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Utilization of Pomegranate Peels for Improving Quality Attributes of Refrigerated Beef Burger

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ABSTRACT

This investigation was amid to evaluate the effect of pomegranate peels powder addition at ratios of 1, 2 and 3% on keeping quality and safety characteristics of prepared beef burger during a storage period at 4±1°C for 12 days. Chemical compositions, physicochemical characteristics, microbiological criteria, cooking quality and sensory characteristics of different prepared beef burger samples were evaluated. The addition of different ratios of pomegranate peels powder caused in high storage stability as significantly *(P≤0.05)* observed reduction in TBARS and TVN values during refrigerated storage compared to control beef burger samples prepared without addition pomegranate peels powder. The microbiological criteria of beef burger samples prepared with pomegranate peels powder were also improved. This could be due to the phenolic compounds present in pomegranate peels, which could act as antioxidant and antimicrobial substances. Prepared beef burger samples containing pomegranate peels powder recorded high cooking quality and sensory characteristics in comparison to control beef burger samples.

Key words: Beef burger; Cooking characteristics; Microbiological criteria; Pomegranate peels; TBARS; TVN; Sensory characteristics

Introduction

Meat and meat products are important sources for protein, fat, essential amino acids, minerals, vitamins and other nutrients. Considered as perishable food, so special care and handling must be exercised. It is necessary to minimize deterioration to prolong the period during which an acceptable level of quality is maintained (Biesalski, 2005 and Devatkal *et al.,* 2014).

Refrigeration storage is usually the most common preservation method for short time storage of fresh meat and meat products. In order to extend refrigerated storage time, antimicrobial and antioxidant additives especially of those synthetic origin, are added to meat products. However, consumers increasingly demand use of natural products as alternative preservatives in foods, as the safety of synthetic additives has been questioned in last years (Imaida *et al.,* 1983).

Oxidation of lipid is one of the major causes of quality deterioration, loss and reduced shelf life of meat products. This may produce changes in meat products quality parameters which adversely affect such as color, flavor, odor, texture and even nutritional value and this limits the shelf-life of meat products (Fernandez *et al.,* 1997). The rate and extent of oxidative deterioration can be reduced through various mechanisms like curing, vacuum packaging, modified atmosphere packaging and most importantly adding synthetic or natural antioxidants to prevent deterioration of meat products during processing and storage.

Fruits and vegetables are rich sources of valuable bioactive substances especially antioxidants (Phillips *et al.,* 1993; Slattery *et al.,* 2000 and Naveena *et al.,* 2008) and can serve as a source of natural antioxidants for meat products due to their high phenolic compounds content (Liz *et al.,* 2013). Food processing by-products may still valuable sources for fibers, pigments, sugars, organic acids, flavors, antibacterial (Sanchez-Zapata, *et al.,* 2011) and antioxidants substances (Balasundram *et al.,* 2005).

Pomegranate (*Punica granatum* L.) peels contain a high concentration of natural antioxidants and considerable as good source of tannins, anthocyanins, and flavonoids (Naveena,, *et al.,* 2008). Devatkal *et al.,* (2010) used kinnow rind powder, pomegranate rind powder, and pomegranate seed powder in raw goat meat, and then prepared cooked goat patties. Goat meat patties were stored at 4±1 °C for 12 d. Incorporation of pomegranate rind powder in goat patties was effective in reducing TBARS formation up

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to 67%. Sensory evaluation of color, appearance, flavor, and overall acceptability indicated no differences ($P \leq 0.05$) 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 efficiency of pomegranate juice, pomegranate rind powder extract as antioxidants in cooked chicken patties during refrigerated storage was observed by Naveena *et al.,* (2008). Liz *et al.,* (2013), reported that the 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.

The antimicrobial activity of pomegranate peels is well documented. For example, pomegranate fruit peels have been widely used in herbal remedies for treating several diseases (Al-Zorkey, 2009). Pomegranate fruit peels extracts were shown to inhibit the growth of several foodborne pathogens including *L. monocytogenes*, *S. aureus, E. coli*, *Y. enterocolitica*, and *B. cereus* (Agourram *et al.,* 2013; Al-Zoreky, 2009 and 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 *S. typhimurium* at the same concentration (Kanatt *et al.,* 2010).

