Annals of Agricultural Science (2015) 60(2), 403–412

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Annals of Agricultural Science

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Quality characteristics of beef sausage containing pomegranate peels during refrigerated storage

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Received 19 October 2015; accepted 28 October 2015
Available online 29 December 2015

KEYWORDS

Beef sausage;
Cooking characteristics;
Microbiological criteria;
Pomegranate peels;
TBA;
TVN;
Sensory characteristics

Abstract This study was performed to evaluate the effect of pomegranate peels powder at concentrations of 1%, 2% and 3% on keeping quality characteristics of prepared beef sausage during a storage period at (4 ± 2 °C) for 12 days. Chemical compositions, physical, physicochemical and chemical characteristics, microbiological criteria, cooking quality and sensory characteristics of different prepared beef sausage samples were determined. The addition of different concentrations of pomegranate peels powder caused high storage stability and reduced values of TBA and TVN of prepared beef sausage samples during refrigerated storage compared to control beef sausage samples. The microbiological criteria of prepared beef sausage samples with pomegranate peels powder were also improved. This could be due to the presence of phenolic compounds in pomegranate peels which could act as antioxidant and antimicrobial substances. Prepared beef sausage samples containing pomegranate peels powder recorded high cooking quality and sensory characteristics in comparison with control beef sausage samples.

Introduction

Meat and meat products are important sources for protein, fat, essential amino acids, minerals, vitamins and other nutrients (Biesalski, 2005). In recent years, much attention has been paid to develop meat and meat products with physiological functions to promote health conditions and prevent the risk of diseases. Oxidation of lipid and auto-oxidation are one of the major causes of quality deterioration and reduction of shelf life of meat products. This may produce changes in meat quality parameters such as color, flavor, odor, texture and even nutritional value (Fernandez et al., 1997). Meat mincing, cooking and other processing steps prior to refrigerated storage disrupt muscle cell membranes facilitating the interaction of unsaturated lipids with pro-oxidant substances such as non-haem iron, accelerating lipid oxidation leading to rapid quality deterioration and development of rancidity (Tichivangana and Morrissey, 1985). The rate and extent of oxidative deterioration can be reduced through various means such as curing, vacuum packaging, modified atmosphere packaging and most importantly addition of synthetic or natural antioxidants. Although synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated

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Peer review under responsibility of Faculty of Agriculture, Ain-Shams University.

http://dx.doi.org/10.1016/j.aoas.2015.10.002

hydroxy anisole (BHA) have been used extensively, recent studies have implicated them to have toxic effects (Lindenschmidt et al., 1986; Shahidi et al., 1992). In response to recent claims that synthetic antioxidants have the potential to cause toxicological effects and consumers' increased interest in purchasing natural products, the meat and poultry industry has been seeking sources of natural antioxidants. Due to their high phenolic compound content, fruits, vegetables and other plant materials provide a good alternative to conventional natural antioxidants, and can serve as a source of natural antioxidants for meat products (Phillips et al., 1993; Slattery et al., 2000; Karre et al., 2013). These antioxidants include fat-soluble vitamins and precursors, such as tocopherols and carotenoids, as well as the water-soluble vitamin ascorbic acid, and flavonoids. Application of plant extracts in meat products as natural antioxidants has been attempted by different researchers. By-products of food processing contain valuable substances such as fibers, pigments, sugars, organic acids, flavors, antibacterial and antioxidants substances (Balasundram et al., 2005). Pomegranate fruit parts contain a high concentration of antioxidants (Sánchez-Zapata et al., 2011). The peel and rind are good sources of tannins, anthocyanins, and flavonoids (Naveena et al., 2008). Devatkal et al., (2010) used kinnow rind powder (KRP), pomegranate rind powder (PRP), and pomegranate seed powder (PSP) in raw goat meat, and prepared cooked goat (80 °C) patties. Goat meat patties were stored for 12 d at (4 ± 1 °C). Incorporation of PRP was effective in reducing TBARS formation up to 67% and *L*-values as color parameter were decreased significantly, but no differences were observed among PSP and control for *L* values. Conversely, redness was reduced when using PRP and PSP compared with the control and KRP-treated goat patties. Sensory evaluation of color, appearance, flavor, and overall acceptability indicated no differences ($p > 0.05$) among the different goat patties. The antioxidant and antimicrobial potential of pomegranate peel and seed extract in chicken products was investigated by Kanatt et al. (2010). The efficacy of pomegranate juice, pomegranate rind powder extract and butylated hydroxyl toluene as antioxidants in cooked chicken patties during refrigerated storage was observed by Naveena et al. (2008). According to Karre et al. (2013) pomegranate components could be used as antioxidants in refrigerated chicken and goat patties. Pomegranate is effective in inhibiting lipid oxidation and does not significantly affect the overall sensory attributes of the finished product. More investigation needs to be conducted for other varieties of meat products with a focus on different storage conditions. The antimicrobial activity of fruit peels is well documented. For example, pomegranate fruit peels have been widely used in herbal remedies for treating several diseases (Al-Zoreky, 2009). Pomegranate fruit peels extracts have been shown to inhibit the growth of several foodborne pathogens including *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Yersinia enterocolitica*, and *Bacillus cereus* (Agourram et al., 2013; Al-Zoreky, 2009; Kanatt et al., 2010). Pomegranate peel extract was more effective against Gram-positive bacteria even at a concentration of 0.01%. However, in the case of Gram-negative bacteria, extract was effective against *Pseudomonas* spp. at a higher concentration of 0.1% and less effective against *E. coli* and *Salmonella typhimurium* at the same concentration (Kanatt et al., 2010).

The current investigation was performed to evaluate the effects of adding various levels of pomegranate peels powder on keeping different quality characteristics of prepared beef sausage.

Materials and methods

Preparation of pomegranate peels

Pomegranate fruits used in preparing pomegranate peel powder were purchased from local markets of Cairo, Egypt. Pomegranate fruits were washed, then peeled and their edible portions were carefully separated. The peels were air-dried in a ventilated oven at 40 °C for 48 h and grounded to a fine powder then packaged in polyethylene bags until used.

