Incorporation of pomegranate rind powder extract and pomegranate juice into frozen burgers: oxidative stability, sensorial and microbiological characteristics

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Abstract This study was aimed to evaluate the antibacterial and antioxidant characteristics of incorporated pomegranate juice (PJ) and pomegranate rind powder extract (PRPE) into meat burgers. The peroxide value, thiobarbituric acid reactive substances, and metmyoglobin content for different burgers during 90 days storage at -18°C were evaluated. Total anthocyanin content, total phenolic content (TPC) and free radical scavenging activity (RSA or IC₅₀) for PJ and PRPE were measured as 18.90 (mg/mL), 4380 ppm, 0.136 (mg/mL) and 0.40 (mg/mL), 5598 ppm, 0.084(mg/mL), respectively. Incorporation of PRPE with a high concentration of TPC resulted in less oxidation of lipid in comparison with other formulations. The highest and lowest scores in the sensory analysis and total acceptance at the 90th day corresponded to burgers containing PJ and control, respectively. Butylated hydroxytoluene may be substituted in whole or part with PJ and PRPE due to their desired effects on burgers' properties.

Keywords Burger · Phenolic components · Oxidation · Storage · Antioxidant and antimicrobial activity · Pomegranate

Introduction

Lipid oxidation of meat products strongly influences their sensory properties including flavor, odor, texture, and color and consequently decreases their shelf life (Gahruie et al. 2017). Minced meat products such as burgers are more encountered to a higher rate of lipid oxidation mostly due to greater exposure of membrane's lipid to oxygen. Although synthetic antioxidants such as BHT (Butylated hydroxytoluene) and BHA (butylated hydroxyanisole) have been used to prevent oxidation (Pateiro et al. 2018), the concerns associated with their adverse health effects, drive the increased demand for application of natural antioxidants in the food products such as burgers.

Some natural ingredients such as grains, oilseeds, honey, vegetables, and fruits derivatives and extracts have been examined as antioxidant agents in different types of meat products (Gahruie et al. 2017; Asl et al. 2018; Bakhtiari et al. 2018; Pilevar et al. 2017). Osada et al. (2000) incorporated apple polyphenols as an antioxidant agent into sausages and observed that the cholesterol and linoleic acid oxidation in sausage was inhibited. They attributed this inhibitory effect to the antioxidant properties of catechin in apple. Also, sensory characteristics and phenolic content of pre-cooked pork sausage containing fruit purees were evaluated by Leheska et al. (2006). They reported that these natural additives increased the phenolic content. Additionally, Brannan (2008) demonstrated the high antioxidant activity of added grape seed extract into chicken meat which can inhibit the formation of the secondary oxidation product of lipids. According to Rojas and Brewer (2008), who examined the antioxidant properties of oregano (OR), grape seed (GS), and rosemary extracts (RE) in frozen vacuum packaged beef and pork, OR, GS, and RE showed similar antioxidant activity. Hernández-Hernández

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et al. (2009) evaluated the antioxidant properties of rosemary and oregano extracts on lipid oxidation and color of raw pork batters. It was demonstrated that rosemary extract showed higher antioxidant activity. They also reported that not only the polyphenols concentration but also the solvent and the method of extraction can affect on the intensity of antioxidant activity.

Pomegranate (*Punica granatum*) is an important fruit cultivated in Iran and northern India and most Mediterranean regions. The pomegranate rind, a by-product of pomegranate juice extraction plants, can be considered as a rich source of phenolic compounds (Khajehei et al. 2015). Application of pomegranate juice and pomegranate rind powder extract as natural antioxidant agents in chicken patties had been investigated (Naveena et al. 2008). Considering their findings, the phenolic content in the patties containing pomegranate juice and pomegranate rind powder extract increased significantly without any changes in sensory properties. Pomegranate rind powder extract showed much higher antioxidant activity than BHT. Devatkal and Naveena (2010) incorporated kinnow (mandarin hybrid) and pomegranate by-product powder into the raw ground goat meat to examine their effects on oxidation and color. The effectiveness of these additives on oxidation inhibitory effect was in the order of pomegranate seed powder > pomegranate rind powder > kinnow rind powder.

