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Antioxidant Effect of Pomegranate Rind, Seed Extracts and Pomegranate Juice on Lipid Oxidation and Some Quality Properties of Cooked Beef Patties

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ABSTRACT

Antioxidant effect of adding pomegranate rind (PR), seed (PS) extracts and pomegranate juice (PJ), on lipid oxidation and cooked beef patties quality were investigated. Total phenolics content and DPPH radical scavenging activity of the extracts were evaluated. TBARS values and antioxidant activity (AOA%) of the cooked patties contained PR, PS and PJ during refrigerated storage at 4 ± 1°C for 9 days were determined and calculated. PR, PS extracts and PJ showed good ability in DPPH and exhibited high level of total phenolics content. Significant reduction in TBARS values for all treated patties was observed during storage compared to control. TBARS values were significantly low in PR, followed by PS and PJ patties. Antioxidant activity of the tested patties was in the order PR > PS > PJ and total phenolic contents increased in treated patties as compared to control. Sensory scores of all patties were acceptable. So, PR, PS extracts and PJ could be utilized as a good natural antioxidant source.

Key words: Antioxidant Activity - Lipid oxidation - Pomegranate juice - Pomegranate rind and seed extracts - Quality Properties.

ABB: Kinnow rind powder (KRP)
Pomegranate rind powder (PRP)
Pomegranate seed powder (PSP)

Introduction

The trend towards convenience foods has resulted in increased production and consumption of ground meat products. During refrigerated storage, microbial growth and oxidative stress are well known to cause lipid oxidation and short shelf-life of refrigerated meat and meat products. Prevention of microbial growth and retarding lipid & protein oxidation during storage and retail display trade are essential aspects to maintain the quality and safety of meat (Vaithiyanathan et al., 2011).

Reduction of lipid oxidation during storage of meat and meat products can be accomplished with antioxidants. Synthetic antioxidant e.g. butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA) and tertiary butyl hydroquinone (TBHQ) were used in extending shelf life of meat. These synthetic preservatives are less preferred over bio-preservatives by the consumers. Bio-preservatives derived from plant extracts are well known to be useful in extending shelf life of foods, by reducing or eliminating survival of pathogenic bacteria and increasing overall quality of food products through inhibition of oxidative rancidity (Naveena et al., 2006). It is well known that majority of the plant extracts contain phenolic compounds as secondary metabolites (Vaithiyanathan et al., 2011). The antioxidant activity exhibited by phenolic compounds has been previously reported by (Naveena et al., 2008; Naveena et al., 2008b; Vaithiyanathan et al., 2009) in fresh as well as in processed meat in terms of reduced lipid oxidation.

The antioxidant potential in food is determined by the antioxidant composition and the antioxidative properties of constituents. By definition, the antioxidant activity (AOA) is the capability of a compound (composition) to inhibit oxidative degradation, e.g. lipid per-oxidation. While the antioxidant capacity gives the information about the duration of antioxidative action, the reactivity characterizes the starting dynamics of antioxidation at a certain concentration of an antioxidant or antioxidant mixture. Individual antioxidants can react as chain-braking of oxidative reactions and the activity is related to the reactivity of the antioxidants to free radicals (Roginsky and Lissi, 2005). For that reason different approaches were applied to asses AOA in food and beverages (Descalzo and Sancho, 2008). Attempts were also made to correlate the parameters and establish the best-fit equations with respect to species variations.

The use of natural antioxidants may extend the shelf life of meat by providing additional barriers to the growth of food borne pathogens; acting as antioxidants and stabilizing meat color while adding characteristic
flavors to the meat. Owing to the partially hydrophobic nature of several phenolic compounds, they are able to work more effectively at the interface of the lipid - and water- compatible portions of meat. This physicochemical property may make them suitable ingredients for use in meat systems (Shan et al., 2009).

Efforts towards identifying the safe and natural sources of antioxidants of plant origin have notably increased in recent years. Compounds obtained from natural sources such as grains, oilseeds, honey, fruits and vegetables have been investigated for their natural antioxidant effects in meat products. Waste products from processing of fruits and vegetables offer a practical and economic source of potent antioxidants that could replace synthetic preservatives (Naveena et al., 2008a). The peel and seed fractions of some fruits have higher bioactivities than the pulp fractions (Tomas-Barberan and Espin 2001; Guo et al., 2003; Balasundram et al., 2006). Potential use of powders and extracts of different fruits and their by-products as natural antioxidants in meat and meat products were studied (Fernandez-Lopez et al., 2004; Brannan 2008; Devatkal and Naveena 2010a).