Preparation of beef sausage

Beef meat samples including boneless neck, chuck and rounds along with associated fats were obtained from local markets of Cairo, Egypt, and used for preparing beef sausage samples. All subcut fat and inter-muscular fat were also included as fat sources. The beef meat and fat tissue were transported to the laboratory using an ice box. Different ingredients used in preparing beef sausage samples e.g. table salt, starch and spices mixture such as black pepper, red pepper, nut meg and ginger were obtained from local market of Cairo, Egypt.

Beef sausage samples were prepared according to the method described by Zaika et al. (1978), using the ingredients listed in Table 1. Meat and fat tissues were cut into pieces of about egg-size and frozen at −18 °C for 24 h. The frozen meat and fat were ground to particles of about a rice size, then the ingredients were blended to prepare sausage mixture emulsion, which was then stuffed by hand into mutton casings, and then the casings were closed and chipped (Shehata, 1989). The natural mutton casings were obtained from the slaughterhouse of Cairo and prepared according to El-Deep (1987). For evaluation the effect of pomegranate peel powder as natural preservatives prepared dried pomegranate peel powder was used in concentration of 1%, 2% and 3% of total beef sausage recipe. To evaluate their antioxidant and antimicrobial effects of different prepared sausage samples, T1 (zero % pomegranate peel powder), T2, T3 and T4 (1%, 2% and 3% pomegranate peel powder, respectively) were packaged in polyethylene packages and stored at 4 °C ± 2 for 12 days. Samples were taken at 3, 6, 9 and 12 days interval and subjected to different analysis mentioned below.

Table 1 Ingredients used in manufacturing beef sausage.

Ingredients	Amount (g)	Spices mixture	Amount (g)
Lean meat	70.0	Black pepper	30.0
Fat tissues	12.0	Red pepper	8.0
Sodium chloride	2.3	Cumin	15.0
Water (as ice)	9.3	Nutmeg	8.0
Starch	3.0	All spices	15.0
Garlic	1.0	Cloves	8.0
Onion	1.2	Ginger	8.0
Spices mixture	1.2	Coriander	8.0

Chemical analysis

Moisture, protein (total nitrogen × 6.25), ash and lipid contents of different prepared beef sausage samples were determined according to A.O.A.C. (2012). Carbohydrates (nitrogen free extract) were determined by differences.

Total volatile nitrogen (TVN)

Total volatile nitrogen content of different beef sausage samples was performed according to the method of Harold et al. (1987).

Thiobarbituric acid value (TBA)

The thiobarbituric acid values of different beef sausage samples were determined by the distillation method outlined by Harold et al. (1987).

Water holding capacity

Water holding capacity (WHC) and plasticity of different beef sausage samples were measured according to the method of Volovinskaya and Kelmen (1962).

pH value

The pH values of different beef sausage samples were determined according to the method described by Defreitas et al. (1997) as follows: a known weight of beef sausage sample (30 g) was blended with 100 ml distilled water and the pH of the slurry was then measured using a pH meter (HANNA-Instrument, USA).

Cooking loss and cooking yield

Prepared beef sausage samples were weighted before cooking and then allowed to cool after cooking to room temperature. After cooling, the cooked beef sausage samples were reweighted and the cooking loss was calculated according to Lee et al. (2008) as follows:

$$\text{Cooking loss (g/100 g)} = Wr - Wc \times 100/Wr$$

where

Wr: the weight of raw sausage (g).

Wc: the weight of cooked sausage (g).

Cooking yield of different sausage samples was measured by subtracting cooking loss from 100.

Change in beef sausage diameter and length (Shrinkage)

Change in beef sausage diameter and length (Shrinkage) was measured on cooked samples as mentioned by George and Berry (2000) using the following equations:

$$\%\ \text{Shrinkage} = \frac{\text{Uncooked diameter or length (cm)} - \text{Cooked diameter or length (cm)} \times 100}{\text{Uncooked diameter or length (cm)}}$$

Microbiological analysis

Sample preparation

Different beef sausage samples were prepared for microbiological analysis in accordance with ISO 6887-1 (2003), test method of sample preparation (for microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination). Different beef sausage samples of 10 g were weighed in the sterile stomacher zipped bag. Maximum recovery solution (MRD), of 90 ml was added. The sample and (MRD) solution were blended at low speed for 30–60 s in stomacher machine. A dilution series were prepared by transferring 1 ml of the previous dilution to 9 ml of (MRD) solution. For detection of *Salmonella* sp., 25 g of different sausage samples was weighed in the sterile stomacher zipped bag, and 225 ml of buffered peptone water (BPW) was added according to the reference ISO 6579 (2002).

Microbiological analysis

Different beef sausage samples were analyzed for total aerobic bacterial count (ISO 4833-2003), and aerobic spore forming bacteria were determined according to the method described in Compendium of Methods for the Microbiological Examination of Foods (2001). Other methods for microbiolog-

Table 2 Media and incubation conditions used for microbiological analysis.

Microbiological analysis	Incubation conditions		
	Temp (°C)	Time (h)	Growth medium[a]
Total aerobic bacterial count	30	48	Plate count agar
Aerobic spore forming	55	48	Plate count agar
Yeast and mold count	21–25	72–120	Potato dextrose agar
Coliforms group	37	24	Violet Red Bile Lactose agar
Staphylococcus aureus	37	48	Baird parker agar
Salmonella spp.			
Pre-enrichment	37	24	Buffer peptone water
Selective enrichment	42	24	Tetrathionate broth
Isolation	24	24–48	Xylose Lysine Desoxycholate Agar

[a] Oxoid (2006).

ical analysis were carried out for yeast and mold counts (ISO 21527-1, 2- 2008), coliform group (ISO 4832-2006), *S. aureus* counts (ISO 6888-1-2003) and Detection of Salmonella (ISO 6579-2006). All previous tests were used to reflect the microbiological quality of the prepared beef sausage samples. Each specific medium and incubation temperatures are listed in Table 2.