To date, no investigation regarding the antioxidant and antimicrobial effects of pomegranate derivatives incorporated into meat burgers was published. The present study, for the first time, examined the influence of incorporation of pomegranate rind powder extract (PRPE) and pomegranate juice (PJ) into meat burgers on their physico-chemical and sensory characteristics as well as their oxidation stability and bacterial growth during 90 days of storage. Also, the antioxidant activity of introduced formulation was compared with those prepared with BHT as one of the most used synthetic antioxidant agents.

Materials and methods

Materials

Fresh calf thigh meat comprising of 74% water, 21% protein, 3% fat and 2% ash (approximate data provided by the slaughterhouse) was frozen at -40°C for 24 h and then minced by industrial meat grinder (Meat Grinder, Philips, HR2743, Amsterdam, Netherlands). Soy isolate, containing 70% protein, was obtained from Solae Company (Aarhus, Denmark). Pomegranate was obtained from Khajei Ghasrodasht cultivar, Fars. Also, onion, salt, mixed spices, and toasting flour were purchased from a local

market (Shiraz, Iran). All chemicals used for the analysis in analytical grade were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA) and Merck Co. (Darmstadt, Germany).

Preparing pomegranate juice (PJ) and pomegranate rind powder extract (PRPE)

Fresh pomegranate was washed twice with tap water and cut into 4 pieces. A simple stainless-steel mechanical hand press juice extractor was used to press the juice out of arils. The juice was then centrifuged (KUBOTA 5500 Centrifuge, Japan) for 20 min at 300 g to separate the pulps. The pomegranate juice (PJ) was kept frozen at -20°C (maximum 1 week).

To prepare pomegranate rind powder extract (PRPE), pomegranate rind (PR) was manually separated from the arils, washed, shredded into small strips and dried in a cabinet dryer at 50°C for about 20 h. A low-speed mill (Pars Khazar, Iran) was used to carefully prepare powder from the dried strips. The powder was passed through a 60 mesh (250 microns) sieve and then kept refrigerated (maximum 1 week) in dark polyethylene bags. PRPE was prepared by addition of 500 mL of distilled water to 20 g grind powder. The mixture was then left to stand for about 10 min in order to sediment the suspended particles. The supernatant was finally filtered using a filter paper (Whatman No. 1) (Naveena et al. 2008; Vaithyanathan et al. 2011).

Pomegranate chemical parameters

pH and acidity

The pH of pomegranate juice and rind powder extract were determined using a digital pH-meter (Suntex TS-1, Taiwan). The titratable acidity (based on citric acid) of pomegranate juice and pomegranate rind powder extract (PRPE) was measured using 0.1 N NaOH to a pH value of 8.1 (Poyrazoğlu et al. 2002).

Brix and total soluble solid

The total soluble solids (TSS) of pomegranate juice and rind extract were determined using a refractometer (ATAGO Hand Refractometer, Japan) at 20°C based on the °Brix (Khajehei et al. 2015).

Total anthocyanin content (TAC)

To determine total anthocyanin content (TAC) (mg/mL) of PRPE and PJ, a pH differential method as described by AOAC (method 1995-02) was used. This is an applicable method for determination of monomeric anthocyanin and

the results were expressed as cyanidin-3-glucoside (AOAC 1995).

Total phenolic content (TPC)

The total phenolic content (TPC) (ppm) was measured by Folin–Ciocalteu based on the method described by Shaghaghian et al. (2014).

Radical scavenging activity (RSA)

The radical scavenging activity (RSA) (mg/mL) was determined by 2,2-diphenyl-1-picrylhydrazyl free radicals (DPPH[•]) according to the method described by Ravanfar et al. (2016).

