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Utilization Impact of Adding Pomegranate Rind Powder and Red Beet Powder as Natural Antioxidant on Quality Characteristics of Beef Sausage

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Abstract: Various concentrations of pomegranate rind powder (PRP) 1, 2 and 3%, red beet powder (RBP) 1, 3 and 5% and their mixtures PRP: RBP (1:5, 2:3 and 3:1) were added to prepare beef sausage. Total phenolic compounds and DPPH radical scavenging of PRP and RBP were determined. Physiochemical, changes in the diameter and length, cooking yield, total phenolic content, antioxidant activity and sensory analyses were conducted on manufactured sausage. The addition of PRP, RBP and their mixtures caused to improve the WHC, plasticity, diameter and length and increased total phenolic content and their antioxidant activity in raw and cooked beef sausage. On other hand, lightness (L*), redness (a*), yellowness (b*), Hue and choroma were significantly difference ($P \leq 0.05$) affected by the type and concentration of the addition used. Addition PRP, RBP and their mixtures for manufacturing of sausage caused to reduce the TBA values during storage period up to 6 days compared to control and may have beneficial effect due to the presence of phenolic compounds for these natural additives. Products contained 1% and 3% RBP recorded the highest sensory scores among manufactured sausage samples.

Key words: Beef sausage · Pomegranate rind powder (PRP) · Red beet powder (RBP) · Phenolics · DPPH · antioxidant activity · Instrumental color · Sensory evaluation

INTRODUCTION

Nowadays, the interest in the role of dietary antioxidants in human health has promoted research in the field of food science. Fruits are good sources of these bioactive and rich with polyphenol compounds, which base their marketing strategies on antioxidant potency [1]. However, the synthetic antioxidants currently used have been found to exhibit various health effects. The continuous use of such synthetic chemicals may cause health hazards such as teratogenic and carcinogenic effects in laboratory animals and primates [2]. There has been a growing interest in natural ingredients because they have greater application for increasing products. In recent years, search for natural additives, especially of plant origin, has notably increased. Waste products from processing of fruit and vegetables offer a practical and economic source of potent antioxidants that could replace synthetic preservatives [3]. Lipid oxidation in meat is one of the reasons for quality degradation during storage. [4] Lipid oxidation is further responsible for changes in color, flavor, texture and nutritive value of meat [5].

Reduction of lipid oxidation during storage of meat and meat products can be accomplished with synthetic antioxidants like butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), etc. However, reports of adverse health effects of these synthetic chemicals have increased the resistance to use synthetic antioxidants. Therefore, there is a growing interest in natural sources of antioxidants for applications in meat products [6-7].Where, potential use of powders and extracts of different fruits as natural antioxidants in meat and meat products have been studied in recent years, i.e. cherry fruit [8] apple [9] citrus fruit byproducts [10] and green tea leaves [11].Pomegranate peel or rind extract had scavenging activity against super oxide [12]. However, pomegranate is an important source of bioactive compounds and been used for folk medicine for many centuries. It is interesting that pomegranate peels had the highest antioxidant activity and it seems, therefore, that pomegranate peel may be a rich source of natural antioxidants [12-13]. The antioxidant compounds are known for their properties in scavenging free radicals and inhibition lipid oxidation *in vitro* [14-15].

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Also, pomegranate peels have been used as colorant for textiles in the Middle East because of their high tannin and phenolic contents [16] and pomegranate peel extract could effectively protect (after oral administration against CCL_4 induced hepatoxicity, which ROS damage was intensively involved [17]. Use of pomegranate juice and rind powder as a source of natural antioxidant in chicken patties had been investigated [3] cooked goat meat patties had been demonstrated by Devatkal *et al.* [18].

Red beetroots are grown mainly in Europe, parts of Asia, the United States and the Mediterranean region [19]. They are not considered to be a popular vegetable in North America and per capita consumption of beet makes it among the least consumed vegetables [20].The soluble and cell wall Red beet roots associated phenolics as well as betalains, the main pigments in red beet responsible for its reddish-purple hue, are bioactive compounds [21], being their antioxidant capacity beneficial for human health [22-23]. Beet pigments, collectively known as betalains, have been examined as natural colorants in food products such as processed meat, ice cream, baked goods, candies and yogurt [24-25]. Betalains have been shown to confer free-radical scavenging and allied antioxidant activities [26-27]. Red beet is a good source of natural antioxidants [20, 22, 28]. With respect to their antioxidant activity [20] ranked beets among the ten most potent vegetables. Red beets possess high antiradical and antioxidant activity [22, 28, 29]. Betanin from red beets was found to inhibit lipid peroxidation in membranes and used as natural colorants to enhance the redness of different products such as tomato products in pizzas, sausages, cooked ham, sauces and arrange of dessert products [30]. On the other hand, the extract of red beet root is approved as food colorant with name of red (beet root, betalain or betanin (E162) Directive 95/45/Ec). There has been an increasing trend towards the replacement of synthetic colorants by natural pigments fruits and vegetable extracts in recent years. This trend can be attributed to the safety and health benefits of natural pigments and to a strong consumer demand for more natural products [31].

Therefore, the main objective of present work was to produce a sausage contained dried powder of peel pomegranate and/or red beet and their mixtures with different proportions and studying its effects as antioxidants. Also, physicochemical and sensory properties in beef sausage were undertaken.

MATERIALS AND METHODS

1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) and Folin-Ciocalteus phenol reagent were purchased from Sigma chemical company (St Louis, MO, USA) and other chemicals used in this investigation were of analytical grade. About 15 kg of deboned beef meat was obtained from local retail meat market. Meat samples were stored at 4°C for approximately 4 h before use. Fresh meat samples were obtained separately for each of the replications. Fresh red beet (*Beta vulgaris*) and pomegranate (*Punica granatum*) were obtained from El- Obour market, El-Obour city, Cairo, Egypt, at season 2010. Preparation of pomegranate rind powder (PRP) and red beet powder (RBP): Mature pomegranate fruits and red beet roots were washed. Pomegranate fruits were cut manually and peeled off. The rind (pomegranate peel) thus obtained and red beet were cut into small pieces using a sharp knife and dried in an air circulatory oven (WT-binder Tuttlingen /Germany) at 60 °C for 48 h. Dried pieces were cooled and powdered in a heavy duty kitchen grinder. The powder of each was sieved using a 60 mesh sieve and packed into 100g units in high density polyethylene bags and stored at room temperature for further use.