Therefore, the objective of the present work was to evaluate the effects of adding various levels of dried pomegranate peels, as natural preservatives, on different quality attributes of prepared refrigerated beef burger.

Materials and Methods

Materials:

Mature pomegranate fruits were used in preparing dried pomegranate peel powder; also, beef meat samples lean meat including boneless neck and chuck along with associated fats and different ingredients used in preparing beef burger samples under investigation e.g. table salt, starch and spices mixture were purchased from local markets in Cairo, Egypt during 2014.

Methods:

Preparation of dried pomegranate peels powder:

Pomegranate fruits were washed then cut manually, and the peels of pomegranate fruits were manually removed and their edible portions were carefully separated. The peels were dried in an air-oven drier (DHG-9140A; Yiheng Instrument Co., Ltd., Shanghai, China) at 40±1 °C for 48 h. The dried peels were grounded to a fine powder and passed through a 30-mesh sieve then packed in polyethylene bags until used (Qin *et al.,* 2013).

Preparation of beef burger:

Beef burger samples were prepared according to the method described by Ziprin *et al.,* (1981), with the ingredients listed in table (1).

Table 1: Ingredients used in prepared beef burger:

Ingredients	Amount	Spices mixture	Amount
Lean meat	70.0 g	Black pepper	42.0 g
Fat tissues	12.0 g	Red pepper	1.0 g
Sodium chloride	2.3 g	Cummins	25.0 g
Water (as ice)	10.0 g	Nutmeg	2.0 g
Starch	2.0 g	All spice	19.0 g
Garlic	0.5 g	Cloves	2.0 g
Onion	2.0 g	Cubeb	2.0 g
Spices mixture	1.2 g	Cardamom	2.0 g
		Coriander	5.0 g

Meat and fat tissues were cut into pieces of about egg size and grounded to particles of about a rice size and mixed with the other ingredients listed in table (1). For evaluating the effect of pomegranate peel

powder as natural preservatives, prepared dried pomegranate peel powder was used at ratios of 1, 2 and 3% of total beef burger recipe. One centimeter thick burger style pieces of 50 g weight and 8.5 cm diameter were formed by a manually operated forming machine (NOAW- Italy). Different prepared samples were stacked with waxed paper between them, placed in polyethylene bags and refrigerated at 4±1°C for 12 days.

Analytical methods:

Chemical analysis:

Moisture, protein (total nitrogen x 6.25), ash and lipid contents of different prepared beef burger samples were determined according to A.O.A.C. (2012). Total carbohydrate was calculated by differences as:

$$\text{Total carbohydrate} = 100 - (\text{protein} + \text{fat} + \text{Ash})$$

Physicochemical quality criteria of prepared beef burger:

pH value measurement:

The pH values of different prepared beef burger samples were determined according to the method described by Qin *et al.,* (2013). Ten grams of sample was blended with 50 ml distilled water for 60 sec. in homogenizer and the pH of the slurry was then measured using a pH meter (HANNA-Instrument, USA).

Total volatile nitrogen (TVN):

Total volatile nitrogen content of different prepared beef burger samples were performed according to the method described by Harold *et al.,* (1987) using macrokjeldahl procedure. Ten grams of minced beef burger samples were placed in a 500 ml kjeldahl flask with 300 ml distilled water and 2.0 g magnesium oxide and a drop or two of antifoam solution were added, then the mixture was distilled and the ammonia was received in boric acid solution 4.0% where the formed ammonia borate was titrated by hydrochloric acid solution (0.01N) in the presence of mixed indicator (bromocrysol green / methyl red) and the results were calculated as mg nitrogen (TVN) per 100 g sample.

