The Effect of Orange Peel Oil on Physicochemical, Microbiological and Sensory Properties of Plum Fruit Roll Ups

Mahshid Zamankhani¹*, Moshkan Abdolahi²

1- PhD. Student, Department of Food Science & Technology, North of Tehran, Branch, Islamic Azad University, Tehran, Iran
* Corresponding author (mahshidzamankhani@yahoo.com)
2- MSc. Graduated, Department of Food Science & Technology, Damghan, Branch, Islamic Azad University, Damghan, Iran

Abstract
By increasing consumer awareness about food safety and quality, there is a high demand for the preservative (synthetic)-free foods and use of natural products as preservatives. Plants are the main source of antimicrobials and contain many essential oils that have preservation effect against different microorganisms. The effect of natural orange peel oil on the chemical and microbiological and sensory properties of plum fruit roll ups was investigated during 15 days. The result of the Tensile strength tests plum fruit roll ups showed that adding orange peel to plum fruit roll ups significantly (P<0.05) increased the tensile strength. At the end of storage of all samples, with orange peel oil the lowest pH content and highest acidity level were observed in the sample with 0.5% orange peel oil and no mold and yeast were observed in any of the samples. The sample with 0.5% had a maximum amount of lactic acid bacteria and the sample with 0.1% orange peel oil had a minimum amount of lactic acid bacteria. The results showed that sample with 0.5% orange peel oil had minimum number of total counts of bacteria and Control sample had a maximum amount of total counts of bacteria. The analysis of variance did not show any significant difference in plum fruit roll ups. According to the results of the analysis of variance, the highest texture, taste and aroma score belonged to the control sample. From the results, it can be deduced that due to the antimicrobial properties of orange peel essence and its positive effects on tissue characteristics, it can be used as a natural preservative in the plum fruit roll ups.

Introduction
This specie belongs to Rosaceae family that comprises other plants that also produce edible fruits such as peach (Prunus persica), cherries (Prunus cerasus and Prunus avium) and apricot (Prunus insitia) (Stephen, 1983). Thought the original application of plums is for direct utilization, there are many other applications for it in the culinary and industry, such as in the production of plum juice, plum puree, mixtures with cereal or even ground meat, and for the expansion of products that replace fat in baking (Stacewicz-Sapuntzakis, Bowen, Hussain, Damayanti-Wood, & Farnsworth, 2001).
Plums are also used as a therapy for diverse illnesses. Recent studies also showed clinical document of its activity in the remedy of constipation, osteoporosis, hypertension and dyslipidemia (Stacewicz-Sapuntzakis et al., 2001). It was also shown that fibres from plums lowered plasma and liver lipids (Tinker, Davis, & Schneeman, 1994) and perhaps were the maximum important ingredients responsible for satiety boost by plums use (Farajian, Katsagani, & Zampelas, 2010). Carbohydrates are the principal macronutrient in plums, and include 62.7% of their whole weight (Stacewicz-Sapuntzakis et al., 2001). Despite this numerous content and their importance for some biological functions attributed to plums, up to now, just the presence of a xyloglucan was reported in plums. Essential oils (EO’s) and extracts taken from plenty plants have recently achieved a great amicability and scientific concern. Phenolic compounds present in essential oils have been identified as bioactive ingredients with antimicrobial activity. Maximum plant phenolic compounds are classified as generally identified as Safe (GRAS) substances, so they could be used to inhibit growth of multitude pathogenic and spoilage microorganisms in foods (Burt, 2004; Nedorostova, Kloucek, Kokoska, Stolcova, & Pulkrabek, 2009). However, EOs antimicrobial efficacy in foods is usually achieved at higher concentrations, which many times entail a sensory impact, caused by altering the natural taste and/or odor of the food by exceeding the passable flavor and/or odor thresholds (Nazer, Kobilinsky, Tholozan, & Dubois-Brissonnet, 2005). A small number studies have been published regarding prohibition of microorganisms by the vapor-phase generated by EO’s (Inouye, Uchida, Maruyama, Yamaguchi, & Abe, 2006; López, Sánchez, Batlle, & Nerín, 2007; Nielsen & Rios, 2000; Suppakul, Miltz, Sonneveld, & Bigger, 2003), pointing out that EOs applied in vapor phase could be impressive against foodborne pathogens and spoilage microorganisms at relatively lower concentrations than when applied in liquid phase, therewith causing less effect on sensory properties (Tyagi & Malik, 2011). López et al. (2007) established the antifungal activity of Mexican oregano EO by vapor contact on Aspergillus flavus. Farhat et al. (2011) reports that orange peel accounts for nearly 45% of the total bulk with considerable content of it available as a product after orange processing that make environmental problems, exclusively water pollution, due to the presence of biomaterials such as EO, pectin, and sugars. Citrus spp. EO’s are present in plentiful quantities and it is known that can have an antimicrobial result against both bacteria and fungi (Chanthaphon, Chanthachum, & Hongpattarakere, 2008; Jafari et al., 2011). Orange peel involves of epidermis covering the exocarp consisting of irregular parenchymatous cells, which are wholly enclosing many glands or oil sacs (Lin, Sheu, Hsu, & Tsai, 2010).

