
<td>(1%)</td>
<td>±0.12±0.10**</td>
<td>±0.05±0.04*</td>
</tr>
<tr>
<td>Lemon peel</td>
<td>±0.35±0.38</td>
<td>±0.39±0.39</td>
</tr>
<tr>
<td>(0.5%)</td>
<td>±0.07±0.08*</td>
<td>±0.05±0.06**</td>
</tr>
<tr>
<td>Lemon peel</td>
<td>±0.36±0.37</td>
<td>±0.38±0.38</td>
</tr>
<tr>
<td>(1%)</td>
<td>±0.36±0.37</td>
<td>±0.50±0.54</td>
</tr>
<tr>
<td></td>
<td>Oil treated at 60 °C</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>±0.13±0.16</td>
<td>±0.14±0.20</td>
</tr>
<tr>
<td>Orange peel</td>
<td>±0.05±0.10*</td>
<td>±0.08±0.06**</td>
</tr>
<tr>
<td>(0.5%)</td>
<td>±0.33±0.40</td>
<td>±0.41±0.41</td>
</tr>
<tr>
<td>Orange peel</td>
<td>±0.11±0.09**</td>
<td>±0.09±0.05*</td>
</tr>
<tr>
<td>(1%)</td>
<td>±0.34±0.46</td>
<td>±0.42±0.42</td>
</tr>
<tr>
<td>Lemon peel</td>
<td>±0.05±0.03**</td>
<td>±0.12±0.03**</td>
</tr>
<tr>
<td>Lemon peel</td>
<td>±0.39±0.43</td>
<td>±0.45±0.48</td>
</tr>
<tr>
<td>(0.5%)</td>
<td>±0.08±0.06**</td>
<td>±0.05±0.03*</td>
</tr>
<tr>
<td>Orange peel</td>
<td>±0.35±0.36</td>
<td>±0.45±0.45</td>
</tr>
<tr>
<td>(1%)</td>
<td>±0.13±0.03**</td>
<td>±0.06±0.04**</td>
</tr>
<tr>
<td>Lemon peel</td>
<td>±0.36±0.40</td>
<td>±0.50±0.52</td>
</tr>
<tr>
<td>Lemon peel</td>
<td>±0.10±0.05**</td>
<td>±0.05±0.03*</td>
</tr>
</tbody>
</table>

*Statistically significant differences

and lime peels exhibited better antioxidant abilities in oils heated at 60 °C, where, in comparison to control, FFA were much lower at the end of storage period. Lemon peel was also effective in heated samples as an antioxidant, in comparison to what was observed in unheated samples.

Oil heated at 80 °C also showed similar trend with respect to the FFA content. As the days of storage increased, a gradual increasing trend in FFA content was seen, but the level of increase was found to be negligible. Oil samples treated with 0.5 g orange peel powder showed a relatively lower FFA content. The range of FFAs observed for oil samples with 0.5 and 1.0 g of orange peel and lemon peel were 0.05-0.16, 0.07-0.13, 0.09-0.12 and 0.06-0.13, respectively. Samples subjected to heating at 100 °C also showed still lower FFA content. Among these, the oil treated with 1.0 g of orange peel powder showed lesser FFA values ranging from 0.04-0.15%. Slightly higher increases in FFA were noted for samples treated with 1.0 g lemon peel powder with values in the range of 0.09-0.15%.

PV is a measure of the amount of peroxides formed
in fats and oils through autoxidation and oxidation processes. In fact, the term PV is an indirect measure of the degree of initial oxidation of fats and oils. Control sample (sunflower oil) without heat treatment and without the addition of citrus peel powders indicated a greater increase in the PV through the days of storage reaching up to 11.01 meq/kg at the end of 30 days. In comparison, orange and lime peel added samples showed an increase up to 8.79-10.1 meq/kg. Similar observations were made in oil heated up to 60 °C. At 80 and 100 °C, though the samples heated with orange and lime peel showed a slightly higher range of values, they were still lower than the control sample. In all cases, orange peel was more effective than lemon peel in affording protection against oxidation. For sunflower oil treated at 60 °C, statistical analysis for FFA content demonstrated mild to extremely significant differences for the samples stored with 1.0 g of orange peel powder. Similarly, for lemon peel powder on 0th day, the difference was noticed to be statistically significant, while for 10th day there was no significant difference.