Burger preparation and storage

The formulation of burger consisted of beef ground meat (after removing visible adipose and connective tissues) (85%) and the rest which comprised by soy protein isolate (SPI) (3%), salt (0.99%), water (1.19%), spices (0.2%), breadcrumbs (2.99%) and minced onion (8.57%). Soy protein isolate (SPI) was then added to the mixture (meat, salt, water, and minced onion) and then mixed for 15 min to obtain a homogenous paste. Breadcrumbs and spices were added in the next step. Finally, three groups of samples were formulated with either PJ or PRPE or BHT (all of them in the concentration of 100 ppm) and thoroughly mixed to prepare homogeneous pastes. A burger maker was used to shape the mixture into patties approximately 100 g (9 cm internal diameter and 5 mm thickness). Burgers (2 patties per treatment) were then packed in light-resistant polyethylene containers and frozen at − 18 °C. Analyses were performed at 15-day intervals for three months as triplicates. Before each analysis, burgers were thawed at + 4 °C and mixed thoroughly for 30 s (Afshari et al. 2017).

Burger chemical composition

Protein, moisture, fat, carbohydrate and ash contents of burgers were determined as described by Hashemi Gahrue et al. (2017).

pH

Burger sample (5 g) was mixed with 25 mL deionized water for 2 min, and the pH was measured at 25 °C using a digital pH meter (Suntex TS-1, Taiwan) equipped with a probe-type combined electrode (Ingold). This is done by direct immersion of the electrode into the prepared mixture (Hashemi Gahrue et al. 2017).

Color evaluation

Color properties of burgers were measured as L* (brightness), a* (redness-greenness) and b* (yellowness-blueness) after thawing by a method described by Baeghbali et al. (2016).

Lipid oxidation

Peroxide value Peroxide value was determined based on the method recommended by Egan et al. (1991) and expressed in mmol of O₂ per kg of the sample.

Thiobarbituric acid reactive substances (TBARS) Lipid oxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) and then reported as mg of malonaldehyde (MDA) per kg of burger (Zhang et al. 2016).

Metmyoglobin content

A method proposed by An et al. (2010) with slight modification was used to determine the metmyoglobin contents. At first, 2 g of burger and 10 mL of phosphate buffer (0.04 M, pH 6.8) were mixed and homogenized at 1 °C for 1 h. The sample was then centrifuged (KUBOTA 5100 Centrifuge, Japan) at 3500 g and 4 °C for 30 min. Finally, the mixture was filtered through filter paper (Whatman No. 1). The absorbance (spectrophotometer, spec 1650PC, Shimadzu, Japan) of the filtered mixture was determined at 525, 572 and 700 nm. Metmyoglobin content (%) was calculated according to the Eq. (1):

$$\begin{aligned} \text{Metmyoglobin content (\%)} \\ = [1.395 - [(A_{572} - A_{700}) / (A_{525} - A_{700})]] \times 100. \end{aligned} \quad (1)$$

Total aerobic bacterial count

Total aerobic bacteria were measured every 15 days according to the method described by Aliakbarlu and Khalili Sadaghiani (2015).

Sensory evaluation

Sensory evaluation has been performed by 15 trained panelists aged between 20 and 35, from the Department of Food Science and Technology at Shiraz University. Panelists were selected based on their previous experience in sensory evaluation of burgers. Burgers were cooked at 140 °C in an oven to reach the core temperature to 75 °C and kept warm in an oven for 3–8 min before tasting by the

panelists. Each panelist randomly tested three pieces of burgers and asked to give a numerical value between 1 (the least palatable or very bad) and 5 (the most palatable or very good) for the following attributes: taste, texture properties, appearance, and odor. Finally, each panelist was asked to give an overall score from 1 (dislike very much) to 5 (like very much) for the overall acceptability of different burgers (Dehghani et al. 2018). The burgers were served as warm ($\sim 38^\circ\text{C}$), and unsalted crackers and mineral water were used to clean the palate between experiments.

Statistical analysis

All experiments were performed in triplicates. Data were analyzed using one-way analysis of variance (ANOVA) at a significance level of 5%. Duncan's multiple range tests were used to check for significant differences using the SAS[®] software (ver. 9.1, SAS Institute Inc., Cary, NC, USA.).