The pomegranate is one of the oldest known edible fruits. Nowadays, pomegranate is an important commercial fruit crop that is widely cultivated in parts of Asia, North Africa, the Mediterranean, and the Middle East (Sarkhosh et al., 2006). Pomegranate (Punica granatum L.) fruit is widely used in the food and process industries due to its excellent nutritional and health value and as a raw material for the manufacture of secondary products such as, dyes, and cosmetics (Opara et al., 2009). In addition, to the medicinal use of dried products, the fruit is consumed directly as fresh arils or juice and is used in the food industry in the manufacture of jellies, concentrates, and flavoring and coloring agents. In particular, there has been renewed global interest on the functional and nutriceutical benefits of pomegranate fruit (Sumner et al., 2005) and have potent antioxidant properties (Lansky and Newman 2007). Pomegranate is an important source of bioactive compounds and has been used in folk medicine for many centuries. Moreover, pomegranate juice has been demonstrated to be high in its antioxidant activity and is effective in prevention of atherosclerosis, low-density lipoprotein oxidation, prostate cancer, platelet aggregation and various cardiovascular diseases, and may be helpful against heart disease, Alzheimer’s disease (Adhami and Mukhtar 2006; Eghdami et al., 2011). More recently, positive effects on fat reduction were reported using the pomegranate and its extracts as well to explore the effects of the pomegranate in obesity with various mechanisms have been proposed as to how these different extracts help in fat reduction (Al-Muammar and Khan 2012).

Most pomegranate fruit parts are known to possess enormous antioxidant activity. Pomegranate peel as agro-industrial by-products is a good source of phenolic compounds that have very potent antioxidant and antimicrobial activity. The unutilized peel of pomegranate can be used commercially in the food industry as potential natural preservative (Kanatt et al., 2010). Pomegranate rind is an inedible part/by-product obtained during processing of pomegranate juice. The rind was reported to be as a rich source of tannins, anthocyanins and flavonoids (Ozkal and Dinc 1994). Recently the use of pomegranate juice and rind powder as a source of natural anti-oxidant in chicken patties has been investigated by Naveena et al., (2008a). Antioxidant effect of pomegranate rind extract in cooked goat meat patties had been also demonstrated by Devatkal et al., (2010b). It has been reported that in spite of there are extensive data existing in favor of use of polyphenols from green tea, rosemary, berry fruits, thyme, sage, and other herbs as natural antioxidants (Bozkurt 2006; Pokorny 1991), the interest in the antioxidant properties of polyphenols from pomegranate appeared (Naveena et al., 2008b).

Growing awareness and concern about the quality and safety of meat have led to numerous developments in meat preservation. Meat industry is increasingly seeking for natural solutions to minimize oxidative rancidity and extend the shelf life of meat products. Therefore, the use of natural preservatives to replace the respective synthetics is preferable and may be of great interest to the meat industry. So, the objectives of the present study aimed to investigate the effect of adding pomegranate rind, seed extracts and pomegranate juice as natural antioxidant on lipid oxidation, (TBARS values) color parameters, sensory attributes, pH, cooking yield % and antioxidant activity (AOA%) of the cooked beef patty samples.

**Materials and Methods**

1.1 Materials:

Fresh deboned beef meat was obtained from local market, Cairo, Egypt. Meat was stored at 4 ± 1 °C for approximately 4 h before use. Fresh pomegranate fruit was purchased from local supermarket, Cairo, Egypt.

1, 1, 3, 3-tetraethoxypropane and 1,1-diphenyl-2-picrylhydrazyl were purchased from Sigma chemical company (St Louis, Mo, USA). All chemicals used in this study were of analytical grade.