Sensory evaluation

The sensory evaluation was determined by warm serving of different prepared beef sausage samples after cooking using seven scale evaluations (appearance, color, texture, tenderness, juiciness, flavor and overall acceptability). The samples were coded with three digit random numbers and the order of presentation was made using random permutation. All necessary precautions were taken to ensure that each panelist made an independent judgment (Aminah, 2000).

Statistical analysis

Data were expressed as the mean values of three replicates and standard deviations were statistically analyzed by performing analysis of variance technique using the statistical Analysis System according to SAS (2000). Differences among means were compared using Duncan's multiple range test at significant level 95% ($P \leqslant 0.05$).

Table 3 Proximate chemical compositions of beef sausage prepared with different concentrations of pomegranate peels powder during cold storage at 4 ± 2 °C for 12 days.

Storage period (days)	T1	T2	T3	T4
Moisture (%)				
Zero	61.89 ± 0.44^{aA}	60.65 ± 0.65^{aB}	60.02 ± 0.60^{aB}	58.82 ± 0.47^{aC}
3	59.45 ± 0.44^{bA}	58.41 ± 0.65^{bB}	57.94 ± 0.60^{bB}	56.84 ± 0.47^{bC}
6	56.30 ± 0.44^{cA}	55.66 ± 0.65^{cAB}	55.52 ± 0.60^{cAB}	54.76 ± 0.47^{cB}
9	52.96 ± 0.62^{dA}	53.09 ± 0.65^{dA}	53.33 ± 0.59^{dA}	52.88 ± 0.46^{dA}
12	50.24 ± 0.86^{eB}	51.23 ± 0.65^{eAB}	51.68 ± 0.59^{eA}	51.13 ± 0.51^{eAB}
R^2	0.925	0.895	0.787	0.846
Protein (%)				
Zero	15.85 ± 0.19^{aB}	16.17 ± 0.16^{aAB}	16.23 ± 0.31^{aAB}	16.32 ± 0.15^{aA}
3	14.94 ± 0.44^{bB}	15.74 ± 0.16^{aA}	15.83 ± 0.30^{abA}	15.95 ± 0.15^{bA}
6	14.30 ± 0.44^{bB}	15.18 ± 0.16^{bA}	15.35 ± 0.29^{bA}	15.52 ± 0.15^{cA}
9	13.54 ± 0.44^{cC}	14.47 ± 0.16^{cB}	14.79 ± 0.27^{cAB}	15.06 ± 0.15^{dA}
12	12.72 ± 0.44^{dC}	13.72 ± 0.16^{dB}	14.18 ± 0.27^{dAB}	14.56 ± 0.12^{eA}
R^2	0.916	0.861	0.856	0.916
Ash (%)				
Zero	2.82 ± 0.13^{dA}	2.98 ± 0.12^{dA}	3.10 ± 0.19^{dA}	3.09 ± 0.12^{eA}
3	3.29 ± 0.10^{cA}	3.18 ± 0.12^{dA}	3.35 ± 0.16^{dA}	3.43 ± 0.15^{dA}
6	3.71 ± 0.10^{bAB}	3.57 ± 0.12^{cB}	3.77 ± 0.14^{cAB}	$3.88 \pm 0.15^{c\ A}$
9	4.33 ± 0.22^{aA}	4.03 ± 0.12^{bA}	4.26 ± 0.14^{bA}	4.32 ± 0.12^{bA}
12	4.51 ± 0.40^{aA}	4.50 ± 0.12^{aA}	4.77 ± 0.13^{aA}	4.85 ± 0.12^{eA}
R^2	0.908	0.978	0.843	0.923
Fat (%)				
Zero	16.23 ± 0.31^{cB}	16.32 ± 0.13^{eAB}	16.65 ± 0.26^{dAB}	16.73 ± 0.16^{Da}
3	16.35 ± 0.32^{cB}	16.59 ± 0.13^{dAB}	16.88 ± 0.28^{cdA}	16.94 ± 0.17^{Da}
6	16.75 ± 0.35^{bcB}	16.91 ± 0.13^{cAB}	17.17 ± 0.28^{bcAB}	17.25 ± 0.11^{cA}
9	17.17 ± 0.36^{abA}	17.28 ± 0.13^{bA}	17.51 ± 0.25^{abA}	17.54 ± 0.11^{Ba}
12	17.63 ± 0.36^{aA}	17.69 ± 0.13^{aA}	17.94 ± 0.26^{aA}	17.87 ± 0.10^{aA}
R^2	0.974	0.977	0.861	0.969
Total carbohydrates (%)				
Zero	3.29 ± 0.43^{eB}	3.88 ± 0.36^{cB}	4.00 ± 0.15^{cB}	5.04 ± 0.63^{eA}
3	5.97 ± 0.79^{dA}	5.41 ± 1.76^{cA}	6.00 ± 0.16^{dA}	6.91 ± 0.69^{dA}
6	8.91 ± 0.83^{cA}	8.68 ± 0.61^{bA}	8.19 ± 0.16^{cA}	8.59 ± 0.63^{cA}
9	12.01 ± 1.01^{bA}	11.13 ± 0.60^{aBA}	10.10 ± 0.18^{bB}	10.19 ± 0.53^{bB}
12	14.97 ± 0.80^{aA}	12.86 ± 0.62^{aB}	11.30 ± 0.39^{aC}	11.58 ± 0.58^{aC}
R^2	0.842	0.918	0.905	0.887

Where
T1: Control sample without pomegranate peels powder
T2: Prepared with (1%) pomegranate peels powder
T3 Prepared with (2%) pomegranate peels powder
T4: Prepared with (3%) pomegranate peels powder

Means of triplicate ± Standard Deviation (SD).
Means followed by different small letters in the same column (effect of storage period) are significantly by Duncan's multiple test ($p \leqslant 0.05$).
Means followed by different capital letters in the same raw (effect of treatments) are significantly by Duncan's multiple test ($p \leqslant 0.05$).

