Research Article

Oxidative Stability, Color, and Physicochemical and Sensorial Properties of Raw Stacked and Ground Meat Treated with Shahpouri Orange Juice

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Shahpouri orange juice (SOJ) is a rich source of bioactive compounds including flavonoids and phenolic acids. However, limited studies have been done to determine its effect on stacked and ground meat quality. The study was performed to determine and compare the effects of 0, 200, 400, 600, and 800 ppm SOJ with 200 ppm BHA on stacked and ground beef quality. The flavonoid compounds of SOJ were quantified as well as its antioxidant activity. Surface color, pH, lipid oxidation (peroxide value (PV) and thiobarbituric acid (TBA)), and sensorial properties of stacked and ground beef were determined at a day of SOJ incorporation and then after 6 days of storage at 4°C. The addition of SOJ affected pH compared to the control sample. Incorporating SOJ in stacked and ground meat improved redness and decreased lipid oxidation (PV and TBA) during storage compared with control. SOJ at 800 ppm improved overall sensorial properties after 6 days of storage. These results suggested that SOJ could be used as a natural antioxidant in stacked and ground meat to limit lipid oxidation and discoloration.

1. Introduction

Meat and meat products undergo quality loss due to oxy-myoglobin (OxyMb) and lipid oxidation, as well as microbial growth. These phenomena cause adverse effects on the nutritional, taste, color, and textural properties of the meat [1, 2]. Toxic compounds can also be produced as a result of oxidation and microbial activity. Grinding meat makes it more prone to chemical oxidation due to the changes in the integrity of the muscle cells membrane and more exposure of fats to oxygen [3], which cause more facilitated interactions of unsaturated fatty acids and prooxidant molecules followed by free radicals production and rancidizing the meat fat [4]. The application of antioxidant and antimicrobial components is a suitable method for improving the quality of meat products.

Synthetic and natural antioxidants can control the lipid oxidation in meat and meat products and improve their quality [1]. The international regulations support the application of a limited amount of synthetic antioxidants to protect meat and meat products from oxidation, due to confirmed severe impact on human health [5]. Therefore, focus on the application of natural antioxidants sourced from fruits, vegetables, herbs, and spices increased. The efforts continue among scientists to find new natural sources of antioxidants that potentially could be added to human food [5–7]. Fruits juice is a good source of antioxidant components for application in meat products.

The people of any age would supply the nutritional needs of their body through consuming the whole fruit or the juice [8]. Based on the report of the United States Department of Agriculture (USDA), orange juice is the most popular produced fruit juice with more than 50% of the international world trade market [9, 10]. The dominant antioxidant compound in orange fruit juice is ascorbic acid or vitamin C followed by flavanone and phenolic acids [11]. Besides the nutritional value and health effects, orange juice phenolic compounds also play a significant role in improving other

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food flavor and color [12]. Based on our study there is not any related research on the application of orange juice in stacked and ground meat.

Citrus is one of the most important fruit groups produced in the world which have different characteristics such as the longer maturation period, keeping fruits on the tree after maturity, and the different qualities of the nutritional values. Citrus cultivation is mainly carried out between 40° north latitude and 40° south latitude in the world [13–15]. Although its homeland is reported to be tropical and semitropical regions, production is mainly concentrated in the subtropical climatic zones [16, 17].

Phenolic compounds are secondary plant metabolites that can enter the human diet through the consumption of vegetal products. Importantly, a wide range of health-related activities has been reported for phenolic compounds. In this sense, the phenolic compounds present in citrus fruits have received attention due to their antioxidant, anti-inflammatory, and cardioprotective activities [18–20]. Shahpouri orange is an orange variety harvested in Iran. Due to the high content of flavonoids, Shahpouri orange juice (SOJ) can be considered as a good source of natural antioxidants in the food industry. The main aim of this study was to evaluate the incorporation of SOJ into stacked and ground meat for the first time and investigate its effect on lipid oxidation, color parameters, pH, and sensorial properties.

2. Material and Methods

2.1. Materials. Fresh harvested Shahpouri orange fruits were obtained from a local supermarket in November 2019 (Shiraz, Iran). Acetic acid, chloroform, potassium iodide, sodium thiosulphate, sodium acetate, sodium carbonate, hydrochloric acid, ethanol, methanol, potassium chloride, calcium chloride, dehydrate barium chloride, iron (II) sulphate, ammonium thiocyanate, starch, n-hexane, and plate count agar were purchased from Merck (Darmstadt, Germany). Other chemicals such as 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Tris buffer, Folin–Ciocalteu reagent, thiobarbituric acid, trichloroacetic acid, and BHA were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Orange Juice Extraction. The Shahpouri orange fruit was stored at 20–25°C under 70–90% relative humidity until further action. The orange fruits were juiced (Model 4161; Braun, De’Longhi Braun Household GmbH, Hungary) and used for further selected quality tests [21].