Sausage manufacture: The method of Aleson-Carbonell *et al.* [32] was applied with some modification to prepare sausage as follows: Three independed replicates of each sausage were made. Sausages were manufactured with traditional formula (only beef meat percentages add up to 100% and percentages of other ingredients are related to beef meat) 49 % lean beef meat, 40 % beef fat, 5% iced water (wt/wt), 2% sodium chloride (wt/wt), 3% starch (wt/wt) andl% spices mixture. This original mixture was split, mixing well and divided into batches, pomegranate rind powder (PRP), red beet powder (RBP) and mixture of them were added to the batches in different forms and amounts: 0, 1, 2 and 3% of PRP; 0, 1, 3,5 and 5% of RBP and 5:1, 3:2 and 1:3% for the mixture of RBP:PRP. The meat and fat cut were ground with meat grinder, mixed with other ingredients and stuffed in mutton casings of 20 to 23 mm diameter. The three replicates of each manufactured sausage were divided into tow groups. The first group was stored in refrigerator at 5±1°C for 6 days, while the second group were divided into two parts, first part were cooked in an oven at 200°C for 20 min. cooled at room temperature then sensory evaluation was conducted. The total phenolic content, pH, DPPH radical scavenging activity and instrumental color were analyzed in fresh manufactured sausages except TBA values, which were analyzed during the storage up to 6days.

Total phenolic for PRP, RBP and samples of manufactured sausage were estimated according the method described by Escarpa and Gonzalez [33] as follows: One g of PRP, RBP and 10 g of sausage samples were individually mixed with 100 ml boiled distilled water

and left for an hour. The extracts obtained by filtration were analyzed for total phenolic content using Folin-Ciocalteus (F-C) assay. 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 (1 N) reagent and 1.25 ml sodium carbonate solution (20%). The tubes were vortexed and the absorbance recorded at 725 nm after 40 min by using a Hatachi U - 1900 spectrophotometer (Kyoto, Japan). The amount of total phenolics was calculated as gallic acid equivalent from the calibration curve using standard gallic acid solution (0.1 mg/ml) [34]. The results were expressed as milligrams of gallic acid equivalents per gram powder (mg GAE/g powder) by reference to the gallic acid calibration curve (r^2=0.99). The ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by PRP, RBP and their mixture for manufactured sausage were estimated by the method of Singh *et al.* [16]. Each extract of PRP, RBP and their mixture for manufactured sausage (100i l) which previously obtained were diluted with 0.1 M Tris - HCl buffer (pH 7.4) and mixed with 1 ml of DPPH (0.0044g/100 ml) 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 Hitachi U-1900 spectrophotometer (Kyoto, Japan). The scavenging activity was calculated by the following equation:

$$\text{Scavenging activity \%} = (\text{Absorbance}_{\text{Blank}} - \text{Absorbance}_{\text{Sample}} / \text{Absorbance}_{\text{Blank}}) \times 100$$

Thiobarabituric acid values (TBA) were colorimeterically determined in minced samples of manufactured sausage as mentioned by Harold *et al.* [35]. Cooking yield was determined by the method of George and Berry [36] for cooked sausage in boiling water for 10 min. then calculating weight difference of sausage before and after cooking as follows:

$$\text{Cooking yield} = (\text{cooked weight} / \text{raw weight}) \times 100$$

Change of diameter and length of manufactured sausage samples were measured in cooked samples with the method described by George and Berry [36] using the following equations:

$$\text{Change in sausage diameter (\%)} = [\text{Raw sausage diameter (cm)} - \text{Cooked sausage diameter (cm)}] / \text{Raw sausage diameter (cm)} \times 100$$

$$\text{Change in sausage length (\%)} = [\text{Raw sausage length (cm)} - \text{Cooked sausage length (cm)}] / \text{Raw sausage length (cm)} \times 100$$

Water holding capacity (WHC) and plasticity were measured by the filter press method according to the method described by Volovinskaya and Kelmen [37]. Water Retention and pH of manufactured sausage samples were determined for each sample according to A.O.A.C. [38]. Instrumental Color evaluation for raw and cooked sausage: Color parameters of the raw and cooked sausage were determined according to the tri-stimulus color system described by Francis [39] using spectrophotometer (MOM, 100D, Hungary). Color coordinates X, Y and Z were converted to corresponding Hunter L*, a* and b* color coordinates according to formula given by manufacturer. L* value indicates lightness or darkness in a scale from 100 to 0, a* value represents the coordinates of greenness (-) and redness (+), while the b* value signifies change from blueness (-) to yellowness (+). Hue angle (θ) is defined as a color wheel, with red-purple at angle of 0° and 360°, yellow at 90°, bluish-green at 180° and blue at 270°. Hue angles (θ) were calculated from: $\theta = \tan^{-1}(b/a)$. On the other hand, the color intensity or chroma (C) represents color saturation or purity, calculated as follows: $C = (a^2+b^2)^{1/2}$

Sensory Evaluation: Ten Panelists were recruited from the staff and students of food science, Fac. of Agric. Ain shams university. Panelists were chosen on the basis of previous experience in consuming sausage. Testing was initiated after the panel agreed on the specifications [40]. During evaluation, the panelists were situated in private booths under incandescent light. Each panelist evaluated 3 replicates of all manufactured cooked samples; the sample presentation order was randomized for each panelist. Tap water was provided between samples to cleanse the palate [32]. The sensory attributes measured and their descriptors were the appearance, color, flavor, juiciness and overall acceptability. At the end of the test, panelists were asked to give a score for each parameter from 0 to 10. The ethical principles of the evaluation were followed by the sensory analysis [41]

Statistical Analysis: Each parameter was tested in triplicate. Conventional statistical methods were used to calculate means and standard deviation. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range to determine differences ($P \leq 0.05$) to discover the significant differences between the levels of the main factor [42]. The statistical analysis was done using SAS program [43].