Thiobarbituric acid reactive substances (TBARS) value:

The thiobarbituric acid reactive substances values of different prepared beef burger samples were determined by the distillation method outlined by Harold *et al.,* (1987). Ten grams of samples were macerated with 50 ml distilled water for 2 min and then transferred to a distillation tube. The cup that used for blending was washed with additional 47.5 ml distilled water, which was added to the same distillation tube with 2.5 ml 4.0 N HCl and a few drops of antifoam silicone agent was added (Fisher Scientific, Loughborough, UK). The mixture was heated by direct flame, so that 50 ml distillate was collected during 10 minutes from the time of boiling. Five ml of distillate were added into a dry stopper tube, and then 5 ml of TBA reagent (0.2883 g of thiobarbituric acid in 100 ml 90% glacial acetic acid) were added, the tubes were closed and shaken, then heated in boiling water bath for 30 minutes. Similarly, blank tube was prepared using 5 ml of TBA reagent with 5 ml of distilled water. The tubes were then cooled in water for 10 minutes and the absorbance (A) was measured against a blank at 538 nm using digital spectrophotometer (JENWAY- Model 6105, England) and TBA value as mg malonaldehyde/ Kg sample was calculated by multiplying the absorbance (A) by 7.8.

Physical quality criteria of prepared beef burger:

The water holding capacity (WHC) and plasticity of different prepared beef burger samples were evaluated according to the filter press method as described by Voloviskaya and Merkoolova (1958) as follows: 0.3 g of the beef burger sample was put above an ashless filter paper (Whatman No. 41) and pressed for 10 minutes using a 1 kg weight. After pressing, two zones were formed on the filter paper; the outer zones resulted from secretion of water from samples and the internal zones resembled the area of

pressed meat. The formed two zones were measured by a planimeter (KOIZUMI Digital Planimeter PLACOM KP-92) in cm^2. Results were presented in (cm^2 / 0.3 g sample).

Cooking quality criteria of prepared beef burger:

Cooking loss and cooking yield:

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

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

Where; Wr: the weight of raw beef burger (g) and Wc: the weight of cooked beef burger (g).

Cooking yield of different beef burger samples were measured by reduction of cooking loss from 100.

Change of beef burger diameter (Shrinkage):

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

$$\%\text{Shrinkage} = \frac{\text{Uncooked diameter (cm)–Cooked diameter (cm)}}{\text{Uncooked diameter (cm)}} \text{X100}$$

Examination of microbiological quality criteria:

Sample preparation:

Different prepared beef burger samples were taken immediately after processing and during refrigerated storage. Samples were prepared for microbiological analysis in accordance with ISO 6887-1 (2003) test method for sample preparation titled: (Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination). Different samples of 10 g were weighed out from 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 to 60 seconds in stomacher machine. Dilution series were prepared by transferring 1ml of the previous dilution to 9ml of MRD solution. Preparation of samples for detection of *Salmonella sp*., was carried out as follows: 25g of different meat products samples were weighed out from the sterile stomacher zipped bag. Buffered peptone water (BPW), of 225 ml was added in accordance with the reference ISO 6579 (2006).

Microbiological examination methods:

Different prepared beef burger samples were examined for total aerobic bacterial count according to (ISO 4833-2003), while aerobic spore forming bacteria were determined according to the method described in compendium method for food microbiology (APHA, 2001). Other methods for microbiological analysis were carried out for yeast and mold (ISO 21527-1, 2-2008), coliform group (ISO 4832-2006), *Staphylococcus aureus* counts (ISO 6888-1-2003) and detection of Salmonella presence (ISO 6579-2006). All previous tests were used to reflect the microbiological quality of the prepared beef burger samples. The appropriate dilutions were plated onto duplicate plates. Plates were incubated; thin results were expressed as (log cfu /g of samples).

Sensory evaluation:

Different prepared beef burger samples were subjected to sensory evaluation by ten panelists from staff members of Food Science Department, Faculty of Agriculture, Ain Shams University. The sensory evaluation was carried out by serving warm different prepared beef burger samples after cooking using seven scale evaluations (Appearance, color, texture, tenderness, juiciness, flavor and over all acceptability). The samples were coded with three digit random numbers and the order of presentation was made using random permutation (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, (2008). Differences among means were compared using Duncan's multiple range tests at significant level 95% ($P \leq 0.05$).