2.2 Preparation of powders and extracts:

Mature pomegranate fruits were washed and cut manually to separate the rind and seeds. The rind thus obtained cut into small pieces using a sharp knife and dried in an air circulatory tray drier (WT-bimder
Tuttlingen / Germany) at 60 ºC for 48 h. Dried pieces were cooled and powdered in a kitchen grinder and sieved using a 60 mesh sieve, and packed into 100 g units in high density polyethylene bags and stored at room temperature until extraction. Similarly, powder from pomegranate seeds was prepared by drying the pomegranate fruit seeds in a tray drier and grinding in a kitchen grinder and sieving. About 10 g of each dried rind and seed powder was mixed with 100 ml boiled distilled water and left for 1 h. The extracts obtained by filtration were analyzed for total phenolic content and DPPH radical scavenging activity. Freshly prepared extract was used for each replication.

2.3. Preparation of pomegranate juice:

Fresh pomegranate fruits were washed and cut into four pieces. The seeds/arils were separated manually and extracted using a kitchen juicer (Model MJW176P, Panasonic, Osaka, Japan). The freshly prepared juice was analyzed for total phenolics content, DPPH radical scavenging activity; and added to meat samples.

2.4. Preparation of meat samples:

Chilled meat was minced twice using a meat grinder (Hobart4B22-2, Ohio, USA) and divided into equal four meat parts to provide four treatment beef patty samples. A control sample was formulated without adding any extract. The other three treatment samples were prepared by adding the tested extracts to minced beef and assigned as follows: PR (10 ml extract of pomegranate rind powder; PS (10 ml extract of pomegranate seed powder) and PJ (10 ml of pomegranate juice) / 100g meat for each. Sodium chloride 2% (w/w) was added to all samples. The volume of PJ, PR and PS extracts were replaced with distilled water in control sample. Immediately, the four meat samples were thoroughly hand-mixed well. The homogenized meat mixtures were shaped into patties using a meat former and cooked in a hot air oven until the internal temperature reached 80ºC. After cooling to room temperature, the patties were packaged in polyethylene bags and stored at 4± 1ºC for 9 days. Total phenolic content, pH, cooking yield, instrumental color and sensory attributes were analyzed in beef patty samples on first day of storage (zero time). TBARS values were determined in the patty samples for 0, 3, 6 and 9 days of storage period and antioxidant activity (AOA %) was calculated.

2.5. ANALYSIS:

2.5.1. DPPH radical scavenging activity:

The ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by pomegranate juice (PJ), pomegranate rind (PR) and seed (PS) was estimated by the method of Singh et al., (2002). PJ and extracts of PR and PS were diluted with 0.1 M Tris-HCl buffer (pH 7.4) [Tris = (hydroxymethyl) amino methane] and mixed with 1 ml of DPPH (250 l M) with vigorous shaking. The reaction mixture was stored in the dark at room temperature for 20 min and then absorbance was measured at 517 nm using a UV-VIS (Model: PG Instruments Ltd., UK). The scavenging activity was calculated by the following equation:

\[
\text{Scavenging activity} \% = \left( \frac{\text{Absorbance}_{\text{Blank}} - \text{Absorbance}_{\text{Sample}}}{\text{Absorbance}_{\text{Blank}}} \right) \times 100
\]

2.5.2 Total phenolics content:

The pomegranate juice (PJ) and extracts of pomegranate rind (PR), seed (PS) and cooked meat patties (5 g of cooked patty was homogenized with 25 ml of 70% acetone and kept overnight for extraction at refrigeration temperature) were analyzed for total phenolic content using the Folin-Ciocalteus (F-C) assay (Negi and Jayaprakasha 2003; Naik et al., 2008). Suitable aliquots of extracts were taken in a test tube and the volume was made to 0.5 ml with distilled water followed by the addition of 0.25 ml F-C (1N) reagent and 1.25 ml sodium carbonate solution (20%). The tubes were vortexed and the absorbance recorded at 725 nm after 40 min. The amount of total phenolics was calculated as tannic acid equivalent from the calibration curve using 0.1 mg/ml of standard tannic acid solution.