2.3. SOJ Phenolic Compounds. The phenolic compounds were quantified using high-performance liquid chromatography (HPLC, PLATIN blue KNAUER, Germany) Gülçü [22]. ODS-2 C18 column (4 × 250 mm), combined with Sphere-image 80–5 precolumn (CA, German), was used for this purpose. A gradient of methanol-water (75:25) and acetonitrile/dichloromethane/methanol (70:20:10) were used as mobile phases A and B, respectively. The setting for mobile phase gradient was 0% B; 5 min, 5% B; 20 min, 60% B; 40 min, 60% B; 50 min, 100% B; 60 min, 100% B; and 70 min, 0% B at 1 ml/min flow rate. Phenolic compounds were identified and quantified through comparison with the standards. The standard calibration curves were built using a minimum of four concentration levels of each standard, with coefficients of correlation ranging from 0.995 to 0.999. The phenolic compounds were reported as mg of component per gram of dried SOJ.

2.4. Antioxidant Properties of SOJ

2.4.1. Reducing Power. Juice (50–500 µg) was mixed with 1 mL of distilled water and added into 5 mL of phosphate buffer (0.2 M, pH 6.6) and 1% aqueous solution of potassium ferricyanide (50: 50 v/v). The final mixture was incubated at 50°C for 30 min. After the addition of 2.5 mL of trichloroacetic acid (10%), the mixture was centrifuged at 3500 rpm for 10 min. Then, 2.5 mL of the supernatant was added into a mixture of distilled water and FeCl3 (0.1%) in a 5:1 ratio, respectively. The absorbance of the final solution was measured using a spectrophotometer at 700 nm (Spec 1650PC, Shimadzu, Japan) [23].

2.4.2. Radical Scavenging Activity (RSA). 0.1 mL of SOJ sample was blended with 3.9 mL of a DPPH methanolic solution (25 mg/L). The sample solution was kept in dark for 30 min at 25°C and the absorbance was measured at 517 nm using a spectrophotometer (Spec 1650PC, Shimadzu, Japan) [24]. The percent of DPPH° inhibition (%) was calculated as follows:

\[
I_\% = \frac{(A_s - A_b)}{A_s} \times 100
\]

where \(A_s\) and \(A_b\) represent blank and sample absorbance, respectively.

2.5. Sample Preparation. Fresh cow meat samples were bought from a local market (Shiraz, Iran) and kept in sealed polystyrene ice-boxes to transport to the laboratory. Then, meat was minced using a meat grinder (Philips, HR2743, Amsterdam, Netherlands). Stacked meat samples were cut in the same size with 1 cm diameter. The SOJ sample (0, 200, 400, 600, and 800 ppm) and BHT (200 ppm) were incorporated into the ground meat while mixing. The stacked meat sample was immersed and covered by SOJ at different concentrations (0, 200, 400, 600, and 800 ppm) and BHT (200 ppm) for 4 min.

2.6. Physicochemical Properties of Meat

2.6.1. pH. 10 g of meat sample was mixed and homogenized with 50 mL deionized water for 1 min. A digital pH meter
Secondary lipid oxidation products were mixed with 2 mL TBAsolution (0.1% w/v in double-distilled water) and then centrifuged at 3000 g for 10 min. Afterward, 2 mL of upper phase was separated, and the absorbance was read at 510 nm (Spec 1650PC, Shimadzu, Japan). PV was quantified according to the method described by Turgut et al. [27].

2.6.3. TBA. Secondary lipid oxidation products were monitored by measuring thiobarbituric acid reactive substances (TBARS) values as the method described by Turgut et al. [27]. 4 g of meat sample was mixed with 20 mL TCA solution (0.1% w/v in double-distilled water) and then centrifuged at 3000 g for 10 min. Afterward, 2 mL of upper phase was separated, mixed with 2 mL TBA solution (0.1% w/v in double-distilled water), and then heated in a boiling water bath at 100°C for 30 min. After cooling to room temperature, the absorbance was read at 520 nm using a spectrophotometer (Spec 1650PC, Shimadzu, Japan). The TBARS values were calculated as mg of malonaldehyde per kg of the sample according to the standard curve of 1, 5, 10, 20, 50, and 100 μL of thiocyanate/Fe2+. The samples were kept for 20 min at 25°C and the absorbance was read at 510 nm (Spec 1650PC, Shimadzu, Japan). PV was quantified according to an obtained standard curve from cumene hydroperoxide. Peroxide value was reported based on meq of O₂ per kg of lipid.