Result and discussion

Proximate chemical composition

Results of proximate chemical composition of different beef sausage samples prepared by the addition of 1%, 2% and 3% of pomegranate peels powder and stored at 4 °C ± 2 for 12 days are given in Table 3. Significant decrease ($p \leqslant 0.05$) in moisture content of prepared beef sausage samples could be noticed as a result of addition of pomegranate peels powder. The moisture content of control beef sausage samples (0% pomegranate peels powder) was 61.89% as compared with 60.65%, 60.02% and 58.82% for beef samples prepared with addition of 1%, 2% and 3% of pomegranate peels powder at the beginning of storage period, respectively. As the storage period increased the moisture content of different prepared beef sausage samples significantly decreased ($p \leqslant 0.05$), with the observation that, the control beef sausage sample showed the highest decline in moisture content reaching 50.24% as compared with 51.23%, 51.68% and 51.13% for prepared beef sausage samples containing of 1%, 2% and 3% of pomegranate peels powder at the end of the storage period, respectively. The loss in moisture content during cold storage of sausage could be referred to moisture vapor migration from the surfaces of sausage samples as a result of difference in water vapor pressure with the surrounding cold air. From the observed results it could be noticed that, the addition of different concentrations of pomegranate peels powder led to lower reduction in moisture content and improving the water holding capacity because of a lower loss rate in moisture content of beef sausage samples treated with pomegranate peels powder. With respect to protein content of different beef sausage samples, it could be observed that addition of pomegranate peels had no significant effect ($p \geqslant 0.05$), since the protein content of different prepared samples was around 16.00%. The aforementioned results may be explained by the lower protein content of pomegranate peels powder added as a fiber source and thus had no significant impact on the protein content of prepared beef sausage samples.

In the same time, the protein content of prepared beef sausage samples during storage period had the same observed trend of moisture content, since the storage period increased, and the protein content of different prepared beef sausage samples was significantly decreased ($p \leqslant 0.05$), with the observation that, the control beef sausage sample showed the highest decline of protein content with value of 12.72% compared to 13.72%, 14.18% and 14.56% for prepared beef samples with addition of 1%, 2% and 3% of pomegranate peels powder at the end of the storage period, respectively. The reduction of protein content of prepared beef sausage samples during storage period could be explained by the loss of soluble and volatile amino compounds associated protein with the loss of water content of beef sausage samples. Ash content of prepared beef sausage samples at the beginning of storage period was not significantly affected by the addition of different concentrations of pomegranate peels powder as its values were around 3.00%. At the same time, as the storage period increased, the ash content of different prepared beef sausage samples was non-significantly increased ($p \geqslant 0.05$) since the values of ash content were 4.51%, 4.50%, 4.77% and 4.85% for prepared beef sausage samples containing 0%, 1%, 2% and 3% of pomegranate peels powder, respectively. Similar results of ash content were observed for the fat content of different prepared beef sausage samples, as addition of pomegranate peels powder did not significantly affect ($p \geqslant 0.05$) the fat content of prepared beef sausage samples and as the storage period increased, the fat content of different prepared beef sausage samples was increased. The increased fat content of stored beef sausage samples may be explained by the autolysis of lipoprotein to protein and lipid which led to increased ether extract (fat content). Addition of pomegranate powder during the preparing of beef sausage led to significant increase ($p \leqslant 0.05$) in total carbohydrates values as their values were 3.29%, 3.88%, 4.00% and 5.04% for prepared beef sausage samples containing 0%, 1%, 2% and 3% of pomegranate peels

Table 4 Physical characteristics of beef sausage prepared with different concentrations of pomegranate peels powder during cold storage at 4 ± 2 °C for 12 days.

Storage period (days)	T1	T2	T3	T4
Water holding capacity WHC (cm^2/0.3 g)				
Zero	5.77 ± 0.26^{abE}	5.49 ± 0.14^{abE}	5.73 ± 0.18^{abE}	5.88 ± 0.25^{aE}
3	6.06 ± 0.26^{aE}	5.98 ± 0.39^{aE}	6.17 ± 0.31^{aE}	6.28 ± 0.35^{aE}
6	6.79 ± 0.39^{abD}	7.32 ± 0.27^{aD}	7.41 ± 0.46^{aD}	7.46 ± 0.44^{aD}
9	8.50 ± 0.25^{aC}	8.35 ± 0.34^{aC}	8.39 ± 0.32^{aC}	8.42 ± 0.22^{aC}
12	9.28 ± 0.57^{aB}	9.69 ± 0.39^{aB}	9.75 ± 0.20^{aB}	9.77 ± 0.12^{aB}
R^2	0.954	0.978	0.937	0.985
Plasticity (cm^2/0.3 g)				
Zero	5.07 ± 0.52^{aA}	4.95 ± 0.46^{aA}	5.13 ± 0.31^{aA}	5.48 ± 0.25^{aA}
3	4.67 ± 0.28^{aAB}	4.62 ± 0.39^{aAB}	5.07 ± 0.49^{aA}	5.17 ± 0.42^{aAB}
6	4.15 ± 0.33^{bB}	4.45 ± 0.14^{abABC}	4.72 ± 0.13^{abAB}	4.83 ± 0.39^{aBC}
9	3.25 ± 0.19^{bC}	4.21 ± 0.21^{aBCD}	4.53 ± 0.37^{aAB}	4.56 ± 0.18^{aAB}
12	3.04 ± 0.51^{bC}	3.95 ± 0.24^{aCD}	4.25 ± 0.19^{aBC}	4.32 ± 0.40^{aCD}
R^2	0.964	0.981	0.953	0.917

Means of triplicate ± Standard Deviation (SD).
Means followed by different small letters in the same column (effect of storage period) are significantly by Duncan's multiple test ($p \leqslant 0.05$).
Means followed by different capital letters in the same raw (effect of treatments) are significantly by Duncan's multiple test ($p \leqslant 0.05$).

Table 5 Physicochemical and chemical quality criteria of beef sausage prepared with different concentrations of pomegranate peels powder during cold storage at 4 ± 2 °C for 12 days.