RESULTS AND DISCUSSION

Moisture Content, WHC and Plasticity of Manufactured Sausage: The moisture content, WHC and plasticity of manufactured sausage contained different proportion of powders from pomegranate rind powder (PRP) and red

Table 1: The moisture content, water holding capacity (WHC) and plasticity of raw sausage with different ratios of powders from pomegranate rind powder (PRP), red beet powder(RBP) and their mixtures.

Treatments	Moisture (%)	WHC	Plasticity
Control	71.27 ± 0.49^{a}	1.27 ± 0.14^{e}	3.86 ± 0.06^{c}
PRP (%)			
1	66.60 ± 3.50^{bc}	1.51 ± 0.26^{e}	4.72 ± 0.33^{b}
2	66.87 ± 0.25^{bc}	10.59 ± 1.11^{a}	4.07 ± 0.06^{c}
3	64.58 ± 0.27^{c}	7.45 ± 0.11^{b}	4.01 ± 0.11^{c}
RBP (%)			
1	67.97 ± 0.41^{b}	1.62 ± 0.61^{e}	5.58 ± 0.42^{a}
3	68.95 ± 0.70^{ba}	1.08 ± 0.11^{e}	4.01 ± 0.11^{c}
5	67.42 ± 1.03^{b}	2.53 ± 0.27^{d}	4.01 ± 0.11^{c}
PRP: RBP (%)			
1:5	68.65 ± 1.47^{b}	8.25 ± 0.51^{b}	2.90 ± 0.06^{d}
2:3	67.14 ± 0.91^{bc}	6.51 ± 0.19^{c}	3.23 ± 0.39^{d}
3:1	66.70 ± 1.47^{bc}	10.08 ± 0.83^{a}	3.94 ± 0.06^{c}

Data are the mean ± SD, n = 3, Mean values in the same column bearing the same superscript do not differ significantly (P ≤ 0.05).

Table 2: The pH, change in diameter, length and cooking yield of manufactured sausage with add different ratios of pomegranate rind powders (PRP), red beet powders (RBP) and their mixtures.

	pH				
Treatments	Raw Sausage	Cooked sausage	Change in diameter (%)	Change in length (%)	Cooking yield (%)
Control	6.23 ± 0.01^{b}	6.24 ± 0.01^{e}	1.82 ± 0.61^{c}	8.17 ± 0.39^{bac}	89.44 ± 2.35^{ba}
PRP (%)					
1	5.97 ± 0.02^{c}	6.16 ± 0.01^{f}	4.30 ± 1.37^{c}	6.89 ± 0.97^{bac}	83.02 ± 0.52^{d}
2	5.76 ± 0.02^{e}	5.92 ± 0.01^{h}	10.92 ± 3.87^{ba}	10.89 ± 5.25^{ba}	72.31 ± 1.43^{e}
3	5.43 ± 0.01^{g}	5.91 ± 0.01^{h}	16.44 ± 5.39^{a}	7.09 ± 2.30^{bac}	66.34 ± 1.32^{f}
RBP (%)					
1	6.23 ± 0.02^{b}	6.53 ± 0.02^{b}	6.55 ± 4.74^{bc}	5.53 ± 2.88^{bac}	90.12 ± 0.26^{a}
3	6.25 ± 0.02^{b}	6.34 ± 0.01^{d}	6.29 ± 0.99^{bc}	4.20 ± 0.74^{bc}	89.01 ± 0.88^{ba}
5	6.27 ± 0.01^{a}	6.55 ± 0.02^{a}	7.43 ± 4.63^{bc}	1.33 ± 0.79^{c}	86.44 ± 0.77^{bc}
PRP: RBP (%)					
1:5	5.98 ± 0.01^{c}	6.36 ± 0.01^{c}	3.65 ± 2.39^{c}	3.14 ± 0.15^{bc}	84.71 ± 0.79^{dc}
2:3	5.81 ± 0.01^{d}	5.95 ± 0.01^{g}	6.19 ± 3.35^{bc}	12.83 ± 10.62^{a}	70.18 ± 3.90^{e}
3:1	5.46 ± 0.01^{f}	5.71 ± 0.01^{i}	13.79 ± 2.16^{a}	7.78 ± 2.17^{bac}	65.09 ± 1.29^{f}

Data are the mean ± SD, n = 3, Mean values in the same column bearing the same superscript do not differ

beet powder (RBP) and their mixtures are illustrated in Table 1. Results indicated that, a significant decrease (P=0.05) for moisture content of manufactured sausage contained PRP, RBP and mixture of PRPRBP at and any levels used compared to control was noticed. Where, the moisture content was 71.27 ± 0.49 for control sample sausage compared with the range of 64.58 ± 0.27 and 68.95 ± 0.70 for all manufactured samples. The most important difference between control and other tested sausage in the presence of PRP and mixture of PRP and RBP was the increase (P ≤ 0.05) in WHC when using 2.0 and / or 3.0% PRP and mixture of PRP and RBP at ratios 3:1, 1:5 and 2:3, respectively. While, the sausage contained different concentration of RBP was little significant difference in WHC (P ≤ 0.05) compared to control. The measurement of plasticity for manufactured sausage indicated that, no significant difference in plasticity for samples contained PRP with levels 2.0 and 3.0%, RBP with levels 3.0 and 5.0% and 3:1 for mixture of PRP and RBP was observed but the sample contained 1.0% RBP recorded the highest and best level of plasticity than all tested samples. Fore instance, adding PRP and RBP caused to improve the plasticity of processed sausage.

pH, (diameter and length) and Cooking yield of Manufactured Sausage: There were no significant difference in pH values for sausage contained PRP and RBP but adding RBP at levels of 1.0, 3.0 and 5.0 % in manufacturing sausages samples caused to little or no significant difference in pH compared to control sample (Table 2). However, the pH values for manufactured sausage contained the mixture from PRP: RBP with ratios of 1:5, 2:3 and 3:1 were less than both the pH of control sample and that contained any concentration of RBP.