2.6.2. PV. The PV values of samples were measured according to the method of Alizadeh-Sani et al. [26]. 0.3 mL of samples was added to 1.5 mL of isooctane/2-propanone solution (3:2 (v/v)) and mixed for 30 s. The mixture was then centrifuged at 2000 g for 2 min. 200 μL of clear upper phase was blended with 2.8 mL of methanol: 1-butanol (2:1 (v/v)) and 30 μL of thiocyanate/Fe2+. The samples were kept for 20 min at 25°C and the absorbance was read at 510 nm (Spec 1650PC, Shimadzu, Japan). PV was quantified according to the method described by Turgut et al. [27].

2.6.4. Color Properties. The color of the sample was determined using the method described by Hosseini et al. [28]. Photos were taken using a digital camera (Canon Powershot A540, 6 megapixels resolution) at certain conditions under natural daylight source (6500K) and then saved as JPG format. The values of color parameters including L* (lightness), a* (redness-greenness), and b* (yellowness-blueness) of each meat sample were determined using Adobe Photoshop® CS6.

2.6.5. Sensorial Properties. Organoleptic characteristics were studied by 20 male and female trained panelists aged between 23 and 28 years, from the Department of Food Science and Technology (Shiraz University). Panelists were selected according to their previous experiences in evaluating different formulations [29].

2.7. Statistical Analysis. The average values were analyzed using a one-way analysis of variance (ANOVA) at a significance level of 5%. The significant differences among average values were investigated through Duncan’s multiple range tests using SAS software (ver. 9.1, SAS Institute Inc., Cary, NC, USA.).

3. Results and Discussion

3.1. Orange Juice Properties

3.1.1. Phenolic Compounds. The dominant quantified flavonoids and phenolic acids were hesperidin, narirutin, chlorogenic acid, naringin, caffeic acid, and p-coumaric acid with 152.20, 35.23, 25.02, 11.99, 11.88, and 5.54 mg/g dried SOJ, respectively (Table 1). The obtained results were in accordance with the results reported by Vanamala et al. [30] and Fusco et al. [31] and confirmed; hesperidin and narirutin are the main flavonoid compounds in orange juice obtained from various orange cultivars.

3.1.2. Radical Scavenging Activity and Reducing the Power of SOJ. The results of radical scavenging activity (%) of SOJ are reported in Table 2. The results showed that, with an increase in the concentration of SOJ from 985 to 3940 ppm, radical scavenging activity significantly increased from 24 to 51%. The reducing power of Shahpouri orange juice in comparison with BHA is reported in Table 2. The results showed that BHA had higher reducing power than SOJ and the 200 ppm BHA and 400 ppm SOJ showed similar reducing power. The significant level of antioxidant activity in orange juice is related to its bioactive compounds such as carotenoids, vitamin C, phenolic acids, and flavonoids [32, 33].

3.2. Meat Sample Properties

3.2.1. pH. The pH of stacked and ground meat samples at a day of SOJ incorporation and after 6 days of storage at 4°C are reported in Table 4. The results showed that the pH of stacked and ground meat samples was increased in control samples during storage. An increase in pH is associated with the accumulation of ammonia caused by the degradation of proteins and amino acids [34]. THE addition SOJ and BHA had significant effects on keeping the pH of meat samples at a lower level compared with control sample. However, the results indicated that there were no significant differences between SOJ and BHA. Similar results were reported on the effects of different antioxidants on the meat pH by Amiri et al. [35] and Hashemi Gahruie et al. [29].
respectively [37–40], and apple peel-based edible coating, burger patties, raw pork patties, and in raw beef patties, berries, blackcurrant extract, and artichoke extract in pork products during chilled storage including Mediterranean using plant extract rich in natural antioxidant in meat for this purpose. Some researchers reported successfully anthocyanin compounds are usually used in meat products j+˘erefore, plant extracts rich in phenolic compounds and compounds and anthocyanin by blocking the processing of stacked meat. samples showed higher lipid oxidation in comparison with sample containing 200ppm BHA. Also ground meat 400ppm of SOJ had a similar antioxidant activity with a control. j+˘he results showed that a sample containing samples from lipid oxidation. j+˘he results showed that a sample containing 600ppm of SOJ had a similar anti- samples in comparison with control. It is indicating that the SOJ protected the samples from lipid oxidation. The results showed that a sample containing 600ppm of SOJ had a similar anti- oxidant activity with a sample containing 200ppm BHA. The antioxidant effect of SOJ can be attributed to its bioactive compounds such as flavonoids (mainly hes- peridin), phenolic acids, and ascorbic acid [48, 49]. The antioxidant effect of SOJ can be attributed to its bioactive compounds such as flavonoids (mainly hes-peridin), phenolic acids, and ascorbic acid [48, 49]. Similar resultswere reported by Villalobos-Delgado et al. [46] and Firuzi et al. [50] on lipid oxidation of treated meat with different types of vegetable extract.