Storage period (days)	T1	T2	T3	T4
pH values				
Zero	7.12 ± 0.03abA	7.14 ± 0.02Aa	7.11 ± 0.02abA	7.15 ± 0.02aA
3	6.95 ± 0.08aB	6.96 ± 0.05Ab	6.84 ± 0.07abB	6.76 ± 0.17bB
6	6.63 ± 0.05aC	6.57 ± 0.04abC	6.54 ± 0.10abC	6.50 ± 0.03bcC
9	6.53 ± 0.06aD	6.49 ± 0.11abC	6.46 ± 0.08abC	6.37 ± 0.01bC
12	6.47 ± 0.05aD	6.38 ± 0.09Bd	6.29 ± 0.02cD	6.22 ± 0.05cD
R^2	0.997	0.984	0.915	0.973
TBA as (mg of malonaldhyde/kg sample)				
Zero	0.237 ± 0.810 ^{eA}	0.232 ± 0.043 ^{dA}	0.233 ± 0.071 ^{dA}	0.235 ± 0.024 ^{cdA}
3	0.381 ± 0.091 ^{dA}	0.281 ± 0.074 ^{dB}	0.262 ± 0.064 ^{cdB}	0.251 ± 0.087 ^{cdB}
6	0.616 ± 0.078 ^{cA}	0.343 ± 0.061 ^{cB}	0.316 ± 0.049 ^{cB}	0.287 ± 0.066 ^{cC}
9	0.985 ± 0.057 ^{bA}	0.509 ± 0.055 ^{bB}	0.496 ± 0.035 ^{bB}	0.441 ± 0.026 ^{bBC}
12	1.024 ± 0.039 ^{aA}	0.711 ± 0.037 ^{aB}	0.581 ± 0.042 ^{aC}	0.542 ± 0.071 ^{aC}
R^2	0.894	0.927	0.975	0.918
T.V.N as (mg nitrogen /100 g sample)				
Zero	8.38 ± 0.45^{eA}	8.08 ± 0.74^{eAB}	8.04 ± 0.22^{eB}	8.00 ± 0.45^{eC}
3	14.29 ± 0.61^{dA}	13.63 ± 0.25^{dAB}	13.44 ± 0.57^{dAB}	13.42 ± 0.35^{dAB}
6	23.40 ± 0.37^{cA}	18.69 ± 0.37^{cB}	17.135 ± 0.41^{cBC}	16.88 ± 0.26^{cC}
9	31.10 ± 0.18^{bA}	23.27 ± 0.31^{bB}	22.54 ± 0.38^{bBC}	22.32 ± 0.18^{bBC}
12	36.51 ± 0.28^{aA}	26.07 ± 0.47^{aB}	25.04 ± 0.27^{aBC}	24.87 ± 0.21^{aC}
R^2	0.874	0.933	0.875	0.927

Means of triplicate ± Standard Deviation (SD).
Means followed by different small letters in the same column (effect of storage period) are significantly by Duncan's multiple test ($p \leqslant 0.05$).
Means followed by different capital letters in the same raw (effect of treatments) are significantly by Duncan's multiple test ($p \leqslant 0.05$).

powder at the beginning storage period, respectively. In the same time, as the storage period increased, the values of total carbohydrates were significantly increased.

Physical characteristics

Results of the relationship between additions of pomegranate peels powder and the physical characteristics e.g. water holding capacity (WHC) and plasticity of prepared beef sausage samples are presented in Table 4. The addition of different concentrations of pomegranate peels powder had no significant effect ($p \geqslant 0.05$) on the WHC of different prepared beef sausage samples. As the storage period increased, the WHC of different prepared beef sausage was non-significantly decreased ($p \geqslant 0.05$) during all storage periods. Concerning the plasticity of different prepared beef sausage samples, addition of different concentrations of pomegranate peels powder had no significant effect ($p \geqslant 0.05$) with little improvement of plasticity of prepared beef sausage samples containing pomegranate peels powder as compared to control beef sausage sample (0% pomegranate peels powder). In the same time, the storage period did not significantly affect ($p \geqslant 0.05$) the plasticity of prepared beef sausage samples containing 1%, 2% and 3% of pomegranate peels powder. On the contrary, the plasticity of control beef sausage samples was significantly affected and decreased during storage period. The aforementioned results may be explained by the small amount of pomegranate peels powder added during the preparation of beef sausage (1%, 2% and 3%) and the aim of addition was primarily targeting their effect as natural preservatives.

Table 6 Microbiological criteria of beef sausage prepared with different concentrations of pomegranate peels powder during cold storage at 4 ± 2 °C for 12 days.

Storage period (days)	T1	T2	T3	T4
Total plate counts (log cfu/g)				
Zero	3.28	3.36	3.28	3.98
3	3.38	3.34	3.15	2.70
6	3.79	2.98	2.81	2.65
9	4.15	2.93	2.72	2.59
12	5.36	2.93	2.71	2.54
Yeast and mold counts (log cfu/g)				
Zero	2.00	2.30	2.20	2.28
3	3.40	2.18	2.18	1.90
6	3.62	2.18	1.93	1.80
9	4.70	2.11	1.70	1.65
12	4.76	2.08	⩽1	⩽1
Spore forming bacteria counts (log cfu/g)				
Zero	1.81	1.81	1.82	1.90
3	1.88	1.74	1.74	1.70
6	1.95	1.70	1.54	1.54
9	2.15	1.70	1.18	0.70
12	2.30	⩽1	⩽1	⩽1
Coliform counts (log cfu/g)				
Zero	1.86	1.80	2.00	2.00
3	2.28	1.88	1.90	1.88
6	2.30	1.81	1.88	1.73
9	2.36	1.77	1.74	1.54
12	3.65	1.74	1.70	1.52

Table 7 Cooking characteristics of beef sausage prepared with different concentrations of pomegranate peels powder during cold storage at 4 ± 2 °C for 12 days.