The present study was undertaken to determine the potential of various peel oil orange as an antimicrobial agent against microorganisms such as mold and yeast and lactic acid bacteria and total counts of bacteria; an attempt to formulate as natural food preservatives.

Materials and methods
Fruit materials
Fresh plums were collected from a garden in Shahmirzad, Semnan, Iran, and transported directly to the laboratory for tests. Fruits were selected according to their size, colour and appearance, discarding the ones with defects and physiological disorders. Then, the selected plums were sanitized with chlorinated water (200 ppm sodium hypochlorite) for 3 min and left to get dry at room temperature for about 1 h. Plums later got boiled and were filtered out. Further the peel and core were deleted. Later, according to the
formulations, the orange peel oil was added to the plum puree. This content, then, has been distributed on a tray and left to be dried in the sun.

**Preparation of orange peel oil**
Orange oil has been acquired from orange peels by the process of steam distillation (Harbone, 1998), using the Clevenger apparatus (Pyrex UK). The peels were placed in the round bottom flask and filled with water to about three quarter full. This flask was connected to distillation apparatus. Water was filled into the trap arm in order for the oil to condense on the surface of the water. The heating mantle supplied the necessary heat and as the water in the flask boiled, steam containing the volatile orange oil got into the neck of the flask and condensed on the layer of water in the graduated trap arm. The distillation procedure was continued up to the point where there was no more diversity in continuous readings of the oil volume. The process then followed by draining off the oil which was successively dehydrated over anhydrous sodium sulphate (BDH). The density of the oil was distinguished according to the weight: volume ratio (Ayoola et al., 2008)

**Sampling**
For all tested factors, determinations were performed on 1 and 15 days of storage at Laboratory temperature, on four separate samples in three replicates.

**Formulations of plum fruit roll ups**
Fruit roll ups (~100 g) were prepared with plum puree. Four samples were prepared according to following formulations:

A control sample, one with 0.1% peel oil orange, second with 0.2% peel oil orange, and the last one with 0.5% peel oil orange (Table 1). For all tested factors, determinations were performed on 1 and 15 days of Laboratory temperature storage on four separate samples in three replicates.

<table>
<thead>
<tr>
<th>Ingredients (g)</th>
<th>Fruit 1</th>
<th>Fruit 2</th>
<th>Fruit 3</th>
<th>Fruit 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>plum powder</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Orange peel oil</td>
<td>0</td>
<td>10</td>
<td>20</td>
<td>50</td>
</tr>
</tbody>
</table>

| Fruit 1: control fruit; Fruit 2: with 0.1% orange peel oil; Fruit 3: with 0.2% orange peel oil; Fruit 4: with 0.5% orange peel oil.

After mixing the material, the fruit roll ups were shaped into plates. These roll ups were placed in laminated bags and left to be used and investigated after 1, 15 days of storage at Laboratory temperature. From each sample three replicates were made.

**Microbial tests**
For microbial tests, a 10 g sample of fruit roll ups was aseptically weighted. Next samples were homogenized with 90 mL of a sterile solution of 0.1% (w/v) peptone water (Razi serum), for 2 min at 20-25 °C in a Masticator blender (Pause International, Iran), thus making a 1:10 dilution. Serial 10-fold dilutions were set by mixing 1 mL of the earlier dilution with 9 mL of 0.1% (w/v) sterile peptone water. Lactic acid bacteria were counted using duplicate 1 mL volumes of suitable dilutions in overlaid pour plates of MRS agar, incubated inverted at 30 °C for 3 days. For yeast and mold counts, duplicate 0.2 mL volumes of suitable dilutions were spread on to the dried surface of prepoured plates of yeast glucose chloramphenicol agar (YGC, Quelab, Canada), which were incubated at 25 °C for 5 days. Eventually, total viable counts were specified with using 1 mL of suitable dilutions on pour plates of plate count agar (Quelab, Canada) incubated at 32 °C for 3 days. After incubation, plates with 30-300 colonies were counted. The microbiological data were transformed into logarithms of the number of colony forming units (CFU/g).