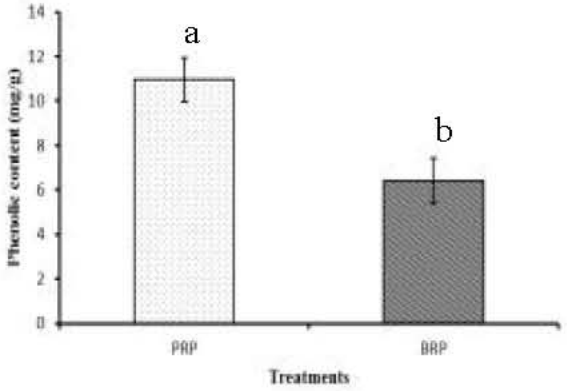

Fig. 1: Total phenolics content (mg/g) of pomegranate rind powder (PRP) and red beet powder (RBP).

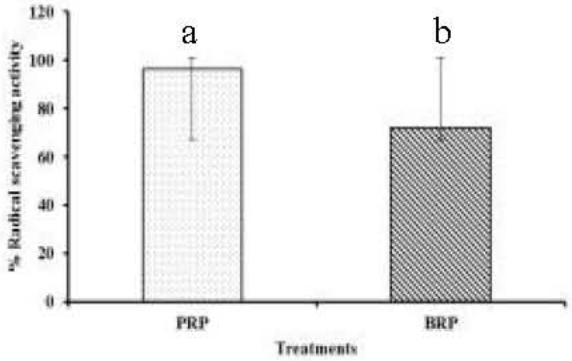

Fig. 2: The DPPH radical scavenging activity (%) of extracts of pomegranate rind powder (PRP) and red beet powder (RBP).

Therefore, sausage samples which contained PRP and/or mixture of PRP: RBP could be the same pH values but less than both control and that which contained different ratios of RBP. These results are in agreement with Naveena *et al.* [3] they reported no significant difference in pH of cooked chicken patties due to addition of pomegranate juice extract. Consequently, there were a slightly significant difference in diameter of processed sausage contained of different levels of PRP, RBP and their mixtures comparing with control sample. The diameter was higher in the presence of 3.0% (10.52 ± 3.87) PRP, followed by mixture of 3:1 (13.75 ± 2.16), PRP and RBP, 2.0% (10.52 ± 3.87) PRP, 5% (7.43 ± 4.63) RBP, but no significant difference for samples contained RBP was observed. Therefore, the addition of 3% PRP and/or the mixture of 3:1 PRP: RBP caused to improve and increase the diameter of sausage. On the other hand, the length percentages of processed sausage were less than control owing to use RBP at levels of 5%. Results also indicated that, a good correlation between cooking yield and processed sausage contained different levels of PRP from1.0 to 3.0%, 1.0 to 5.0% of RBP and their mixtures.

Total Phenolic Content and DDPH Free Radical Activity (% Scavenging Activity) for Manufactured Sausage: Total phenolic contents were 10.96 and 6.57 mg GAE/g for PRP and RBP as shown from Fig 1. Pomegranate peel had been shown to be rich in polyphenols [44]. The total phenolic content (mg GAE/g) was increased gradually by increasing the level of PRP and RBP in manufactured sausage either in raw and/or cooked samples comparing to control sample are illustrated in Table 3. The higher level of phenolic compounds may indicate this product is nutritionally enhanced due to the rind powder that was added [45]. The raw manufactured samples contained 1:5, 2:3 and 3:1 for mixture of PRP, RBP recorded 7.42 ± 0.02, 5.42 ± 0.03 and 4.89 ±0.02, respectively. While, the corresponding results were 5.82 ± 0.02, 4.43± 0.04 and 4.07 ± 0.05 for cooked samples. Therefore, adding PRP, RBP and mixtures of PRP,RBP caused to increase the total phenolic content in manufactured sausage. The antioxidant properties of phenolic compounds were very well documented [18]. A significant relation between phenolic content and antioxidant effect of pomegranate peel extracts has been reported by Negi and Jayaprakasha [46]. Naveena *et al.* [3] they observed significantly higher antioxidant effect for PRP compared to pomegranate juice and BHT in cooked patties during refrigerated storage. Similarly Li *et al.* [12] observed strong antioxidant effect of PRP and pulp. The DPPH was used as free radical to evaluate antioxidant activity present in natural sources [47]. The DPPH radical scavenging activity % for PRP and RBP are illustrated in Fig. 2. PRP had higher scavenging activity (96.24) than RBP (72.11%). On the other hand, The DPPH free radical scavenging activity (DPPH %) as a result of adding different ratio of PRP, RBP and their mixtures in manufactured sausage are shown in Table 3. All used ratios of powders of PRP,RBP and the mixture of PRP+RBP for manufactured sausage samples showed an excellent ability in radical scavenging activity in raw sausage samples (59.24 - 88.71%) contained any level of PRP,RBP and their mixtures compared to control sample (26.16%). Among these used powder 3% PRP had significantly ($P \leq 0.05$) greater free radical scavenging activity than other tested samples. These results are similar with Negi and Jayaprakasha [46] reported radical

Table 3: Total phenolic content and antioxidant activity of raw and cooked sausage as affected by addition different ratios of pomegranate rind powder, beet root powder and their mixtures.