Storage period (days)	T1	T2	T3	T4
Cooking loss (%)				
Zero	17.11 ± 0.18^{eA}	15.33 ± 0.37^{dB}	14.95 ± 0.24^{dC}	12.49 ± 0.42^{dD}
3	18.87 ± 0.23^{dA}	16.83 ± 0.32^{cdB}	15.96 ± 0.31^{cdC}	13.54 ± 0.17^{cdD}
6	21.08 ± 0.45^{cA}	17.63 ± 0.41^{cB}	16.86 ± 0.37^{cC}	14.96 ± 0.32^{cD}
9	23.37 ± 0.33^{bA}	19.35 ± 0.28^{bB}	18.35 ± 0.25^{bC}	16.18 ± 0.15^{bD}
12	28.67 ± 0.54^{aA}	22.95 ± 0.19^{aB}	20.69 ± 0.22^{aC}	18.44 ± 0.41^{aD}
R^2	0.914	0.894	0.927	0.942
Cooking yield (%)				
Zero	82.9 ± 0.18^{aC}	84.67 ± 0.37^{abB}	85.05 ± 0.24^{aB}	87.51 ± 0.42^{aA}
3	81.13 ± 0.23^{aC}	83.17 ± 0.32^{bBC}	84.04 ± 0.31^{aB}	86.46 ± 0.17^{abA}
6	78.92 ± 0.45^{bC}	82.37 ± 0.41^{bcBC}	83.14 ± 0.37^{bB}	85.04 ± 0.32^{bA}
9	76.63 ± 0.33^{cC}	80.67 ± 0.28^{cBC}	81.66 ± 0.25^{cB}	83.82 ± 0.15^{cA}
12	71.33 ± 0.54^{dD}	77.05 ± 0.19^{cdC}	79.31 ± 0.22^{dB}	81.56 ± 0.41^{dA}
R^2	0.935	0.974	0.911	0.907
Change in diameter (%)				
Zero	6.82 ± 0.12^{cA}	6.53 ± 0.27^{dB}	5.95 ± 0.18^{dC}	4.93 ± 0.28^{cD}
3	10.32 ± 0.18^{bA}	8.68 ± 0.41^{cB}	7.18 ± 0.26^{cC}	5.74 ± 0.41^{bD}
6	11.67 ± 0.24^{abA}	9.11 ± 0.36^{bcB}	7.66 ± 0.37^{bC}	5.93 ± 0.38^{bD}
9	11.93 ± 0.31^{abA}	9.72 ± 0.29^{bB}	8.13 ± 0.21^{abC}	6.43 ± 0.29^{abD}
12	12.36 ± 0.27^{aA}	10.23 ± 0.41^{aB}	8.89 ± 0.17^{aC}	7.12 ± 0.22^{aD}
R^2	0.964	0.934	0.978	0.952
Change in length (%)				
Zero	8.87 ± 0.25^{dA}	7.94 ± 0.33^{dB}	5.87 ± 0.35^{dC}	5.46 ± 0.27^{cD}
3	11.67 ± 0.31^{cA}	10.24 ± 0.28^{cB}	9.47 ± 0.12^{cBC}	8.34 ± 0.36^{dC}
6	13.41 ± 0.41^{bcA}	11.66 ± 0.16^{cB}	11.31 ± 0.37^{bBC}	10.81 ± 0.25^{cC}
9	15.22 ± 0.46^{bA}	14.92 ± 0.31^{bAB}	14.26 ± 0.19^{aAB}	13.61 ± 0.19^{bB}
12	17.21 ± 0.23^{aA}	15.21 ± 0.16^{aB}	14.67 ± 0.11^{aC}	14.08 ± 0.38^{aCD}
R^2	0.971	0.954	0.974	0.939

Means of triplicate ± Standard Deviation (SD).
Means followed by different small letters in the same column (effect of storage period) are significantly by Duncan's multiple test ($p \leqslant 0.05$).
Means followed by different capital letters in the same raw (effect of treatments) are significantly by Duncan's multiple test ($p \leqslant 0.05$).

Physicochemical and chemical quality criteria

Physicochemical (pH values) and chemical (TBA as mg of malonaldhyde/kg sample and TVN as mg nitrogen/g sample) as quality criteria are presented in Table 5. There were no significant differences ($p \geqslant 0.05$) in pH values of different prepared beef sausage samples containing 0%, 1%, 2% and 3% of pomegranate peels powder. During storage period, the pH values of prepared beef sausage samples were decreased with little significant effect ($p \leqslant 0.05$) for beef sausage samples containing different concentrations of pomegranate peels powder. On the contrary, pH of the control beef sausage sample (0% pomegranate peels powder) was decreased during storage period without significant effect ($p \geqslant 0.05$). The slight decreases in pH values during cold storage period in different sausage samples might be attributed to the breakdown of glycogen with the formation of lactic acid.

TBA values (mg of malonaldhyde/kg sample) as a criterion of oxidation of lipid content of meat products are presented in Table 5. The positive effect of addition of pomegranate peels powder as a natural antioxidant was noticed with significant differences ($p \geqslant 0.05$) in TBA values of prepared beef sausage samples containing 1%, 2% and 3% pomegranate peels powder in comparison with control beef sausage sample. During the storage period, a significant difference ($p \geqslant 0.05$) was observed for TBA values of prepared beef sausage samples with a gradual significant increase in TBA values for all prepared beef sausage samples. In the same time, addition of pomegranate peels powder reduced the rate of increase of TBA values, especially at concentration of 2% and 3%. TBA values were 0.237, 0.232, 0.233 and 0.235 mg of malonaldhyde/kg sample for prepared beef sausage samples containing 0%, 1%, 2%, and 3% pomegranate peels powder, respectively in the initial of storage period and reached 1.024, 0.711, 0.581 and 0.542 mg of malonaldhyde/kg sample for the aforementioned treatments, respectively, at the end of storage period (after 12 days of storage at 4 ± 2 °C). Therefore, pomegranate peels powder could be used as a natural antioxidant for preventing lipid oxidation in meat products. This result could be correlated to the presence of phenolic compounds in pomegranate peels powder. The aforementioned results are in agreement with the results of Naveena et al. (2008) and Devatkal et al. (2010) as well as El-Gharably and Ashoush (2011). They reported that pomegranate peels powder improved the storage stability of meat products especially at refrigerated storage by reducing the

rate of lipid oxidation expressed as TBA values of prepared samples.