**pH determination**
The pH of the samples was measured after homogenization with distilled water at a 2:8 ratio using a digital pH meter (3510 pH Meter, Jenway, England).

**Ash determination**
Ash percentage was computed by weight loss experimented by the sample (5 g) maintained in a muffle furnace (Carbolite RWF1200, Hope Valley, England) into a porcelain capsule at 700 °C until constant weight.

\[
\text{Ash \%} = \frac{\text{Plant weight empty} - \text{Plant weight of ash}}{\text{Weight of sample}} \times 100
\]

**Tissue measurement**
All texture measurements were undertaken using an Instron materials testing machine (Model Testometric, Rochdale, England) and 4 individual fruit per treatment were chosen for each of the three tests.

**Brix measurement**
10 g sample of the plum fruit roll ups that were homogenized in 50 mL of distilled water; the mixture was filtered and 50 mL of the filtered mixture were taken to Brix, using a Mettler automatic Tritator (Model KRUSS, Optronic, Germany). TSS was measured directly from the filtered residue, using an Abbe digital refractometer (E-Inginst Electron Corp., USA) and expressed as Brix.

**Sensory analysis**
The sensory panel evaluation was conducted with 30 panelists selected between members of the students in the Department of Agriculture, Azad University of Damghan at day 15 of storage. The casing was removed and then, samples were cut in slices of approximately 4 mm thickness and finally samples were grilled at 170° C for 10 min and served on white plastic dishes. Samples were separately. A quantitative descriptive analysis (QDA) was used for evaluating aroma, taste, textural, overall acceptability. A seven-point hedonic scoring scale (7: excellent; 6: very good; 5: good; 4: moderate; 3: slightly bad; 2: bad and 1: very much bad) was employed for evaluation of burgers. Water was used to clean the palates and remove residual flavours, at the beginning of the session and in between samples.

**Statistical analysis**
All data were analyzed using the General Linear model of ANOVA with treatment and time as factors, all statistical analyses were conducted by use of the SPSS statistical package (SPSS 16.00), after normality and homogeneity of variances were confirmed. Differences between means were determined by the least remarkable difference test, and significance was well-defined at \( P<0.05 \) (with Duncan’s Multiple Range Test).

**Results and discussion**

**pH values**
Changes of pH values during the 15-days storage period are presented in Fig. (1). In the total sample after 15 days of storage at laboratory temperature, pH values increased. This is associated mostly with increase of Gram-negative bacteria populations (Verma & Sahoo, 2000), such as *Enterobacteriaceae* and *Pseudomonads*, as well as yeasts and molds, which cause protein and amino acid degradation, resulting in formation of ammonia and consequent pH increase (Nychas, Drosinos, & Board, 1998). At the end of 15 days storage of samples in laboratory temperature, sample with the 0.5 orange peel oil (4.01) had the maximum and control sample (3.41) had the minimum pH values. Lower values of pH indicate that some fermentation occurs during storage of these products, although no sugars are usually added to our samples with orange peel oil. Carbohydrates contained in fruits, could be used as substrates for LAB metabolism, resulting in production of organic acids and lower values of pH (Papadima & Bloukas, 1999).
Acidity

Due to the fact that the acidity and pH levels have inverse correlation, results of variance analyses for acidity at the end of storage (15 days) showed that the sample with 0.5% orange peel oil had the minimum and control sample had the maximum level of acidity (Fig. 2).

Microbiological analysis

Lactic acid bacteria

Results related to the microbiological analyses of the samples of fruit roll ups with orange peel oil during the 15-days storage period are presented in Fig. (3). The counts of all determined microbiological indicators were significantly ($P<0.05$) affected by the addition of the natural antimicrobial and especially samples with 0.2% orange peel oil. All microbial groups increased in the control fruit roll ups. Increasing trends of different extents were also observed in samples of the remaining treatments for total viable counts, lactic acid bacteria (LAB), yeasts and molds. According to the results of variance analysis for counts of lactic acid bacteria (LAB), values were significantly affected ($P<0.05$) by concentration and time of storage. Comparison of the data showed that sample with 0.1% orange peel oil (2.36) showed the lowest counts of lactic acid bacteria (LAB) and the control sample and sample with 0.5 orange peel oil showed the highest count of lactic acid bacteria (LAB).