Results and discussion

Physicochemical properties of pomegranate juice (PJ) and pomegranate rind powder extract (PRPE)

Table 1 summarized some of the physicochemical properties of PJ and PRPE. The titratable acidity of PJ and PRPE were 1.10 and 0.32, respectively. The higher acidic character of pomegranate juice in comparison with pomegranate rind powder extract was expectable. Also, the Brix of pomegranate juice was noted as higher while compared with pomegranate rind extract (18.26 PG and 3.26 PRPE). Orak et al. (2012) evaluated the antioxidant activities of juice, peel, and the seed of pomegranate (*Punica granatum* L.) and their correlation with total phenolic, tannin, anthocyanin, and flavonoid contents. They reported acidity of juice is between 0.85 and 3.01% and acidity of peel is between 1.48 and 3.66%, also brix of juice 14.6–17.2. In a previous study, Vardin and Abbasoglu (2004) classified the pomegranate juices according to acidity as the following

classification; sour (acidity higher 2%), sour–sweet (acidity between 1 and 2%), sweet (acidity lower than 1%).

TAC, TPC and antioxidant activity of PJ and PRPE

As is shown in Table 1, PJ contained a considerable amount (18.9 mg/mL) of anthocyanin as compared to PRPE (0.4 mg/mL). The high concentration of anthocyanin in PJ was confirmed previously by Baeghbali et al. (2016).

TPC in PJ and PRPE were 4380 and 5598 ppm, respectively. Aviram et al. (2000) reported the phenolic content of PJ in different varieties of pomegranate. Anthocyanins, catechin, gallic and ellagic acids and ellagic tannins (soluble phenolic compounds) varied between 0.2 and 1.0% depending on the variety. According to Madrigal-Carballo et al. (2009), an increase in the phenolic content, significantly increased the antioxidant activity.

IC₅₀ value of PJ and PRPE were 0.136 and 0.084 mg/mL, respectively. IC₅₀ shows the concentration of inhibitor at which the response (or binding) is reduced by 50%. The lower the IC₅₀, the higher is the antioxidant capacity of an agent. The excessive antioxidant capacity of pomegranate can be associated with its high phenolic content. Interestingly, this property in rind extract was higher than the juice. The findings of Negi et al. (2003) confirmed the high antioxidant activity in different pomegranate peel extracts (in different solvents). Comparison between pomegranate rind powder (PRP), kinnow rind powder (KRP), and pomegranate seed powder (PSP) showed that the pomegranate rind powder and PSP had the highest phenolic content and free radical scavenging, respectively (Devatkal and Naveena 2010).

Proximate composition of burgers

The amount of protein in the formulation was calculated based on the 84.7% content of tight calf meat and soy isolate (containing 70% protein). Except for the meat fat, there was no fat added to the formulation. The moisture, protein, fat and ash content in prepared burgers were measured as 69.1%, 24.86%, 4.35%, and 2.03%, respectively. Based on the recommended levels by the Institute of

Table 1 Chemical analysis, total anthocyanins content (TAC), total phenolic contents (TPC) and antioxidant activity of pomegranate juice (PJ) and pomegranate rind powder extract (PRPE)

Sample	pH	Acidity (% citric acid)	TSS (°Brix)	TAC (mg/mL)	TPC (ppm)	IC ₅₀ (mg/mL)
PJ	3.39 $\pm$ 0.01 ^b	1.10 $\pm$ 0.00 ^a	18.26 $\pm$ 0.05 ^a	18.90 $\pm$ 0.62 ^a	4380 $\pm$ 39.40 ^b	0.136 $\pm$ 0.005 ^a
PRPE	3.61 $\pm$ 0.00 ^a	0.32 $\pm$ 0.00 ^b	3.26 $\pm$ 0.05 ^b	0.40 $\pm$ 0.02 ^b	5598 $\pm$ 52.5 ^a	0.084 $\pm$ 0.010 ^b

Data represent averages of three independent repeats $\pm$ standard errors. The data were analyzed by ANOVA at $P \leq 0.05$ significance. In each column, means with different letters (a and b) are significantly different. Antioxidant activity indicated as IC₅₀. IC₅₀ is the sample concentration providing 50% inhibition