2.5.3. Thiobarbituric acid reactive substances (TBARS) measurement:

The TBARS values were determined spectrophotometrically according to Byun et al., (2001). Patty samples were analyzed for the optimum concentration of each extract homogenized patty sample (2g) was taken and TBARS were extracted twice with 10 ml of 0.4M perchloric acid. Extracts were collected and made up to 25ml
with 0.4M perchloric acid and then centrifuged for 5 min at 1790g. After centrifugation, 1ml of extract was poured into a glass stoppard test-tube. A TBARS reagent (5ml) was added and the extract was heated in a boiling water bath for 35 min. After cooling under tap-water, the absorbance of the sample was read against the appropriate blank at 538nm. A standard curve was prepared using 1,1,3,3-tetraethoxypropane (TEP).

2.5.4. Antioxidant activity (AOA %):

The antioxidant potential expressed in terms of percentage of antioxidant activity (AOA %) was calculated by the following equation (Wijewickreme and Kitts 1998).

\[
\text{AOA } \% = \left( \frac{\text{TBARS value of the control} - \text{TBARS of the test sample}}{\text{TBARS value of the control}} \right) \times 100
\]

2.5.5. pH determination:

The pH of cooked patty was determined by blending 10 g sample with 100 ml distilled water for 1 min in a blender. The pH values were measured using a standardized electrode attached to a digital pH meter (HAANA, hI902 meter, Germany).

2.5.6. Cooking yield %:

Cooking yield was determined according to Alleson-Carbonell et al., (2005) by dividing cooked product weight by the raw uncooked weight and multiplying by 100.

2.5.7. Instrumental color measurement:

Color of each tested patties was measured using a Hunter Lab. scan XE color-meter (Hunter Lab. Inc., Reston, VA, USA) calibrated with a white standard tile: (X = 77.26, Y = 81.94 and Z = 88.14). Commission International d’Eclairage (CIE): L* (lightness), a* (redness) and b* (yellowness) saturation index were measured. Reflectance measurements were collected at 10 nm increments using illuminate A (Podolak et al. 1997) and three random readings per sample were recorded. Hue and chroma of meat were calculated by using the formula (tan^(-1) b/a) and \((a^2 + b^2)^{1/2}\), respectively, where \(a\) = red unit and \(b\) = yellow unit according to Bochi et al. (2008). The redness index \((a*/b*)\) was determined as described by Chen et al., (1997).

2.5.8. Sensory evaluation:

The sensory attributes (appearance, juiciness flavor, and overall acceptability) of the beef patty samples were evaluated using a ten-point numerical scale, where, ten corresponded to ‘components characteristic of the highest quality’. The panel consisted of 10 members of the staff who were familiar with meat characteristics. Patties were pre warmed before serving and water was served for rinsing the mouth between samples (Kanatt et al., 2010).

2.5.9 Statistical analysis:

The conventional statistical methods were used to calculate means and standard deviations, All the measurements were replicated three times and the data are presented as mean +SD. The effects of natural antioxidant extracts addition and storage period were analyzed and the obtained data were subjected to analysis of variance (ANOVA) according to PC_STAT,Version IA Copyright 1985, the University of Georgia (PC-STAT,1985).

Results and Discussion

3.1. Total phenolic content and radical scavenging activity (DPPH):

Total phenolic content and radical scavenging activity of pomegranate rind (PR), pomegranate seed (PS) extracts and pomegranate juice (PJ) are illustrated in Table1. Generally, The PR, PS extracts and PJ showed a good ability in radical scavenging activity and high content of total phenolics. Total phenolic content was significantly (P < 0.05) higher in PR followed by PS then PJ. Phenolic compounds, secondary metabolites products by the plants, are generally responsible for the antioxidant activity of many fruits and vegetables (Kanatt et al., 2010). They are important molecules contribute to antioxidant and pharmacological properties
Most pomegranate fruit parts are known to contain higher polyphenolic compounds (Ozkal and Dinc, 1994). Phenolics present in pomegranate fruit juice may act in a similar fashion as reductones by donating the electrons and reacting with free radicals to convert them to more stable product and to terminate free radical chain reaction. Protein precipitable phenolics, is a feature that comes from the chemical property (protein binding) of the polyphenolic compounds present in the extract of pomegranate fruit juice (Vaithiyananthan et al., 2011). Pomegranate by-products (rind and seeds) have substantial amount of phenolic compounds and significant free radical scavenging activity (Devatkal et al., 2010b).