Results and Discussion

Proximate chemical composition of prepared beef burger:

Results of proximate chemical composition of different beef burger samples prepared with addition of 1, 2 and 3% dried pomegranate peels powder and stored at 4±1°C for 12 days were given in table (2). The obtained data showed a significant ($P \leq 0.05$) decrease in moisture content of prepared beef burger samples with increasing the concentration of incorporation of pomegranate peels powder and also during different storage periods. The moisture content of control sample which prepared without pomegranate peels powder was 66.17% compared to 63.42%, 61.80% and 59.13% for beef burger samples prepared with 1%, 2% and 3% of pomegranate peels powder at the beginning of refrigerated storage period, respectively. In the same time, the control sample showed the highest decline in moisture content was 54.93% as compared with 56.30%, 54.03% and 52.32% for prepared beef burger samples with the aforementioned treatments at the end of the storage period, respectively. These results are in agreement with those of Hayes *et al.*, (2013) and Verma *et al.,* (2013).

Gibriel *et al.,* (2007); Devatkal *et al.,* (2014) and Qin *et al.,* (2013) reported that, as the ability of the protein to bind moisture decreases, the moisture loss increases. From the observed results, it could be noticed that, the addition of different ratios of pomegranate peels powder led to lower reduction in moisture content and improving the water holding capacity which resulted in lower loss rate in moisture content of beef burger samples treated with the addition of pomegranate peels powder.

Regarding the protein content of different prepared beef burger samples, it could be noticed that the protein content values of all samples ranged from 15.74 to 16.36% at zero time ranged from 13.20 to 14.77% after 12 days of refrigerated storage at 4±1 °C.

However, protein content of beef burger samples prepared with 2 and 3% of pomegranate peels powder showed relatively higher protein content after 12 days of refrigerated storage, being 14.33 and 14.77%; respectively. In the same time, the protein content of different prepared beef burger samples had analogy trend as that observed for moisture content. As the storage period increased, the protein content was significantly ($P \leq 0.05$) decreased, with the observation that, the control beef burger sample showed the lowest protein content value. The decrease in protein content of treated beef burger samples during storage period could be explained by the loss of soluble protein associated with the loss of water content of beef burger and may be associated to activity of proteolytic bacterial enzymes. In conclusion, raw beef burger prepared with pomegranate peels powder showed higher protein content than the control samples after 12 days of storage. With respect to the obtained data, it could be observed that addition of pomegranate peels had a significant ($P \leq 0.05$) effect. The aforementioned results may be explained by the antimicrobial effect of pomegranate peels powder thus had keeping and protecting effect against the bacterial growth and the action of their proteolytic enzymes, which in turn, reflect the significant impact of pomegranate peels powder on the protein content of prepared beef burger. Similar findings were reported by Taludkar and Sharma (2009). They observed a decrease in the protein content of chicken meat patties incorporated with wheat and oat bran.

Ash content values of all beef burger samples ranged from 2.46 to 2.81% at zero time and from 3.76 to 4.11% after 12 days of refrigerated storage. However, ash content of prepared beef burger at the beginning of storage period was slightly affected by the addition of different ratios of pomegranate peels powder as its values were around 2.66% for all samples. At the same time, as the storage period increased, the ash content of different prepared beef burger showed a slight significant ($P \leq 0.05$) increased, since the values of ash content were 4.11, 3.76, 3.85 and 4.04 for prepared beef burger samples containing 0, 1, 2 and 3% of pomegranate peels powder, respectively. This may be attributed to the higher mineral content or ash content of pomegranate peels powder in compared to beef meat. Similar results were reported by Verma *et al.,* (2013).

Table 2: Proximate chemical composition of beef burger samples prepared with different ratios of pomegranate peels powder during refrigerated storage at 4±1°C for 12 days.