Che Man and Jasvir (2000) determined the effect of rosemary and sage extracts (04%) on frying performance of refined, bleached and deodorized palmolein during deep fat frying in comparison to unheated oils over a duration of 6 days. The results indicated that on day one, the PV for rosemary and sage treated oils were 2.18 and 1.98 meq/kg, respectively, while that of control was 6.55 meq/kg. In accordance with this study, on day 6, the values were 7.22, 6.95 and 11.5 for rosemary, sage and control, respectively. The lower PV of samples treated with either rosemary or sage was due to the antioxidative activity of both the materials. Another study by Chang et al. (1977) reported that rosemary extract was found to be effective when applied to steamed lard, chicken fat, sunflower oil or corn oil at level of 0.02% and reduced PV by about 50%. It was comparable to commercial antioxidants such as BHA, BHT, propyl gallate and citric acid especially when added to animal fat.

Antioxidant efficacy of leafy vegetable powders on thermal stability of oils was studied by Shyamala et al. (2007). Refined groundnut oil and sunflower oil were used as substrate for this study. Four types of green leafy vegetables, namely cabbage, coriander leaves, hongone and spinach were employed for determining their ability to prevent lipid peroxidation. About 2.0 g of dry leafy vegetable powder/100 mL oil were added. Oil samples without additive were used as the control. All samples were subjected to heating and accelerated storage conditions and tested for PV during and at the end of 4 weeks. Results showed that heating and storage accelerated the peroxidation in oils with all green leafy vegetables accounting for a protective effect. The effect was more pronounced in sunflower oil than the groundnut oil. It was also concluded that among the leafy vegetables, cabbage was most effective in preventing lipid peroxidation.

The peroxide value (PV) and free fatty acid (FFA) content of groundnut oil subjected to various degrees of heat treatment with the incorporation of orange and lemon peel powder is compiled in Table 3. From the results on the changes in FFA and PV during storage of unrefined groundnut oil subjected for 60 °C, 80 °C and 100 °C heat treatment with and without the addition of peel powders, it is apparent that the FFA values obtained for the control samples of groundnut oil were slightly higher than those obtained for sunflower oil. For the oil samples treated at 60 °C with 0.5 g of orange peel powder, the FFA was in the range of 0.35-0.45%. Samples treated with 1.0 g of orange peel powder exhibited a relatively lower FFA than those treated with 0.5 and 1.0 g of lemon peel powder. Samples treated at 80 °C also followed a similar trend and FFA for 1.0 g orange peel powder treated samples were much lesser than others. Addition of 0.5 and 1.0 g of lemon peel powder was found to stabilize the FFA which was evident from the data over storage duration.

The increase in PV of control sample was very marginal up to 20th day storage and PVs were in the range of 8.23-8.59 meq/kg, whereas in samples stored up to 30 days, a steady increase was observed (9.65 meq/kg). For samples with 0.5 g and 1.0 g of orange peel, the PVs were in the range of 7.73-8.83 meq/kg and 7.25-8.06 meq/kg respectively during the entire 30 days of storage. In samples treated with 0.5 g lemon peel, the PV ranged between 7.22-8.65 meq/kg and for 1.0 g lemon peel powder, it was 7.52-8.65 meq/kg. Oil samples treated at 60 °C indicated relatively lower PVs compared with unheated oil samples. Samples treated with 1.0 g of orange peel powder were found to inhibit peroxidation and thereby resulting in lower production of PV (7.3-7.86 meq/kg). Control samples had the highest increase in PV (7.5-11.24 meq/kg). Samples treated at 80 °C also exhibited a similar trend in PVs.

Groundnut oil samples heated at 100 °C showed appreciable reduction in PV for samples treated with 1.0 g of orange peel powder (7.34-9.29 meq/kg). On the addition of 0.5 g lemon peel powder, a comparatively higher increase in PV was seen (8.95-10.6 meq/kg). Overall results indicated that groundnut oil samples treated with 1.0 g of orange peel powder exhibited a highest level of efficiency by preventing the deterioration of the stored samples. With the results obtained through the present investigation, it seems that orange peel can be considered to have the highest level of oxidation inhibitory capacity since in all the heat treated samples it showed the minimal levels of increment in PVs among all the treated groundnut oil samples.

4. Concluding remarks

The present investigation demonstrated that between the two peel powders studied, orange peel had higher antioxidant activity than lemon peel powder. This could be attributed to the overall bioactive
components present in the peels. When compared with the calculated absolute values of FFA and PV, lemon peel exhibited a higher antioxidant capacity in heated and stored oil samples. Taking into account the achieved results, the orange peel and lemon peel can be used as natural antioxidants to prevent peroxidation in heated and stored oils.

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