Total volatile nitrogen (TVN) content is widely used as an indicator for protein decomposition caused by microorganisms as well as protein breakdown caused by tissue proteolytic enzymes during storage (Gibriel et al., 2007). The obtained data in Table 5 indicate that, TVN content was gradually and significantly increased ($p \leqslant 0.05$) during storage of different prepared beef sausage samples. Results also revealed that, the control beef sausage sample (0% pomegranate peels powder) had significantly ($p \leqslant 0.05$) the highest TVN content in every period of storage, being 8.38 mg TVN/100 g sample at the beginning of the storage period, and continued to increase reaching 36.51 mg TVN/100 g sample after 12 days. On the other hand, the corresponding TVN value for different prepared beef sausage samples containing different concentrations of pomegranate peels powder (1%, 2% and 3%) had lower TVN content at the initial of storage period (8.08, 8.04 and 8.00 mg/100 g, respectively), and at the end of the storage period after 12 days they were changed to 26.07, 25.04 and 24.87 mg/100 g, respectively. These results indicated the significant ($p \leqslant 0.05$) positive effect of addition of pomegranate peels powder on the inhibition of microbial growth especially proteolytic microorganisms that cause the breakdown of protein resulting in volatile nitrogen compounds. The increase in TVN during cold storage of sausage meat samples might be attributed to the breakdown of nitrogenous substances by microbial activity. These results are in agreement with those of Madkour et al. (2000) and Gibriel et al. (2007).

Microbiological criteria

Results of the effect of addition of different concentration of pomegranate peels powder as natural preservative in preparing

Table 8 Sensory characteristics of beef sausage prepared with different concentrations of pomegranate peels powder during cold storage at 4 ± 2 °C for 12 days.

Storage period (days)	T1	T2	T3	T4
Appearance				
Zero	8.30 ± 0.67^{aC}	9.50 ± 0.53^{aA}	8.30 ± 0.95^{aC}	9.10 ± 0.32^{aB}
3	8.30 ± 0.82^{aB}	8.60 ± 0.70^{bAB}	8.20 ± 0.42^{aC}	8.90 ± 0.57^{aA}
6	7.10 ± 0.74^{bC}	8.70 ± 0.48^{bA}	7.50 ± 0.53^{bBC}	8.70 ± 0.48^{aA}
9	6.90 ± 0.57^{bC}	8.30 ± 0.45^{bcA}	7.30 ± 0.66^{bcB}	8.10 ± 0.57^{bAB}
12	6.20 ± 0.42^{cC}	7.90 ± 0.32^{cA}	6.80 ± 0.42^{cB}	7.70 ± 0.46^{bAB}
Color				
Zero	7.94 ± 0.52^{aD}	9.60 ± 0.70^{aA}	8.70 ± 0.94^{aC}	9.40 ± 0.63^{aB}
3	7.90 ± 0.57^{aC}	9.60 ± 0.52^{aA}	8.30 ± 0.67^{ab}	9.20 ± 0.63^{abAB}
6	7.30 ± 0.67^{abC}	9.20 ± 0.42^{aA}	8.00 ± 0.67^{bB}	9.10 ± 0.57^{abA}
9	7.20 ± 0.42^{bD}	8.70 ± 0.48^{bA}	7.80 ± 0.63^{bBC}	8.40 ± 0.70^{bA}
12	6.50 ± 0.53^{cD}	8.40 ± 0.52^{bA}	7.30 ± 0.48^{cBC}	7.80 ± 0.79^{cB}
Tenderness				
Zero	8.10 ± 0.74^{aC}	9.60 ± 0.52^{aA}	8.60 ± 0.70^{aB}	9.30 ± 0.67^{aAB}
3	7.90 ± 0.57^{abC}	9.40 ± 0.52^{abA}	8.50 ± 0.53^{aB}	9.20 ± 0.79^{aAB}
6	7.40 ± 0.52^{bC}	9.20 ± 0.42^{abA}	8.10 ± 0.32^{abB}	8.90 ± 0.57^{aA}
9	6.90 ± 0.57^{cD}	8.90 ± 0.57^{bA}	7.80 ± 0.63^{bcBC}	8.20 ± 0.63^{bB}
12	6.20 ± 0.63^{dC}	8.30 ± 0.67^{cA}	7.30 ± 0.67^{cB}	7.80 ± 0.63^{bAB}
Juiciness				
Zero	8.40 ± 0.84^{aC}	9.90 ± 0.32^{aA}	8.80 ± 0.92^{aB}	9.30 ± 0.82^{aAB}
3	8.00 ± 0.67^{abC}	9.70 ± 0.48^{abA}	8.70 ± 0.67^{aB}	9.50 ± 0.53^{aA}
6	7.50 ± 0.97^{bD}	9.30 ± 0.48^{bA}	8.30 ± 0.67^{abBC}	9.20 ± 0.42^{aA}
9	7.20 ± 0.79^{bcC}	8.60 ± 0.52^{cA}	7.90 ± 0.57^{bcB}	8.50 ± 0.53^{bA}
12	6.80 ± 0.63^{cC}	8.30 ± 0.48^{cA}	7.60 ± 0.70^{cB}	8.00 ± 0.67^{bAB}
Flavor				
Zero	8.50 ± 0.97^{aB}	9.50 ± 0.53^{abA}	8.50 ± 0.71^{abB}	9.00 ± 0.67^{aAB}
3	8.10 ± 0.57^{aC}	9.60 ± 0.52^{aA}	8.70 ± 0.48^{aB}	9.20 ± 0.63^{aAB}
6	7.40 ± 0.52^{bC}	9.10 ± 0.57^{bA}	8.10 ± 0.32^{bcB}	8.70 ± 0.48^{abA}
9	7.00 ± 0.47^{bB}	8.50 ± 0.53^{cA}	7.90 ± 0.32^{cA}	8.10 ± 0.99^{bcA}
12	6.00 ± 0.67^{cC}	7.90 ± 0.32^{dA}	7.00 ± 0.47^{dB}	7.70 ± 0.48^{cA}
Over acceptability				
Zero	8.60 ± 0.84^{aB}	10.00 ± 0.00^{aA}	8.50 ± 0.85^{aB}	9.60 ± 0.70^{aA}
3	8.40 ± 0.52^{aD}	9.50 ± 0.71^{abA}	8.70 ± 0.82^{aBC}	9.20 ± 0.79^{aAB}
6	8.10 ± 0.57^{aC}	9.10 ± 0.74^{bcA}	8.20 ± 0.63^{abC}	8.40 ± 0.70^{bBC}
9	7.10 ± 0.57^{bD}	8.80 ± 0.48^{cdA}	7.80 ± 0.67^{bcC}	8.10 ± 0.57^{bBC}
12	6.30 ± 0.67^{cD}	8.40 ± 0.52^{dA}	7.20 ± 0.42^{cC}	7.90 ± 0.57^{bAB}