Subba, Soumithri, & Rao (1967) also showed inhibitory effect of essential oils of orange and lemon oil examined on bacteria and fungi in nutrient media. Orange oil was observed to be extra effective antimicrobial agent than lemon oil. All other conditions being identical, 2000 ppm of orange oil had result on all the Gram-positive cultures tested, containing the spores of Bacillus subtilis.

Total viable count

According to results of variance analyses for counts of total viable count, values were significantly affected ($P<0.05$) by kind, concentration and time of storage (Fig. 4). At the end of storage (15 days), sample with 0.5% orange peel oil showed the lowest and control sample highest total viable count of microbial load. Citrus essential oils have been reported to contain antibacterial activities against Salmonella typhimurium, E. coli O157:H7, Listeria monocytogenes, Escherichia coli and...
**Vibrio vulnificus** in media (Kim, Marshall, & Wei, 1995) and to *S. typhimurium* on fish cubes (Kim et al., 1995).

![Fig. 4](image.png)

**Fig. 4.** Effect of orange peel oil on Log number of total bacteria of the experimental fruit roll ups

**Yeast and Molds**

According to the results of variance analysis for count of yeast and mold, least number of yeast and molds was found in all of the samples with orange peel oil (Table 2). Most number of yeast and molds were found in control (6.41). Essential oils of lemon, orange, and bergamot were also illustrated to contain bactericidal effect against *Campylobacter jejuni, E. coli O157:H7, L. monocytogenes, Bacillus cereus, Staphylococcus aureus* (Fisher & Phillips, 2006) and *Acrobacter butzlei* (Fisher, Rowe, & Phillips, 2007) in media and on foods. In addition, antifungal actions against *Penicillium digitatum, Penicillium italicum* and yeast, *Saccaromyces cerevisiae* have been reported (Caccioni, Guizzardi, Biondi, Renda, & Ruberto, 1998). Those reports also illustrated little water solubility of citrus essential oil could be overcome by mixing it with an emulsifier (Fisher & Phillips, 2006; Kim et al., 1995; Kim & Shin, 2004).

**Table 2.** Effect of storage time on Log yeast and mold for fruit roll ups treatment

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 1</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control sample</td>
<td>1.33±0.75</td>
<td>1.33±0.75</td>
</tr>
<tr>
<td>Sample with 0.1% orange peel oil</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Sample with 0.2% orange peel oil</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Sample with 0.5% orange peel oil</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Ash analysis**

According to the results of variance analyses, values of ash were significantly not affected (*P*<0.05) by kind, concentration and time of storage. No significant change was observed in the fruit fly ash and the numbers are not much different. High levels of ash represents the material is inappropriate, in this experiment, the amount of ash is normal (Fig. 5).

![Fig. 5](image.png)

**Fig. 5.** Effect of orange peel oil on ash values of the experimental fruit roll ups

**Mechanical properties of fruit roll ups**

**Quality evaluation of fruit roll ups**

Textural properties may serve as an index of maturity or process ability to the food processor and of eating quality to the purchaser. Fruit roll ups produce. Thickness within the test sample: Control sample: 0.63; second sample: 0.52; third sample: 0.47 and the fourth sample was 0.37 mm. Method: The samples were cut in size 1 to 10 cm like tape, the samples were placed between two probes. Turn the machine, the probes were far apart. Expressed at high speed and until the sample is rupture, the amount of elasticity and the force required to rupture the Newton calculated.

**Tensile strength (TS)**

The results of measuring of the tensile strength of tissue for fruit roll ups acquired by measuring apparatus shown in Fig. (6). According to the results of variance analysis, added orange peel oil to plum fruit significantly (*P*<0.05) decreased the tensile strength (TS).
The Effect of Orange Peel Oil on Physicochemical, Microbiological …