	Raw sausage	Cooked sausage	Raw sausage	Cooked sausage
Treatments	----------Total phenolic (mg GAE/g)----------		----------Scavenging activity %----------	
Control	2.94 ± 0.02^{h}	2.46 ± 0.04^{j}	26.16 ± 0.1^{j}	16.51 ± 0.09^{j}
PRP (%)				
1	4.15 ± 0.03^{f}	3.44 ± 0.03^{h}	61.84 ± 0.15^{h}	32.88 ± 0.15^{h}
2	4.38 ± 0.03^{e}	4.64 ± 0.02^{d}	79.79 ± 0.14^{c}	50.84 ± 0.14^{e}
3	5.12 ± 0.03^{c}	4.89 ± 0.04^{c}	88.71 ± 0.19^{a}	59.75 ± 0.19^{b}
BRP (%)				
1	3.57 ± 0.03^{g}	3.24 ± 0.03^{i}	59.24 ± 0.24^{i}	30.28 ± 0.24^{i}
3	4.18 ± 0.03^{f}	3.53 ± 0.02^{g}	64.12 ± 0.24^{g}	35.17 ± 0.24^{g}
5	5.09 ± 0.02^{c}	5.05 ± 0.02^{b}	69.59 ± 0.19^{f}	40.64 ± 0.19^{f}
PRP: BRP (%)				
1:5	7.42 ± 0.02^{a}	5.82 ± 0.02^{a}	70.63 ± 0.29^{e}	51.32 ± 0.29^{d}
2:3	5.42 ± 0.03^{d}	4.43 ± 0.04^{e}	77.38 ± 0.24^{d}	58.08 ± 0.24^{c}
3:1	4.89 ± 0.02^{g}	4.07 ± 0.05^{f}	81.21 ± 0.34^{b}	62.25 ± 0.33^{a}

Data are the mean ± SD, n = 3, Mean values in the same column bearing the same superscript do not differ significantly ($P \leq 0.05$).

Table 4: Effect of adding different concentration of pomegranate rind powder (PRP), beet root powder (RBP) and their mixtures for manufactured sausage on Hunter L*, a*, b*, Hue and Chroma values.

Treatments	L*	a*	b*	Hue	Chroma
Control	41.29 ± 0.09^{e}	3.58 ± 1.71^{g}	11.47 ± 0.16^{b}	72.91 ± 7.51^{a}	12.09 ± 0.63^{f}
PRP (%)					
1	44.02 ± 0.01^{b}	7.43 ± 0.12^{f}	11.26 ± 0.03^{cb}	56.59 ± 0.56^{b}	13.49 ± 0.05^{ed}
2	44.14 ± 0.02^{b}	6.33 ± 0.15^{f}	11.01 ± 0.04^{c}	60.10 ± 0.65^{b}	12.7 ± 0.04^{ef}
3	46.36 ± 0.03^{a}	4.47 ± 0.22^{g}	13.58 ± 0.06^{a}	71.78 ± 0.90^{a}	14.3 ± 0.03^{d}
RBP (%)					
1	34.21 ± 0.02^{e}	15.36 ± 0.07^{e}	6.67 ± 0.02^{d}	23.43 ± 0.28^{c}	16.75 ± 0.06^{c}
3	28.04 ± 0.22^{g}	17.11 ± 0.91^{cd}	1.79 ± 0.36^{g}	6.08 ± 1.31^{e}	17.21 ± 0.87^{c}
5	24.38 ± 0.33^{i}	18.36 ± 1.54^{bc}	0.97 ± 0.52^{h}	3.05 ± 1.65^{e}	18.39 ± 1.51^{b}
PRP: RBP (%)					
1:5	25.11 ± 0.06^{h}	18.59 ± 0.20^{b}	2.42 ± 0.06^{f}	7.33 ± 0.13^{e}	18.75 ± 0.20^{b}
2:3	29.94 ± 0.05^{f}	20.46 ± 0.11^{a}	2.57 ± 0.07^{f}	7.22 ± 0.33^{e}	20.62 ± 0.12^{a}
3:1	35.93 ± 0.02^{d}	15.99 ± 0.08^{de}	5.34 ± 0.05^{e}	18.62 ± 0.27^{e}	16.86 ± 0.08^{c}

Data are the mean ± SD, n = 3, Mean values in the same column bearing the same superscript do not differ significantly ($P \leq 0.05$). L*= lightness, a*= redness, b*= yellowness, hue= $\tan^{-1}$ b*/ a*, chroma= $(a^{*2}+b^{*2})^{1/2}$

scavenging activity of pomegranate seeds and rind powder .In contrast, significantly higher radical scavenging activity was observed in pomegranate rind powder compared to juice [3]. Cooked sausage contained 3% PRP had significantly($P \leq 0.05$) greater free radical scavenging activity followed by3:1 mixture of PRP:RBP, both mixture of PRP,RBP 2:3 and 1:5, 2% PRP, 3% RBP, 1%PRP, 1% RBP and control, respectively. For instance, a sharp increase in radical scavenging activity with increasing the level of PRP up to 3% and /or present with RBP at ratios 3:1PRP: RBP was observed among all raw manufactured or cooked samples. Also, Negi and Jayaprakasha [46] reported a sharp increase in radical scavenging activity with an increase in the concentration of pomegranate peel extracts from 50 to 400 ppm. Reducing properties are generally associated with the presence of reductions [48].