The DPPH was used as a free radical to evaluate antioxidant activity present in natural sources (Schwarz et al., 2001). From the data reported in Table 1, the radical scavenging activity (DPPH %) of PS demonstrated significantly (P < 0.05) greater free radical activity followed by PJ then PR. This agreed with Devatkal et al., (2010b) who found that pomegranate seed powder showed significantly (P < 0.05) greater free radical scavenging activity than pomegranate rind powder. The difference in the antioxidant activity of the peel and seed may be ascribed to their different phenolic compositions as reported by Singh et al., (2002). Significant higher radical scavenging activity was observed by Naveena et al., (2008a) in pomegranate rind powder compared to pomegranate juice. The pomegranate juice is a rich source of polyphenols; however its composition can change depending on the cultivar type, growing region, soil, climate, maturity, cultural practices, storage and processing factors (Labbé et al., 2010). Results in a recent study developed by Zaouaya et al., (2012) to compare the physico-chemical characteristics and antioxidant activity of 13 pomegranate cultivars showed significant correlations between total phenolics content and antioxidant activity, indicated the contribution of phenolic compounds in juice antioxidant capacity, there are linear and significant correlations between total phenolics content and antioxidant activity, indicating significant antioxidant effects of the extracts of pomegranate rind and seeds as well pomegranate fruit juice phenolics (PFJP) might be due to its hydrogen-donating ability of the phenolic hydroxyl groups. In addition, antioxidants are believed to intercept the free radical chain of oxidation and to give hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product that does not initiate or propagate further oxidation of lipids (Sherwin 1998).

### Table 1: Total phenolic content and radical scavenging activity (DPPH) of pomegranate PR, PS extracts and PJ

<table>
<thead>
<tr>
<th>Pomegranate extracts</th>
<th>Total phenolic content (µg/ kg) as tannic acid equivalent</th>
<th>DPPH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR</td>
<td>5104.203 ± 0.000</td>
<td>35.500 ± 0.095</td>
</tr>
<tr>
<td>PS</td>
<td>2767.000 ± 1.000</td>
<td>45.300 ± 0.101</td>
</tr>
<tr>
<td>PJ</td>
<td>2122.305 ± 0.707</td>
<td>38.620 ± 0.016</td>
</tr>
</tbody>
</table>

Mean ± SD= All values determinations ± standard deviation (SD) are mean of triplicate. Mean values in the same column bearing the same superscript do not differ significantly (P < 0.05).

PR= pomegranate rind extract; PS= pomegranate seed extract; PJ= pomegranate juice

Effect of pomegranate rind (PR), pomegranate seed (PS) extracts and pomegranate juice (PJ) on thiobarbituric acid reactive substances (TBARS) values in cooked beef patties are shown in Table 2. All the treatments significantly (P < 0.05) reduced the TBARS values throughout storage compared to the control sample. The lipid oxidation inhibition effect was of highest value (P < 0.05) in beef patty sample contained PR extracts compared to samples contained PS extract and PJ during all storage times. The TBARS values significantly (P < 0.05) increased in control and a gradually increase was noticed also for all treated patties samples throughout the storage period. At the 9th day of storage the increase in control sample was the highest relative to all treated samples. The TBARS values for the treated samples can be arranged as PJ > PS > PR indicating significant antioxidant effects of the extracts of pomegranate rind and seeds as well pomegranate juice used in this study. It was also, observed that the increase in TBARS values in PR treated samples remained the lowest up to 9 days. The large amount of phenolics contained in rind powder extract may cause its strong antioxidant ability (Li et al., 2006). The same authors observed strong antioxidant effect of pomegranate rind (PRP) and pulp. Also, a significant relation between phenolic content and antioxidant effect of pomegranate peel extract has been reported (Negi and Jayaprakasha 2003). A Significant higher antioxidant effect for PRP compared to pomegranate juice and BHT in cooked chicken patties during refrigerated storage was observed (Naveena et al., 2008a).