Storage period (days)	Treatments			
	T1	T2	T3	T4
	Moisture %			
Zero	66.17 ^{aA} ± 0.74	63.42 ^{aB} ± 0.56	61.80 ^{aC} ± 0.62	59.13 ^{aD} ± 0.72
3	62.96 ^{bA} ± 0.57	62.26 ^{bA} ± 0.44	59.89 ^{bB} ± 0.25	56.74 ^{bC} ± 0. 35
6	59.77 ^{cA} ± 0.43	59.71 ^{cA} ± 0.55	58.10 ^{cB} ± 0.19	54.90 ^{cC} ± 0.24
9	57.68 ^{dA} ± 0.45	58.12 ^{dA} ± 0.25	55.97 ^{dB} ± 0.18	53.83 ^{dC} ± 0.50
12	54.93 ^{eB} ± 0.20	56.30 ^{eA} ± 0.16	54.03 ^{eC} ± 0.24	52.32 ^{eD} ± 0.45
	Protein %			
Zero	15.74 ^{aA} ± 0.33	15.80 ^{aA} ± 0.24	15.85 ^{aA} ± 0.39	15.96 ^{aA} ± 0.40
3	15.32 ^{abB} ± 0.34	15.46 ^{aAB} ± 0.24	15.79 ^{abAB} ± 0.39	15.82 ^{aA} ± 0.40
6	14.74 ^{bAB} ± 0.34	15.03 ^{bAB} ± 0.24	15.39 ^{bcAB} ± 0.39	15.63 ^{abA} ±0.40
9	14.03 ^{cC} ± 0.34	14.47 ^{cBC} ± 0.24	14.91 ^{cdAB} ± 0.39	15.20 ^{bcA} ± 0.40
12	13.20 ^{dC} ± 0.34	13.77 ^{dBC} ± 0.24	14.33 ^{dAB} ± 0.39	14.77 ^{cA} ± 0.40
	Ash %			
Zero	2.79 ^{dA} ± 0.15	2.46 ^{dB} ± 0.18	2.60 ^{dAB} ± 0.21	2.81 ^{dA} ± 0.07
3	3.04 ^{cdA} ± 0.15	2. 67 ^{cdB} ± 0.18	2.78 ^{cdAB} ± 0.21	3.09 ^{cA} ± 0. 07
6	3.30 ^{cA} ± 0.15	2.95 ^{cA} ± 0.18	3.09 ^{bcA} ± 0.21	3.33 ^{bcA} ± 0.26
9	3.73 ^{bA} ± 0.15	3.34 ^{bB} ± 0.18	3.40 ^{bB} ± 0.13	3.53 ^{bAB} ± 0.10
12	4.11 ^{aA} ± 0.15	3.76 ^{aB} ± 0.18	3.85 ^{aAB} ± 0.13	4.04 ^{aA} ± 0.10
	Fat %			
Zero	14.67 ^{aB} ± 0.40	14.91 ^{aAB} ± 0.30	15.18 ^{aAB} ± 0.45	15.54 ^{aA} ± 0.27
3	14.90 ^{aAB} ± 0.40	14.73 ^{aB} ± 0.30	15.04 ^{aAB} ± 0.45	15.51 ^{aA} ± 0.36
6	14.63 ^{aB} ± 0.40	14.87 ^{aB} ± 0.37	14.97 ^{aAB} ± 0.49	15.68 ^{aA} ± 0.36
9	15.00 ^{aB} ± 0.41	15.19 ^{aAB} ± 0.37	15.24 ^{aAB} ± 0.47	15.91 ^{aA} ± 0.38
12	15.40 ^{aA} ± 0.40	15.51 ^{aA} ± 0.36	15.49 ^{aA} ± 0.49	16.19 ^{aA} ± 0.38
	Total carbohydrates %			
Zero	1.73 ^{eC} ± 0.55	2.46 ^{dB} ± 0.18	4.28 ^{eB} ± 1.30	6.16 ^{dB} ± 0.23
3	3.78 ^{dC} ± 0.48	4.88 ^{cBC} ± 0.57	6.14 ^{dB} ± 0.58	8.87 ^{cA} ± 0.89
6	7.57 ^{cBC} ± 0.32	7.44 ^{bC} ± 0.41	8.45 ^{cB} ± 0.63	10.46 ^{bA} ± 0.60
9	9.57 ^{bAB} ± 0.32	7.70 ^{bB} ± 2.10	10.49 ^{bA} ± 0.21	11.53 ^{abA} ± 0.99
12	1 2.37 ^{aA} ± 0.51	10.66 ^{aB} ± 0.31	12.30 ^{aA} ± 0.22	12.68 ^{aA} ± 0.77

- 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 ($p \leq 0.05$).
- Means followed by different capital letters in the same raw (effect of treatments) are significantly by Duncan´s multiple test ($p \leq 0.05$).

On the other hand; fat content values of all prepared beef burger samples ranged from 14.67 to 15.54% at zero time of refrigerated storage period and from 15.40 to 16.19% after 12 days. In contrast, the fat content of prepared beef burger samples was increased as the storage period increased. An addition of pomegranate peels powder showed significant ($P \leq 0.05$) effect.