Instrumental Color Evaluation of Manufactured Sausage: Preliminary work with evaluation of instrumental color in manufactured sausage with added different concentrations of PRP, RBP and their mixtures revealed a significantly difference as shown in Table 4. Treatments with different ratios of RBP and mixtures of PRP +RBP reduced ($P \leq 0.05$) the L* (lightness) values compared to control sausage and sausage treated with various ratios of PRP. Meanwhile, there was a little significant increase ($P \leq 0.05$) in L* values for manufactured sausage by rising

the concentration of PRP up to 3.0% but significantly reducing was noticed by increasing the adding ratio of RBP (from 1-5%) on lightness (L*) values. Significant increase in a* (redness) values was observed in various manufactured sausage correlated well with the type and concentration of used either PRP up to 3%, or RBP up to 5% or their used mixtures. However, a significant gradually increasing ($P \leq 0.05$) in a* values were showed by increasing the concentration of both RBP 1-3% and (1:5 and 2:3) of PRP: RBP compared with control sausage. In contrast, significant gradually decreasing in a* values was noticed by increasing the concentration of PRP in manufactured sausage samples compared to control. The significant decrease in a* values indicated the change in color from red to brown which could be due to the formation of metmyoglobin in salt containing treatment [18]. Naveena *et al.* [3] reported that addition of PRP reduced the lightness (L*) value. However they have further found an increase in redness value of chicken patties due to addition of PRP. However, our results indicated that, the addition of PRP, RBP and their mixtures might be responsible for increasing the L* and a* values in manufactured sausage. Consequently, control and both 1.0 and 2.0% PRP treated sausage did not show a significant difference for yellowness (b* value). However, b* values significantly ($P \leq 0.05$) reduced by adding RBP and mixture of PRP+ RBP but this reduction in b* values were very sharp by adding 3.0 and 5% RBP for manufactured sausage. Hue values significantly ($P \leq 0.05$) reduced sharply due to addition of PRP mixing with RBP at ratios of (1:5, 2:3 and 3:5) and /or RBP up to 5%, while, there were no significant difference for hue values between control and sample contained 3.0% PRP. On other hand, a little significant difference ($P \leq 0.05$) for chroma values were noticed between control and adding PRP up to 3.0%. The reason why addition of PRP, RBP and their mixtures for manufactured sausage affected for color parameters may be due to the different contents of anthocyanines and betalains pigments present in PRP and RBP.

There was no significant difference ($P \leq 0.05$) in lightness (L* value) between cooked control sample and samples contained 1, 2 and 3% PRP (Table 5). But, L* value significantly ($P \leq 0.05$) decreased gradually in samples contained 1, 3 and 5% RBP than control. On the other hand, a* values were greater significantly difference ($P \leq 0.05$) among samples contained RBP depending on the used ratio, where the sample contained 3% had recorded the highest a*values followed by 5% and 1%, respectively. Yellowness (b* values) significantly ($P \leq 0.05$) increased gradually by increasing the concentration of PRP or the mixture of PRP: RBP compared to control. Whereas, no significantly difference between control sample and cooked sample contained 3% RBP was showed. The yellowness (b^*) was affected by the type and concentration of used additives.

Also, results in Table 5 revealed that, a good correlation between the type and concentration of used additives for cooked sausage. Addition of PRP increased Hue values but adding any concentration of RBP (1-5%) and mixtures of PRP: RBP with ratios of 1:5 and 2:3 caused to decline hue values. In contrast, cooked sausage

Table 5: Effect of adding different concentrations of pomegranate rind powder (PRP), beet root powder (RBP) and their mixtures for internal parts of cooked manufactured sausage on Hunter L^*, a^*, b^*, Hue and Chroma values

Treatment	L^*	a^*	b^*	Hue	Chroma
Control	47.2 ± 0.07^{b}	5.93 ± 0.08^{g}	8.15 ± 0.02^{f}	54.00 ± 0.41^{d}	10.08 ± 0.05^{j}
PRP (%)					
1	47.16 ± 0.01^{b}	4.74 ± 0.05^{h}	10.48 ± 0.01^{e}	65.68 ± 0.24^{c}	11.50 ± 0.03^{i}
2	46.99 ± 0.03^{c}	4.10 ± 0.03^{i}	11.15 ± 0.03^{d}	69.81 ± 0.18^{a}	11.89 ± 0.02^{h}
3	47.49 ± 0.08^{a}	5.58 ± 0.15^{g}	13.44 ± 0.02^{a}	67.48 ± 0.62^{b}	14.55 ± 0.04^{f}
RBP (%)					
1	39.98 ± 0.03^{e}	10.91 ± 0.03^{e}	6.62 ± 0.02^{h}	31.31 ± 0.12^{g}	12.76 ± 0.02^{g}
3	37.5 ± 0.16^{f}	18.87 ± 0.05^{b}	8.15 ± 0.08^{f}	23.43 ± 0.28^{h}	20.55 ± 0.05^{b}
5	33.69 ± 0.07^{h}	17.16 ± 0.29^{c}	5.23 ± 0.11^{i}	16.99 ± 0.51^{j}	17.94 ± 0.25^{d}
PRP: RBP (%)					
1:5	32.63 ± 0.06^{i}	22.47 ± 0.24^{a}	7.35 ± 0.02^{g}	18.09 ± 0.30^{i}	23.64 ± 0.22^{a}
2:3	36.99 ± 0.10^{g}	15.99 ± 0.55^{d}	11.87 ± 0.20^{c}	36.69 ± 1.32^{f}	19.92 ± 0.32^{c}
3:1	43.52 ± 0.04^{d}	10.18 ± 0.01^{f}	12.92 ± 0.18^{b}	51.78 ± 0.38^{e}	16.45 ± 0.15^{e}

Data are the mean ± SD, n = 3, Mean values in the same column bearing the same superscript do not differ significantly ($P \leq 0.05$). L^* = lightness, a^* = redness, b^* = yellowness, hue = $\tan^{-1} b^*/a^*$, chroma = $(a^{*2}+b^{*2})^{1/2}$

Table 6: Effect of adding different concentration of pomegranate rind powder (PRP), beet root powder (RBP) and their mixtures for surface layers of cooked manufactured sausage on Hunter L*, a*, b*, Hue and Chroma values