Standards and Industrial Research of Iran (ISIRI 2016), the maximum allowable amounts of moist, protein, fat and ash for burgers containing 85% meat are 70%, 13.7%, 19%, and 2.5%, respectively. Gahruie et al. (2017) reported that beef burgers contain 59% moisture, 12% fat, 19% protein, 8% carbohydrate and 2% ash.

pH value of burgers

The changes in the pH value of burgers during 90 days of storage at -18°C is shown in Fig. 1. Because of the acidic nature of PJ and PRPE, the following order regarding the pH can be proposed: control > PJ and PRPE > BHT. In a similar study, the addition of BHT to chicken patties formulation reduced the pH value more than other patties containing PJ, PRPE, and control (Naveena et al. 2008). The pH value of control and burgers containing BHT were constant until the 45th day and then reduced. Moreover, in the burgers containing PJ and PRPE, the reduction in the pH observed after 60 days. The noted differences (the sharp decline in pH that occurred faster in control and sample containing BHT in comparison to the burgers containing PJ and PRPE) can be attributed to the antimicrobial effect of PJ and PRPE. Microbial growth occurred later in burgers containing PJ and PRER, and so the pH reduction (arisen due to microbial growth) occurred faster in the control burger and burger containing BHT (without any added antimicrobial agent).

Peroxide value of burgers

According to Fig. 2a peroxide value did not show a linear trend and had decreased and increased several times. The reductions can be the result of peroxide decomposition, and the increases may be the result of peroxide reproduction. The decrease in the peroxide value of all burgers was observed until the 45th day. Afterward, it was increased

Fig. 1 pH changes of meat burgers during 90 days of storage at -18°C (C: control, PJ: pomegranate juice, PRPE: pomegranate rind powder extract, BHT: butylated hydroxytoluene)

Fig. 2 Changes in peroxide value (a), MDA (mg/kg) (b) and metmyoglobin content (%) (c) of meat burgers during 90 days of storage at -18°C (C: control, PJ: pomegranate juice, PRPE: pomegranate rind powder extract, BHT: butylated hydroxytoluene)

and maximized on the 75th day. There was another reduction on the 90th day. The measured peroxide value of the control group was higher than those measured for other burgers. The value of 1.273 Meq O_2/kg on the 75th day for the control burgers was much higher than those measured for burgers containing PJ and PRPE. The incorporation of PJ and PRPE, as well as the synthetic antioxidant BHT, caused a reduction in the peroxide formation. The lowest peroxide value during the storage time (except at the 75th day) belonged to burgers containing PRPE and then, on 15th, 30th and 45th days of storage was related to burgers incorporated by PJ and on 60th, 75th and 90th days of storage belonged to samples containing BHT. Qin et al.

(2013) reported that the presence of antioxidants including BHT, PJ powder, PRPE, and pomegranate seed powder (PSP) in raw ground pork meat caused the PV to reduce. Based on their results, the inhibitory effect of PRPE on peroxide formation was greater than PSP and PJ powder.

Thiobarbituric acid value of burgers

Thiobarbituric acid (TBA) value of burgers as an index of oxidative stability was determined during 90 days of storage at -18°C . Considering that TBA measurement is a colorimetric method, the red color of PJ can interfere with the result of the test. Hence burgers containing PJ showed high absorbance even on the first day. To overcome this issue and eliminate the effect of the red color due to the presence of PJ, the amount of measured Malondialdehyde (MDA) on the first day was subtracted from MDA measured on following days up to 90th. Both the amount of oxidation, as well as MDA, increased during the elapsing time in all treatments (Fig. 2b). The values of MDA correspond to control group was always the highest except on the 60th day. Incorporation of PJ and PRPE into burgers' formulation resulted in a decrease in lipid oxidation compared to the control due to their antioxidant characteristics. MDA showed a significant increase between each two ($P \leq 0.05$) consecutive measurements. The maximum increase in the values of MDA was evaluated for the control and PJ containing burgers, respectively. No significant difference ($P \leq 0.05$) was observed between MDA formation in burgers containing PRPE and BHT which was due to efficient control of lipid oxidation by PRPE and BHT from the first to 90th day throughout the storage period. Qin et al. (2013) showed that TBA value of cooked chicken patties containing PRPE, PJ and BHT (0.896, 0.763 and 0.203 mg malonaldehyde per kg samples, respectively) was less than control (1.272 mg malonaldehyde per kg samples). The lipid oxidation in cooked chicken patties was substantially prevented ($P < 0.01$) in PRPE treatment to a much greater extent than BHT treatment. According to Devatkal and Naveena (2010), the formation of TBARS in cooked goat meat patties was reduced more effectively by PRPE compared to PSP and kinnow rind powder. Lorenzo et al. (2013) investigated the effect of grape seed and chestnut extract on the physico-chemical, lipid oxidation, microbial and sensory characteristics of dry-fermented sausage. The addition of grape seed and chestnut extract had a significant effect on TBARS as compare to BHT.