### Table 1: Phenolic compounds of Shahpouri orange juice (SOJ).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (mg/g dried SOJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid</td>
<td>25.02 ± 0.97C</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>11.88 ± 0.72D</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>5.54 ± 0.63E</td>
</tr>
<tr>
<td>Narirutin</td>
<td>35.23 ± 0.37B</td>
</tr>
<tr>
<td>Naringin</td>
<td>11.99 ± 0.55D</td>
</tr>
<tr>
<td>Hesperidin</td>
<td>152.20 ± 1.85A</td>
</tr>
</tbody>
</table>

*Data represent mean ± standard deviation of three independent repeats. **Different capital letters in each column indicate significant differences (p < 0.05).

### Table 2: Radical scavenging activity (%) of Shahpouri orange juice (SOJ).

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>RSA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>985</td>
<td>24.00 ± 1.24 C</td>
</tr>
<tr>
<td>1970</td>
<td>39.66 ± 0.72B</td>
</tr>
<tr>
<td>3940</td>
<td>51.00 ± 1.25 A</td>
</tr>
</tbody>
</table>

*Data represent mean ± standard deviation of three independent repeats. **Different capital letters in each column indicate significant differences (p < 0.05).

### Table 3: Reducing power of Shahpouri orange juice (SOJ).

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>BHA</th>
<th>SOJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>0.211 ± 0.002Da</td>
<td>0.101 ± 0.002Db</td>
</tr>
<tr>
<td>400</td>
<td>0.393 ± 0.002Ca</td>
<td>0.201 ± 0.002Cb</td>
</tr>
<tr>
<td>600</td>
<td>0.636 ± 0.001Ba</td>
<td>0.334 ± 0.002Bb</td>
</tr>
<tr>
<td>800</td>
<td>0.847 ± 0.001Aa</td>
<td>0.461 ± 0.001Ab</td>
</tr>
</tbody>
</table>

*Data represent mean ± standard deviation of three independent repeats. **Different capital letters in each column and lowercase ones in each row indicate significant differences (p < 0.05).

3.2.2. Peroxide Value. Peroxide value of stacked and ground meat samples at a day of SOJ incorporation and then after 6 days of storage at 4°C are reported in Table 5. The results showed that the peroxide value of stacked and ground meat samples was increased in control samples after 6 days of storage. The addition of SOJ and BHA had significant effects on the peroxide value of samples in comparison with control. The results showed that a sample containing 400ppm of SOJ had a similar antioxidant activity with a sample containing 200ppm BHA. Also ground meat samples showed higher lipid oxidation in comparison with stacked meat.

The oxidation process can be prevented by phenolic compounds and anthocyanin by blocking the processing of free fatty acids formation and free radicals interaction [36]. Therefore, plant extracts rich in phenolic compounds and anthocyanin compounds are usually used in meat products for this purpose. Some researchers reported successfully using plant extract rich in natural antioxidant in meat products during chilled storage including Mediterranean berries, blackcurrant extract, and artichoke extract in pork burger patties, raw pork patties, and in raw beef patties, respectively [37–40], and apple peel-based edible coating, pomegranate peel extract, and pistachio green hull extract in beef [25,41]. A 75% reduction in the oxidation of ground goat meat by using pomegranate rind powder containing 300 ppm phenolic compounds was also reported by Devatkal and Narsaiah [42].

3.2.3. Secondary Oxidation (TBA). One of the adverse effects of lipid oxidation is changing the meat and meat products flavor, which limits its acceptance [43]. Greene and Cumuze [44] revealed that 0.2–0.6 mg MDA/kg of TBARS caused the oxidized flavor in beef and decreased acceptability of the meat tested by panelist. The threshold of 2 mg MDA/kg beef was reported to be acceptable by Campo et al. [45].