Treatment	L*	a*	b*	Hue	Chroma
Control	31.41 ± 0.05^{e}	5.91 ± 0.15^{f}	8.85 ± 0.03^{ab}	56.18 ± 0.62^{ab}	10.64 ± 0.09^{c}
PRP (%)					
1	38.89 ± 0.02^{b}	5.95 ± 0.19^{f}	7.93 ± 0.06^{c}	53.17 ± 1.21^{b}	9.92 ± 0.07^{e}
2	39.92 ± 0.05^{a}	5.10 ± 0.04^{f}	33.69 ± 0.02^{a}	59.63 ± 0.17^{a}	10.1 ± 0.03^{de}
3	39.25 ± 0.95^{b}	5.29 ± 1.30^{f}	8.71 ± 0.25^{b}	59.69 ± 6.69^{a}	10.46 ± 0.49^{cd}
RBP (%)					
1	32.16 ± 0.05^{d}	12.93 ± 0.07^{cb}	8.97 ± 0.04^{a}	28.52 ± 0.25^{d}	14.69 ± 0.04^{b}
3	25.64 ± 0.03^{f}	8.70 ± 0.45^{e}	6.99 ± 0.07^{d}	12.41 ± 0.95^{f}	8.91 ± 0.43^{f}
5	23.55 ± 0.08^{g}	9.72 ± 0.11^{d}	0.47 ± 0.1^{h}	2.85 ± 0.56^{g}	9.73 ± 0.1^{e}
PRP: RBP (%)					
1:5	25.45 ± 0.06^{f}	16.43 ± 0.14^{a}	2.79 ± 0.04^{f}	9.11 ± 0.49^{f}	16.66 ± 0.14^{a}
2:3	31.39 ± 0.12^{e}	13.49 ± 0.45^{b}	5.95 ± 0.02^{e}	23.78 ± 0.8^{e}	14.75 ± 0.42^{b}
3:1	37.64 ± 0.14^{c}	12.15 ± 0.34^{c}	8.09 ± 0.04^{c}	33.65 ± 0.85^{c}	14.6 ± 0.28^{b}

Data are the mean ± SD, n = 3, Mean values in the same column bearing the same superscript do not differ significantly (P = 0.05). L* = lightness, a* = redness, b* = yellowness, hue= $\tan^{-1}$ b*/ a*, chroma= $(a^{*2}+b^{*2})^{1/2}$

samples contained mixtures of PRP + RBP recorded higher chroma values than all investigated cooked sausage samples. While, another tested samples either contained 3.0% PRP and any concentration of RBP and mixtures of PRP + RBP had significantly difference (P ≤ 0.05) as compared to control. Lightness (L*) , redness (a*), yellowness (b*) hue and chroma were significantly difference (P ≤ 0.05) affected by the type and concentration of adding PRP, RBP and their mixtures for the surface layers of cooked manufactured sausage (Table 6). While, increasing the concentration of PRP in manufactured sausage increased (P ≤ 0.05) the L* values on surface of manufactured sausage compared to control. But L* values significantly (P ≤ 0.05) reduced gradually by increasing the concentration of RBP up to 5.0% and a significant difference for sausage manufactured with adding different ratios of PRP:RBP was observed compared with control. On other hand, redness (a*) in the surface of cooked manufactured sausage did not differ (P ≤ 0.05) between control and different concentrations of PRP, this indicates that, PRP did not have any positive or negative on the b* values of manufactured sausage. Meanwhile, adding of RBP up to 5% and mixtures of PRP: RBP were significantly gradually increased a* values. The changes of L*, a* and b* for cooked manufactured sausage than raw sausage may be explained by the opinion of Mitsumoto *et al.* [49] they have reported the discoloration of chicken meat patties with addition of natural antioxidants like tea catechins. Also, our results are similar with Naveena *et al.* [3], they noted that some other factors could have an effect on meat color parameters such as the fineness of mincing and consequently surface reflection properties. Yellowness (b* values) had no significantly (P≤0.05) difference for sausage manufactured by adding 3.0% PRP, 1.0% RBP and 3: mixture of PRP: RBP compared to control. But there were a significantly (P≤0.05) gradual reduction was observed by increasing the concentration of RBP up to 3.0%. Rojas and Brewer [50] also reported a decrease in b* values of beef patties containing natural antioxidants. They further observed that L* value increased initially and remained constant later on. On the other hand, there was no significant difference (P≤0.05) for both hue and chroma values between control and sausage contained PRP up to 3%. Whereas, increasing the addition of RBP significantly decreased both hue and chroma values in the surface of manufactured sausage compared to control.

Effect of Storage Period on Thiobarabituric Acid (TBA) Values for Manufactured Sausage: Effect of storage period at 5±1°C up to 6 days on TBA values for manufactured sausage is shown in Table 7. The raw manufactured sausage either the control or treated with different ratios of PRP, RBP and their mixtures recorded 0.0 TBA values. These results are in agreement with Devatkal and Naveena [51], they found that control goat meat +2 % salt+2 % Kinnow rind powder(KRP), goat meat+2 % salt+2 % Pomegranate rind powder(PRP) and goat meat +2 % salt+2 % pomegranate seed powder (PSP) recorded zero TBA during initial days of storage.

Table 7: Changes in Thiobarabituric acid (TBA) values (mg of malonaldehyde kg^{-1} meat) for raw sausage contained different levels of pomegranate rind powder (PRP), red beet powder (RBP) and their mixtures during refrigerated storage at (5±1°C).

Treatments	Day 0	Day 2	Day 4	Day 6
Control	0	0.447 ± 0.02^{aC}	0.563 ± 0.03^{aB}	0.678 ± 0.46^{aA}
PRP (%)				
1	0	0.273 ± 0.02^{bC}	0.530 ± 0.03^{bB}	0.545 ± 0.04^{bA}
2	0	0.250 ± 0.02^{cC}	0.445 ± 0.02^{cB}	0.530 ± 0.02^{cA}
3	0	0.233 ± 0.05^{dC}	0.328 ± 0.03^{fA}	0.351 ± 0.02^{fB}
RBP (%)				
1	0	0.126 ± 0.02^{eC}	0.421 ± 0.02^{dB}	0.491 ± 0.03^{dA}
3	0	0.109 ± 0.01^{fC}	0.390 ± 0.02^{eB}	0.437 ± 0.03^{eA}
5	0	0.094 ± 0.08^{gC}	0.234 ± 0.02^{iB}	0.257 ± 0.03^{iA}
PRP: RBP (%)				
1:5	0	0.098 ± 0.02^{gC}	0.242 ± 0.02^{hA}	0.265 ± 0.18^{iB}
2:3	0	0.124 ± 0.02^{eC}	0.250 ± 0.05^{gA}	0.281 ± 0.02^{hB}
3:1	0	0.108 ± 0.03^{fC}	0.251 ± 0.03^{gA}	0.289 ± 0.02^{gB}

Storage conditions within the same treatment (row-wise) bearing the same superscript do not differ significantly ($P \leq 0.05$); each value is a mean ± SD of three replicates.