Metmyoglobin content of burgers

Because of the red color of pomegranate juice, the absorption and the metmyoglobin content measurement in the burgers containing the juice showed higher values than other burgers even on the first day. For this reason, the metmyoglobin content was reported in proportion to its content on the first day. Increase in the metmyoglobin content was observed in all treatments. Moreover, the control sample showed the maximum metmyoglobin content. However, no significant difference in term of metmyoglobin content between pomegranate rind powder extract and BHT incorporated burgers was observed, they were more efficient in keeping the red color as compared with pomegranate juice. There was an increasing trend in metmyoglobin content in the all 4 treatments during 90 days of storage (Fig. 2c). The average increase in metmyoglobin content in control and burgers containing the PJ, the rind extract, and BHT were 21.72%, 19.48%, 17.81%, and 18.81%, respectively. In this research, PRPE and PJ in burgers' formula not only reduced burgers oxidation (and formation of free radicals) but also maintained the favorite color of burgers up until the 90th day. Antioxidant activity of PRPE may be related to its reducing ability and radical scavenging power. Donation of hydrogen from phenolic compounds causes free radical chains to break, and a stable end product can be formed in this way. In similar studies, stabilizing of the color in meat products by incorporation of natural antioxidants obtained from leafy vegetables (butterbur, chamnamul, bok choy, Chinese chives, crown daizy, fatsia, pumpkin, sesame, stonecrop and flowering head of broccoli) was reported by Kim et al. (2013). Prevention or lowering metmyoglobin formation by incorporation of a kimchi powder natural antioxidant into the meat products is also reported by An et al. (2010).

Color of burgers

The brightness factor L^* of burgers in the control group increased during 90 days of storage. L^* for all other groups, however, exhibited a decreasing trend (Fig. 3a). In an industrial production scale, increasing brightness of frozen burgers is an unfavorable phenomenon leading to the non-meat components to look in a higher proportion. This reduction can be the result of dilution of meat pigments because of the presence of non-meat components. In this work, PJ, PRPE and BHT addition to burgers resulted in inhibition of the brightening process. L^* factor of different burgers had a significant difference with each other's ($P < 0.05$). The highest effect was associated with the presence of PRPE, followed by PJ and BHT, respectively. Burgers containing PJ on 75th and 90th days had lower L^* values than others day. The control group of burgers had

Fig. 3 Changes in L^* value (a), a^* value (b) and b^* value (c) of meat burgers during 90 days of storage at -18°C (C: control, PJ: pomegranate juice, PRPE: pomegranate rind powder extract, BHT: butylated hydroxytoluene)

the lowest and the highest values of brightness on the first and 90th days, respectively. Reduction in the lightness of chicken burgers containing grape antioxidant dietary fibers and also their controls during the storage time was reported by Sáyago-Ayerdi et al. (2009).