**Elongation test (EB)**

The results of measuring the elongation of tissue for fruit roll ups acquired by measuring apparatus shown in Fig. (7). According to the results of variance analysis, orange peel oil added to plum fruit roll ups significantly \( (P>0.05) \) increased the elongation. According to the statistics mentioned above, the maximum tensile strength of tissue belonged to the sample 1 (13.37) followed by sample 2 with 0.1\% orange peel oil (10.92), sample 3 with 0.2\% orange peel oil (10.77) and sample 4 with 0.5\% orange peel oil (8.86). However, the results acquired for elongation of tissue changes reversely, which leaves us with the following results: sample 4 with 0.5\% orange peel oil (26.17), sample 3 with 0.2\% orange peel oil (23.12), sample 2 with 0.1\% orange peel oil (22.47) and sample 1 which has no density of orange peel oil and is considered as control sample (19.10). Measured the elongation of tissue for kiwifruit roll up sample. According to the results this characteristic of the kiwifruit roll up was significantly lower than all the other samples. This is probably caused by the type, tissue and density of the fruit used in the formulation of the industrial fruit roll ups. Moreover, the collation of TS results showed that the tensile of tissue for the Kiwifruit sample is significantly higher than other samples. This is perhaps a result of the tissue in this type of fruit roll ups, in which the texture of the Kiwifruit prevents the roll ups to be completely torn apart immediately after the rupture is applied. (Azeredo, Brito, Moreira, Farias, & Bruno, 2006) analyzed the elongation of tissue for the seedless pomegranate and pomegranate roll ups. The results showed that elongation of tissue is significantly lower for the seedless pomegranate sample than the normal pomegranate. This showed that normal pomegranate texture prevents the immediate rupture of the roll ups.

**Sensory evaluation**

You can see in Table (3), most tasting for the control sample (5.93) and sample with 0.2 orange peel oil (5.77). The highest rating was for the texture of the control sample (5.53). According panelist news, lowest score of the tissue belonged to the samples containing 0.5\% orange peel oil. According to variance analysis, highest rating was related to the odour of the control sample (5.93) and the lowest scores belonged to samples with 0.5 orange peel oil. According to variance analysis, the highest rating was for the overall acceptance was for the control sample (5.69) and lowest score of the overall acceptance was for samples containing 0.5\% orange peel oil (4.03).
Table 3. Sensory evaluation of various parameters fruit roll ups on the 15 day of temperature Laboratory.

<table>
<thead>
<tr>
<th></th>
<th>Control sample</th>
<th>Sample with 0.1% orange peel oil</th>
<th>Sample with 0.2% orange peel oil</th>
<th>Sample with 0.5% orange peel oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texture</td>
<td>5.53 ±0.78a</td>
<td>5.33±0.06b</td>
<td>5.37±0.68b</td>
<td>5.17±0.26c</td>
</tr>
<tr>
<td>Flavour</td>
<td>5.93 ±0.25a</td>
<td>5.31±0.07b</td>
<td>5.77±0.57b</td>
<td>4.27±0.48c</td>
</tr>
<tr>
<td>Adour</td>
<td>5.93 ±0.25a</td>
<td>5.80±0.48b</td>
<td>5.37±0.25c</td>
<td>4.03±0.65d</td>
</tr>
<tr>
<td>Overall acceptance</td>
<td>5.69 ±0.66c</td>
<td>5.00±0.36b</td>
<td>4.77±0.74b</td>
<td>4.03±0.92c</td>
</tr>
</tbody>
</table>

Conclusions
The results of the present study demonstrate the effectiveness of orange peel oil, added on microbial growth inhibition, and shelf life extension of fruit roll ups of the during temperature laboratory storage for 15 days. Samples with orange oil peel, which showed the best results, could have a valuable potential for commercial use in order to improve preservation of these products without using other additives. Therefore, using orange peel oil as natural preservatives in plum fruit roll ups is suggested.

References


تأثیر انسان پوست پرتقال بر ویژگی‌های شیمیایی، میکروبی و حسی لواشک آلو
مهمان زمانخانی ۱، مشکان عبداللهی ۲

چکیده

آفزایش غذایی نصرف کیتیوگان از اهدی و کیفیت غذایی، جزء از اشحفاده از ناد غذایی در افزایش قرار دارد. این تحقیق بر تاثیر افزایش ضدمیکروپی انسان پوست پرتقال بر ویژگی‌های شیمیایی، میکروبی و حسی لواشک آلو حاکم می‌باشد که در این تحقیق تأثیرات محافلگی کندگان در برای برخی از نادرانیک بدن انسان پوست پرتقال بر ویژگی‌های شیمیایی، میکروبی و حسی لواشک آلو در مقدار pH بالاترین مقدار در هر هفته و نادرانیک مقدار تر در هر هفته آفریده شده در هر هفته در نزدیکی از نادرانیک هر هفته و نادرانیک مقدار تر در هر هفته آفریده شده در هر هفته. واژه کلیدی: آلو، انسان پوست پرتقال، خاصیت بیماری‌محیطی، میکروبی، حسی لواشک آلو