Table 8: Sensory evaluation of manufacture cooked sausage in relation to adding different ratios of pomegranate rind powder (PRP), red beet powder (RBP) and their mixtures.

Treatments	Appearance	Color	Flavor	Juiciness	Overall palatability
Control	7.83 ± 0.75^{a}	7.00 ± 1.26^{ba}	8.50 ± 0.84^{a}	8.00 ± 1.79^{ba}	8.00 ± 1.38^{ba}
PRP (%)					
1	7.67 ± 0.82^{a}	6.33 ± 1.21^{b}	6.33 ± 0.41^{d}	7.33 ± 0.82^{bac}	6.67 ± 0.82^{bdc}
2	6.83 ± 0.98^{a}	6.67 ± 1.03^{b}	6.67 ± 0.82^{d}	7.00 ± 1.10^{bac}	7.00 ± 0.89^{bdac}
3	6.33 ± 1.21^{a}	6.50 ± 0.55^{b}	6.67 ± 0.82^{d}	6.17 ± 1.33^{c}	6.58 ± 0.92^{dc}
BRP (%)					
1	7.33 ± 2.07^{a}	8.17 ± 1.33^{a}	8.00 ± 1.26^{bac}	8.33 ± 1.51^{a}	8.33 ± 1.51^{a}
3	7.83 ± 1.60^{a}	7.17 ± 0.41^{ba}	8.17 ± 0.75^{ba}	8.17 ± 1.17^{bac}	7.83 ± 0.68^{bac}
5	7.83 ± 1.33^{a}	6.33 ± 0.82^{b}	7.00 ± 1.10^{bdc}	7.67 ± 1.51^{bac}	7.17 ± 0.98^{bdac}
PRP: BRP (%)					
1:5	7.67 ± 1.21^{a}	6.83 ± 0.98^{b}	6.83 ± 0.98^{dc}	7.33 ± 1.37^{bac}	7.17 ± 1.13^{bdac}
2:3	6.50 ± 1.76^{a}	6.50 ± 1.22^{b}	6.17 ± 1.17^{d}	6.00 ± 1.67^{c}	6.27 ± 0.98^{d}
3:1	7.00 ± 1.26^{a}	6.17 ± 0.41^{b}	6.50 ± 0.55^{d}	6.33 ± 1.21^{c}	6.50 ± 0.55^{dc}

Data are the mean ± SD, n = 10, Mean values in the same column bearing the same superscript do not differ significantly ($P \leq 0.05$).

However, a significant difference ($P \leq 0.05$) was observed of TBA during refrigerated storage after 2 days of storage for all tested samples but correlated well with the type and concentration of used additives. Where, after two days of storage gradual significant decrease in TBA values was recorded by increasing the ratios of PRP and RBP than control. On the other hand, a gradual significant increase ($P=0.05$) in TBA values was noticed for all treatments by extending the shelf life up to 6 days. Whereas, the sausage treated with 5% RBP had the lowest TBA value followed by treated with mixture of 1:5 from PRP: RBP and treated with 3% PRP after 2,4 and 6 days respectively compared to greater significant increase ($P \leq 0.05$) in control sample either after 4 days and /or 6 days of storage. Therefore, adding PRP, RBP and their mixtures for manufactured sausage reduced the TBA values by extending the storage period and improving the shelf life compared to control sausage, where, the best concentrations were 3% PRP, 5% RBP and mixture contained 1:5 PRP: RBP. These results are in agreement with Naveena *et al.* [3], they found that, the TBARS values significantly ($P \leq 0.05$) increased in control patties throughout storage, however in treated patties the values increased ($P \leq 0.05$) up to the 5th days of storage only. The increase in TBARS values in rind pomegranate treated samples was very slow and remained lowest up to 15 days. In the recent study Devatkal *et al.* [18] found positive correlation between phenolic compounds of pomegranate and Kinnow by-products extracts and reduced TBARS in cooked goat meat patties.

Sensory Evaluation: Results from sensory evaluation of cooked sausage contained different concentrations of used additives are presented in Table 8. Appearance of all cooked manufactured sausage contained different concentrations of used additives were not effected ($P \leq 0.05$) by the type and/ or concentrations of used PRP, RBP and Their concentrations. But a significant difference ($P \leq 0.05$) between cooked manufactured sausages correlated with type and proportions of addition concentration was noticed. However, most tested sausages recorded no significantly difference ($P \leq 0.05$) between them, respectively. Whereas, there were a significantly difference ($P \leq 0.05$) for flavor among manufactured samples correlated with the type and concentration of used additives were observed. The sausage sample contained 1.0 % RBP recorded the highest score for juiciness among tested samples including control sample and there were a significant difference for juiciness between manufactured samples. On other hand, a slightly significant difference ($P \leq 0.05$) of over all palatability between manufactured samples affected with the type and concentration of PRP, RBP and their mixtures were noticed. However, the sausage contained 1% RBP recorded the highest values for over all palatability than other tested samples. Therefore, the sensory attributes of manufactured sausage was affected and significant relation ($P \leq 0.05$) with the type concentration of used additives. Meanwhile, our results indicated that, the manufactured sausage made with 1% and/or 3% RBP recorded the highest sensory scores of selected parameters.

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