The total antioxidant capacity or activity has been generally recognized as a tool to test the antioxidant potential of a pure compound or a food extract (Aruoma 1996). Antioxidant activity of a food could be a useful index to predict oxidative stability (Sacchetti et al. 2008). Data on the antioxidant activity of cooked beef patties as affected by addition of PR, PS extracts and PJ as antioxidants stored at 4±1°C for 9 days are depicted in Table 2. Within the tested samples, a significant difference between the AOA % as a result of adding the PR, PS and PJ during storage for 9 days was observed. The order of antioxidant activity was PR > PS > PJ. It was also,
noticed that PR extract exhibited a higher AOA% than other patty samples treated with PS and PJ at 0, 3, 6 and 9 days. However, AOA% in PR and PS patties increased (P < 0.05) up to the 6th day then decreased at the 9th day of storage. Worthy to note that, PJ extract exhibited a lowest AOA% in PJ patty samples and decreased at the 6th day of storage then increased at the 9th day (i.e. difference in pattern). This pattern difference may be due to the phenolic content in each of PR, PS extract and PJ. The data indicated that the marked antioxidant activity of RP, PS and PJ seemed to be the result of their radical scavenging activity. The pomegranate peel phenolics may act in a similar fashion as reductones by donating electrons and reacting with free radicals to convert them to more stable products and terminate free radical chain reactions (Negi and Jayaprakasha 2003).

3.3. pH, cooking yield and total phenolic content of cooked beef patties

Table 2: Thiobarbituric acid reactive substances (TBARS) values and Anti-oxidative Activity (AOA %) in the cooked beef patties as affected by addition of Pomegranate PR, PS extracts and PJ

<table>
<thead>
<tr>
<th>TBARS values (mg malonaldehyde/kg meat)</th>
<th>Refrigerated storage at 4±1 ºC (days)</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef samples</td>
<td></td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.803±0.021\textsuperscript{aD}</td>
<td>0.960±0.019\textsuperscript{bC}</td>
<td>1.853±0.021\textsuperscript{bD}</td>
<td>1.963±0.025\textsuperscript{cB}</td>
</tr>
<tr>
<td>PR</td>
<td></td>
<td>0.520±0.027\textsuperscript{aD}</td>
<td>0.570±0.009\textsuperscript{bC}</td>
<td>0.680±0.027\textsuperscript{bD}</td>
<td>0.860±0.027\textsuperscript{cB}</td>
</tr>
<tr>
<td>PS</td>
<td></td>
<td>0.603±0.021\textsuperscript{aC}</td>
<td>0.630±0.010\textsuperscript{bC}</td>
<td>0.710±0.027\textsuperscript{bD}</td>
<td>1.063±0.021\textsuperscript{cA}</td>
</tr>
<tr>
<td>PJ</td>
<td></td>
<td>0.720±0.027\textsuperscript{aD}</td>
<td>0.760±0.027\textsuperscript{bD}</td>
<td>1.520±0.027\textsuperscript{cA}</td>
<td>1.550±0.010\textsuperscript{dA}</td>
</tr>
</tbody>
</table>

Mean ± SD= All values determinations ± standard deviation (SD) are mean of triplicate.
Mean values in the same column bearing the same superscript do not differ significantly (P < 0.05).
Mean values in the same row bearing the same superscript do not differ significantly (P < 0.05).
PR= Beef patty samples with addition of pomegranate rind extract; PS= Beef patty samples with addition of pomegranate seed extract; PJ= Beef patty samples with addition of pomegranate juice

Table 3: pH, cooking yield and total phenolic content of cooked beef patties as affected by addition of pomegranate PR, PS extracts and PJ

<table>
<thead>
<tr>
<th>Beef patty samples</th>
<th>pH values</th>
<th>Cooking yield (%)</th>
<th>Total phenolic content (µg/g as tannic acid eq.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>PR</td>
<td>6.120±0.009 \textsuperscript{a}</td>
<td>84.300±0.031\textsuperscript{a}</td>
<td>1150.333±0.209\textsuperscript{a}</td>
</tr>
<tr>
<td>PS</td>
<td>6.050±0.064 \textsuperscript{a}</td>
<td>82.910±0.000\textsuperscript{b}</td>
<td>581.333±0.153\textsuperscript{b}</td>
</tr>
<tr>
<td>PJ</td>
<td>5.930±0.019 \textsuperscript{a}</td>
<td>84.520±0.000\textsuperscript{a}</td>
<td>447.667±0.153\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Mean ± SD= All values determinations ± standard deviation (SD) are mean of triplicate.
Mean values in the same column bearing the same superscript do not differ significantly (P < 0.05).
PR= Beef patty samples with addition of pomegranate rind extract; PS= Beef patty samples with addition of pomegranate seed extract; PJ= Beef patty samples with addition of pomegranate juice