Means of triplicate ± Standard Deviation (SD).
Means followed by different small letters in the same column (effect of storage period) are significantly by Duncan's multiple test ($p \leqslant 0.05$).
Means followed by different capital letters in the same raw (effect of treatments) are significantly by Duncan's multiple test ($p \leqslant 0.05$).

beef sausage samples and during stored at 4 ± 2 °C for 12 days are presented in Table 6. It should be mentioned that *E. coli*, *Staphylococcus aureus* and *Salmonella* sp. (as pathogenic criteria) were not detected in all prepared beef sausage samples containing 0%, 1%, 2% and 3% of pomegranate peels powder (Data are not given). Beef sausage samples were analyzed for total bacterial counts, yeast and mold counts, spore forming bacterial counts and coliform counts. Total plate count values of different prepared beef sausage ranged from 3.28 to 3.98 log cfu/g at the beginning of storage period, while during the storage period a gradual increase in the total plate count of control beef sausage samples was observed and reached 5.36 log cfu/g at the end of storage period.

On the other hand, the obtained data revealed that, the other prepared beef sausage samples which contained different concentrations of pomegranate peels powder (1%, 2% and 3%) showed a progressive reduction in total bacterial count over the time of storage period; where, total plate counts of prepared beef sausage samples contained 1%, 2% and 3% of pomegranate peels powder reached 2.93, 2.71 and 2.54 log cfu/g. These results could be due to the antimicrobial effect of pomegranate peels powder especially when the concentration of pomegranate peels powder was increased. The aforementioned results were also observed for all other tested microbial criteria, where, counts of yeast and mold, spore forming bacteria and coliform were reduced during storage period for all prepared beef sausage samples containing different concentrations of pomegranate peels powder in comparison with control beef sample prepared without addition of pomegranate peels powder. The aforementioned microbial criteria were gradually increased as the storage period increased. The obtained results could be correlated with the results of TVN as reported in Table 5. The observed results are in agreement with the results of Agourram et al. (2013), Al-Zoreky (2009) and Kanatt et al. (2010), as they evaluated the antimicrobial characteristics of pomegranate peels and they found that pomegranate peels have an inhibition effect against gram positive and gram negative bacteria.

Cooking characteristics

Cooking loss, cooking yield, change in diameter, and change in length (%) of different prepared beef sausage samples containing 0%, 1%, 2% and 3% pomegranate peels powder were evaluated and the results are illustrated in Table 7. In general, cooking yield was significantly increased ($p \leqslant 0.05$) and cooking loss, change in diameter and length were significantly decreased ($p \leqslant 0.05$) in different prepared beef sausage samples containing different concentrations of pomegranate peels powder during the storage period. Values of cooking loss and yield were 17.11%, 15.33%, 14.95%, 12.49% and 82.90%, 84.67%, 85.05%, 87.51% for prepared beef sausage samples contained 0%, 1%, 2% and 3% of pomegranate peels powder, respectively at the beginning of storage period. At the end of storage period, values of cooking loss and yield reached 28.67%, 22.95%, 20.69%, 18.14% and 71.33%, 77.05%, 79.31%, 81.56% for the aforementioned samples, respectively. Similar trend was observed for the other cooking characteristics such as change in diameter and length of different prepared beef sausage samples. The positive effect of addition of pomegranate peels powder in improving the cooking characteristics of prepared beef sausage samples was observed especially as the concentration of pomegranate peels powder was increased. These results could be correlated to the functional properties of pomegranate peels powder as a water binding material which was the most important factor in improving cooking characteristics of meat products.

Sensory characteristics

Sensory characteristics, appearance, color, tenderness, juiciness, flavor and overall acceptability of prepared beef sausage samples containing different concentration of pomegranate peels powder were evaluated and the results are presented in Table 8. It could be noticed that, addition of pomegranate peels powder had a significant effect ($p \leqslant 0.05$) on improving the sensory characteristics of prepared beef sausage samples and increased their acceptability during storage. In general, as concentration of pomegranate peels powder increased the acceptability of prepared beef sausage samples also increased.

Conclusion

The use of pomegranate peels powder at concentrations of 1%, 2% and 3% has proved to be effective as a natural preservative in producing high quality beef sausage samples. Quality criteria (WHC, plasticity, pH, TBA, TVN and total bacterial counts, yeast and mold, spore forming bacteria and coliform) of prepared beef sausage samples were evaluated during storage at 4 °C ± 2 for 12 days and it could be concluded that, addition of different concentration of pomegranate peels powder improved the aforementioned quality criteria. The application of different concentrations of pomegranate peels powder has improved the cooking characteristics e.g. cooking loss, cooking yield, change in diameter and change in length. In the same time, utilization of the investigated pomegranate peels powder could be useful to achieve high stability of beef sausage during refrigerated storage without any negative effects on the sensory characteristics of the product.

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