TBA value of stacked and ground meat samples at a day of SOJ incorporation and after 6 days of storage at 4°C are reported in Table 6. The results showed that the TBA values were increased in all samples especially in control samples. This increase was higher in ground meat samples compared to stacked meat. In this study meat rancidity started at the values of 0.4–0.6 mg of malonaldehyde/kg, which was similar to the result of the previous study on pork meat refrigerated for 7 days [46, 47]. The addition of SOJ and BHA had significant effects on the TBA value of samples in comparison with control. It is indicating that the SOJ protected the samples from lipid oxidation. The results showed that a sample containing 600ppm of SOJ had a similar antioxidant activity with a sample containing 200ppm BHA. The antioxidant effect of SOJ can be attributed to its bioactive compounds such as flavonoids (mainly hes-peridin), phenolic acids, and ascorbic acid [48, 49]. Similar results were reported by Villalobos-Delgado et al. [46] and Firuzi et al. [50] on lipid oxidation of treated meat with different types of vegetable extract.

3.2.4. Color Properties. Color properties ($L^*, a^*, and b^*$) of stacked and ground meat samples at a day of SOJ incorporation and after 6 days of storage at 4°C are reported in Table 7. The results showed that storage time and antioxidant level had no effect on the $L^*$ value of both meat samples. In meat products, higher fat content generally causes more light reflection and consequently brighter color and higher $L^*$ value [51]. The $a^*$ value, indicating redness of samples, decreased for the control sample during the storage time, whereas samples containing either BHA or SOJ did not show any changes. This finding indicated that adding SOJ containing antioxidants could keep the preferred meat red color stable and prevent discoloration.

The red color in meat products is related to OxyMb concentration [52]. Conversion of OxyMb to metmyoglobin (MetMb) causes changes in color from bright red to brown and, consequently, decreases in $a^*$ value. This change in color depends on lipid oxidation and the addition of antioxidants can retard this discoloration process by delaying OxyMb deterioration and slowing down the formation of MetMb [53]. In accordance with our results, it was demonstrated that the
incorporation of 10 mg gallic acid equivalent in pomegranate rind could control color changes in frankfurter during storage at refrigerator [54] which is probably linked with lipid oxidation [55]. There are some reports demonstrating that natural antioxidants may retard color loss by delaying red color deterioration by slowing metmyoglobin formation [56–58].

The obtained $b^*$ value of stacked and ground meat samples was decreased in control samples. The addition of SOJ and BHA had significant effects on the TBA value of samples in comparison with control. The results showed that a sample containing 400 ppm of SOJ had a similar antioxidant activity with a sample containing 200 ppm BHA. Biswas et al. [59] reported that the addition of mint (Mentha spicata) and curry extracts (Murraya koenigii L.) in raw ground pork meat stabilized the red color during 12 days of chilled storage. Besides, Turgut et al. [25] showed that the presence of pomegranate extracts rich in phenolic compounds and anthocyanin in pork patties and meat controlled the color which changed efficiently during chilled storage. Firuzi et al. [50] noted that 10 mg gallic acid equivalent in pomegranate rind limited frankfurter color change during chilled storage. However, some reports showed an increase in $a^*$ value during storage at the refrigerator.

3.2.5. Sensorial Properties. The appearance and color are the most important properties that influence the consumers’ judgment and acceptance, to purchase meat [46]. Overall sensorial properties of stacked and ground meat samples at a day of SOJ incorporation and after 6 days of storage at 4°C are reported in Table 8. Based on the obtained result, the sensorial properties of the sample containing either BHA or SOJ remained stable during the storage time which was due
to the prevention of MetMb formation. Decreasing OxyMb and increasing MetMb in meat induces a sense of staleness and decrease acceptability. In contrast, the control sample showed a significant decrease in sensorial properties [60].

### 4. Conclusion

Incorporation of SOJ in stacked and ground meat decreased lipid oxidation due to the presence of antioxidant compounds in SOJ and prevent decreasing redness during the 6 days of storage at 4°C. It was also revealed that the addition of SOJ at different levels could improve the sensorial properties of samples compared to that of control. Considering these results, SOJ has the potential to be used as a natural additive in meat products to improve their quality during chilled storage.

### Data Availability

All data and analyses are reported in the table and figure.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

### Acknowledgments

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References