Results in Table 3 showed that total phenolic content of cooked beef patty samples treated with PR was significantly higher than those samples treated with PS and PJ. Also, adding PR, PS extracts and PJ caused increase in the total phenolic content in the treated patties samples compared to control sample. Adding of pomegranate rind, red beet powders and mixture of them increased the total phenolic content in beef sausage (El-Gharably and Ashoush 2011). The higher level of phenolics may indicate that the product is nutritionally enhanced due to the fruit juice and rind powder that was added (Leheska et al., 2006).
3.4. Instrumental color evaluation of cooked beef patties:

Instrumental color evaluation (Table 4) revealed a significant (P < 0.05) effect of pomegranate rind (PR), pomegranate seed (PS) extracts and pomegranate juice (PJ) on Hunter color values of all treated cooked beef patty samples. Addition of PR, PS extracts and PJ reduced (P < 0.05) L*(lightness), a*(redness) and b* (Yellowness) color values of all treated patties compared to control sample. Significant reduction in L* (lightness) and a* (redness) values was noticed in patties sample treated with PR extract compared to PS and PJ Patty samples. Whereas, the PR patties was of significant (P < 0.05) higher b* (yellowness) value, followed by PS patties then PJ patties compared with control. Meanwhile, significant reduction in yellowness (b*) value was shown in PJ patties sample followed by PS then PR Patty samples. Thus, overall treatment means indicated that the PR patties had the lowest (P < 0.05) L* and a* values, whereas b*(yellowness) had the highest value compared to other treated patty samples.

A decrease in (b*) values of beef patties containing natural antioxidants has been reported by Rojas and Brewer (2008). Addition of pomegranate rind powder (PRP) reduced the lightness (L*) value on the surface of cooked chicken patties samples compared to control was previously noticed (Naveena et al., 2008a), the same authors have further found an increase in redness value of chicken patties contained pomegranate rind powder (PRP) compared to other studied samples. However, no difference was observed in the internal (a*) values between their samples. This might be due to cooking at high temperature which might have completely denatured the myoglobin and hence no difference was observed in internal (a*) values. With the addition of rind pomegranate (PR) the chicken patties became slightly darker, which might have resulted in lower (L*) values. Also, addition of pomegranate juice (PJ) changed the chicken meat patties from pale raw to grayish color with lower instrumental values. It should be noted that some other factors could have an effect on meat color parameters such as the fineness of mincing and consequently surface reflection properties (Naveena et al., 2008a).

The dark color of pomegranate rind powder (PRP) might be responsible for decrease in L* value in PRP treated goat meat patties was illustrated by Devatkal et al., (2010b). The discoloration of chicken meat patties with addition of natural antioxidants like tea catechins was reported by Mitsumoto et al., (2005).

Results presented in Table 4 showed color intensities among the tested cooked patty samples. The chroma is an expression of the saturation or intensity and clarity of the color Bochi et al. (2008). Chroma was observed, generally, to be of highest value in control sample and of lowest value in cooked beef patty sample contained PJ extract (Table 4).

![Table 4: Instrumental color values of cooked beef patties as affected by addition of Pomegranate PR, PS extracts and PJ](image)

Mean ± SD= All values determinations ± standard deviation (SD) are mean of triplicate.
Mean values in the same column bearing the same superscript do not differ significantly (P < 0.05).

PR= Beef patty samples with addition of pomegranate rind extract; PS= Beef patty samples with addition of pomegranate seed extract; PJ= Beef patty samples with addition of pomegranate juice.