The carbohydrate content values of all samples ranged from 1.73 to 6.16% before storage and from 10.66 to 12.68% after 12 days of storage. However, carbohydrate content of the raw beef burger samples prepared by 3% pomegranate peels powder had the highest carbohydrate content after 12 days of refrigerated storage (12.68%) compared to other prepared samples. Addition of pomegranate powder

during the preparation of beef burger led to significant ($P \leq 0.05$) increasing in total carbohydrates values as their values were 1.73, 2.46, 4.28 and 6.16% for prepared beef burger containing 0, 1, 2 and 3% of pomegranate peels powder at the beginning storage period, respectively. In the same time, as the storage period increased, the values of total carbohydrates were significantly increased.

Physicochemical quality criteria of prepared beef burger:

Physicochemical (pH values) and chemical (TBARS as mg of malonaldhyde / kg sample and TVN as mg nitrogen / 100 g sample) as quality criteria in prepared beef burger samples were determined and the data are presented in Table 3.

Table 3: Physiochemical quality criteria of beef burger samples prepared with different ratios of pomegranate peels powder during refrigerated storage at 4±1˚C for 12 days.

Storage period	Treatments			
(days)	T1	T2	T3	T4
	pH values			
Zero	6.62 ^{abA} ±0.03	6.64 ^{aA} ±0.02	6.61 ^{abA} ±0.02	6.65 ^{aA} ±0.02
3	6.45 ^{aB} ±0.08	6.46 ^{aB} ±0.05	6.34 ^{abB} ±0.07	6.26 ^{bB} ±0.17
6	6.13 ^{aC} ±0.05	6.07 ^{abC} ±0.04	6.04 ^{abC} ±0.10	6.03 ^{bcC} ±0.03
9	6.01 ^{aD} ± 0.06	5.99 ^{abC} ± 0.11	5.86 ^{abC} ± 0.08	5.80 ^{bC} ± 0.01
12	5.97 ^{aD} ± 0.05	5.88 ^{bD} ± 0.09	5.79 ^{cD} ± 0.02	5.72 ^{cD} ± 0.05
	T.V.N as (mg nitrogen /100 g sample)			
Zero	8.89 ^{eA} ± 0.45	8.67 ^{eAB} ± 0.74	8.49 ^{eB} ± 0.22	8.27 ^{eB} ± 0.45
3	14.56 ^{dA} ± 0.61	11.75 ^{dB} ± 0.25	11.65 ^{dB} ± 0.57	11.22 ^{dB} ± 0.35
6	19.75 ^{cA} ± 0.37	15.85 ^{cB} ± 0.37	15.47 ^{cBC}±0.41	15.05 ^{cC} ± 0.26
9	33.57 ^{bA} ± 0.18	18.28 ^{bB} ± 0.31	18.54 ^{bBC} ±0.38	18.17 ^{bBC} ±0.18
12	54.25 ^{aA} ± 0.28	23.65 ^{aB} ± 0.47	21.97 ^{aBC} ±0.27	19.51 ^{aC} ± 0.21
	TBARS as (mg of malonaldhyde / kg sample)			
Zero	0.145 ^{eA} ± 0.810	0.141 ^{eA} ± 0.043	0.137 ^{eA} ± 0.071	0.131 ^{eA} ± 0.024
3	0.276 ^{dA} ± 0.091	0.257 ^{dA} ± 0.074	0.236 ^{dAB} ± 0.064	0.227 ^{dB} ± 0.087
6	0.455 ^{cA} ± 0.078	0.421 ^{cB} ± 0.061	0.409 ^{cC} ± 0.049	0.389 ^{cD} ± 0.066
9	0.741 ^{bA} ± 0.057	0.563 ^{bB} ± 0.055	0.521 ^{bBC} ± 0.035	0.507 ^{bC} ± 0.026
12	1.292 ^{aA} ± 0.039	0.642 ^{aB} ± 0.037	0.617 ^{aBC} ± 0.042	0.568 ^{aC} ± 0.071

- 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 ($p \leq 0.05$).
- Means followed by different capital letters in the same raw (effect of treatments) are significantly by Duncan´s multiple test ($p \leq 0.05$).