Another important factor influencing the consumer acceptance of meat burgers is a^* , the burger's redness. Decreasing a^* shows metmyoglobin accumulation in the product. Based on Fig. 3b, all burgers showed a decrease in a^* factor during the 90 days which is caused by the metmyoglobin formation and burgers browning (darkening)

(Kennedy et al. 2005). On the final day of storage, burgers containing PRPE showed the least decrease in a^* (the highest a^* value), which is another evidence that shows pomegranate rind powder extract is more efficient than PJ in controlling oxidation process.

b^* values have decreased for all burgers during the storage period (Fig. 3c). Burgers containing BHT and PRPE had the maximum and minimum values for b^* , respectively. Combination of yellow and red colors create brown color. That is why the burgers containing BHT showed the maximum intensity of brown color. Comparing the first and 90th days, the control burgers showed the maximum yellowness while burgers containing the PRPE had the minimum yellowness. Devatkal and Naveena (2010) reported that addition of PRPE into cooked goat meat patties formulation caused L value to decrease and a value to increase.

Antibacterial activity

The formulated burgers were also evaluated for the growth of aerobic bacteria during 90 days of storage at -18°C (Fig. 4). As evident in Fig. 4, the aerobic bacterial count has increased during storage, while the antibacterial effect of pomegranate juice and rind extract were confirmed. The maximum microbial count was associated with control burgers following burgers containing BHT. Aerobic bacterial count of burgers containing PJ and PRPE was almost the same up to the 45th day. However, the count for burgers containing PJ showed an increasing trend between 45th and 90th days. Burger containing pomegranate rind powder extract showed the minimum aerobic bacterial count at the end of the storage. Acidic pH of these two burgers is one of the reason confirmed the results. Antibacterial effect of pomegranate and its derivatives could perhaps be due to the presence of metabolites or

Fig. 4 Comparison in total aerobic bacteria count (CFU/g) of meat burgers during 90 days of storage at -18°C (C: control, PJ: pomegranate juice, PRPE: pomegranate rind powder extract, BHT: butylated hydroxytoluene)

antibiotic compounds (Hayrapetyan et al. 2012). Polyphenols are one of them that apply their antimicrobial effect by reaction with sulfhydryl groups of proteins and make them unavailable for microorganisms (Gullon et al. 2016). Pomegranate extract inhibits bacterial protein from secretion. In a study, Al-Zoreky (2009) indicated the antibacterial effect of pomegranate against *Yersinia enterocolitica*, *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus*.

Sensory attributes of burgers

Color, flavor, and texture are the most important sensory attributes which influence the acceptability of meat products by consumers. Sensory analysis of the burgers is shown in Fig. 5a, b. All burgers attained close scores on the first day of the storage; while the color of burgers containing PJ scored the highest values. This is associated with the favorable red color of anthocyanin imparted to burgers by the presence of PJ. On the 90th day, burgers containing PRPE scored the highest values regarding color, flavor, odor, texture and total acceptance. Burgers containing PJ and BHT achieved the second and third place in the list, respectively. Very low acceptability of control sample may

be attributed to the flavor of fat and fat oxidation products. The sourness of PJ in the burger not only was pleasant to the panelists but also might to some extent reduces the fatty flavor. Devatkal and Naveena (2010) revealed that incorporating PRPE into goat meat patties did not have any negative effect on their acceptance.

Conclusion

PRPE, PJ, and BHT as antioxidant agents were incorporated into meat burger formulations and oxidative stability of burgers were measured during 90 days of storage at -18°C . Antimicrobial and sensory properties were also examined during the storage period. The high content of phenolic compounds in the PRPE and PJ offers a good antioxidant activity. Incorporation of PRPE and PJ into burgers formulation helped to prevent lipid oxidation and enhanced the acceptability of the products. Antioxidant capacity of PRPE and its protective effect on lipids in burgers was higher than PJ. Aerobic bacterial growth in burgers containing PJ and PRPE was less than the control sample. Considering the high antioxidant property of pomegranate derivatives as well as consumer's demand for natural preservatives and ingredients, PRPE and PJ exhibited potentials as appropriate substitutes for synthetic antioxidants in the high-fat meat products.

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Fig. 5 Sensory attributes of meat burgers on first day (a), and day 90th (b), (C: control, PJ: pomegranate juice, PRPE: pomegranate rind powder extract, BHT: butylated hydroxytoluene)

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