In addition, data showed no significant difference (P <0.05) in color intensities among the PR and PS cooked beef patty samples, which had high color intensity than the PJ patty sample. The hue of PR sample was significantly more than those of the other two treated samples (PS & PJ) and control. Previously, the chroma value was observed to be lowest in all beefsteak treated samples with the natural antioxidants Lactic acid + clove + Vitamin C and highest in control on zero day (Naveena et al., 2006). No significant difference was reported for both hue and chroma values between control and sausage contained pomegranate rind powder (PRP) up to 3%. Whereas, increasing the addition of rind beet powder (RBP) significantly decreased both Hue and chroma values in the surface of manufactured sausage compared to control (El- Gharably and Ashoush 2011).

The redness index (a*/b* ratio) of PR patty sample was of lower value as compared to all other tested samples (Table 4), while no significant difference was noticed between PS patties and the control samples. Regarding to PJ patty sample an increase was observed and its redness index remained higher than the values of PS sample and the control. This ratio was used as an index of apparent change in redness. The saturation of red color in meat was related to myoglobin concentration (Mitsumoto et al., 2005). Changes in the redness index can be used as an index of pigment changes and the discoloration was mainly caused by oxidation of myoglobin (Devatkal and Naveena, 2010a).
3.5. Sensory Evaluation:

The sensorial criteria (appearance, juiciness, flavor and overall-acceptability) of the tested cooked beef patty samples were evaluated and presented in Table 5. All tested patty samples were equally acceptable as evidenced by the overall acceptability scores. However, there was a slight reduction in PJ patties appearance compared with others. The juiciness scores were increased from 6.13 in control to 6.43 in PR patty sample and no significant difference was observed in PS and PJ treated patties. No significant difference was observed in flavor scores for PR and PJ patties samples and gained the same higher scores than PS and control samples. Furthermore, the data of sensory evaluation showed that all samples scores were considered acceptable.

Addition of extracts of KRP, PRP and PSP did not have any negative effect on sensory attributes of goat meat patties was observed (Devatkal et al., 2010b). Sensory evaluation scores revealed that PR can be incorporated in chicken meat patties without affecting any of the sensory attributes (Naveena et al., 2008b).

Table 5: Sensory evaluation of cooked beef meat as affected by addition of pomegranate PR, PS extracts and PJ

<table>
<thead>
<tr>
<th>Beef patty samples</th>
<th>Appearance</th>
<th>Juiciness</th>
<th>Flavor</th>
<th>Overall-acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Mean ± SD</td>
<td>6.520 ± 0.026 a</td>
<td>6.380 ± 0.019 b</td>
<td>6.750 ± 0.026 a</td>
</tr>
<tr>
<td>PR</td>
<td>Mean ± SD</td>
<td>6.130 ± 0.027 c</td>
<td>6.430 ± 0.009 b</td>
<td>7.060 ± 0.082 b</td>
</tr>
<tr>
<td>PS</td>
<td>Mean ± SD</td>
<td>6.699 ± 0.027 b</td>
<td>6.750 ± 0.030 c</td>
<td>7.120 ± 0.036 a</td>
</tr>
<tr>
<td>PJ</td>
<td>Mean ± SD</td>
<td>7.113 ± 0.021 a</td>
<td>7.460 ± 0.019 c</td>
<td>6.990 ± 0.026 b</td>
</tr>
</tbody>
</table>

Mean ± SD= All values determinations ± standard deviation (SD) are mean of triplicate.

Mean values in the same column bearing the same superscript do not differ significantly (P < 0.05).

Conclusion:

Pomegranate rind and seeds are by-products of pomegranate juice industry. These by-products have substantial amount of phenolic compounds and significant free radical scavenging activity. In this study, incorporation of 10 ml extract of pomegranate rind (PR), pomegranate seeds (PS) and pomegranate juice (PJ) (10ml /100g meat) could protect cooked beef patties against lipid oxidation during refrigerated storage. All extracts were effective for decreasing TBARS values in cooked beef patties. PR extract was noticed more effective in reducing TBARS formation. Also, sensory evaluation scores revealed that PR, PS extracts and PJ can be incorporated in beef patties without affecting their sensory attributes. So, worthy to conclude that extracts of these by-products could be successfully added to meat to function as natural antioxidant. It is important to note that while processing pomegranate into juice, the rind and pulp are discarded, hence the food industry can make use of these by-products as source of natural anti-oxidants in processed meat products.

References


PC-STAT, 1985 Version IA copyright, University of Georgia pharmacy.