The pH values of all samples ranged from 6.61 to 6.65 at zero time and from 5.72 to 5.97 after 12 days of refrigerated storage indicating a significantly ($P \leq 0.05$) decrease during refrigerated storage. There were no significant differences ($P \leq 0.05$) in pH values of different prepared beef burger samples containing 0, 1, 2 and 3% of pomegranate peels powder. During storage period, the pH values of different prepared beef burger samples were decreased with significant effect ($P \leq 0.05$) for samples containing different ratios of pomegranate peels powder. On the contrary, pH values of the control sample, which prepared without pomegranate peels powder was decreased during storage period without significant effect ($P \leq 0.05$). The slight decreases in pH values during refrigerated storage period in different burger samples may be attributed to the breakdown of glycogen with the formation of lactic acid. These results are in accordance with the findings of Madkour *et al.,* (2000); Gibriel *et al.,* (2007); Devatkal *et al.,* (2010) and Qin *et al.,* (2013).

With respect to total volatile nitrogen (TVN) content of different prepared beef burger, it could be noticed that, there was gradually and significantly ($P \leq 0.05$) increase for all beef burger samples during storage period. The results showed that, all beef burger samples had similar TVN values at zero time of storage period ranged from (8.27 to 8.89 mg TVN /100g sample).

Furthermore, the obtained data indicated that, TVN content was significantly ($P \leq 0.05$) increased during refrigerated storage of different samples. Beef burger samples (T1) had a higher increasing rate in TVN content which was 8.89 mg/100 g at zero time of refrigerated storage, and continued to increase to reach 54.25 mg/100 g after 12 days. On other side, the corresponding value for the beef burger sample prepared with pomegranate peels powder (T4) had the lowest TVN value from the beginning of refrigerated storage 8.27 mg/100 g until the end of refrigerated storage period after 12 days (19.51 mg/100 g). The increase in TVN content during refrigerated storage of beef burger samples might be attributed to the break-down of nitrogenous substances by microbial activity. These results are in agreement with those of Madkour *et al.,* (2000) and Gibriel *et al.;* (2007). These results indicated the significant ($P \leq 0.05$) 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.

Thiobarbituric acid reactive substances (TBARS) values of different prepared beef burger samples containing different concentration of pomegranate peels powder are presented in Table 5. The positive effect of addition of pomegranate peels powder as a natural antioxidant source was noticed with significant differences ($P \leq 0.05$) in TBARS values of different prepared beef burger samples containing 1, 2 and 3% pomegranate peels powder compared to the control sample. The results indicated that, all beef burger samples had low TBARS values at zero time of storage. During the storage period, TBARS values tended to significantly ($P \leq 0.05$) increases, and all samples recorded values lower than the critical limit (0.9 mg malonaldhyde /kg sample) as reported by Greene and Cumuze, (1982), except control samples, which showed more than critical limit of TBARS values after 12 days of storage (1.292 mg malonaldhyde/kg), whereas other prepared samples T2, T3 and T4 showed the lowest TBARS values after 12 days of storage (0.642, 0.617and 0.568 mg malonaldhyde/kg; respectively). From the previous results, it can be seen that TBARS values of beef burger prepared with pomegranate peels powder exhibited their useful retardation effect on those deterioration reactions happened in beef burger lipids during storage.

Pomegranate peels or rind powder and pomegranate seeds are reported to possess significant antioxidant activity due to their polyphenolic compounds. Use of pomegranate peels powder as natural antioxidant in chicken and goat meat products had been investigated (Naveena *et al.,* 2008 and Devatkal *et al.* 2010). The aforementioned results are in agreement with the results of Naveena *et al.,* 2008; Devatkal *et al.,* 2010 and 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 TBARS values of prepared samples. The inhibitory effect of pomegranate peels powder on lipid oxidation might be related to its phenolic constituents and other biochemical compounds that mainly contribute to the antioxidant activity, (Zhang *et al.,* 2010 and Jia *et al.,* 2012).

Physical quality criteria of prepared beef burgers:

Results of change in water holding capacity (WHC) of different raw beef burger samples which prepared by addition of different concentration of pomegranate peels powder are illustrated in figure (1).

The addition of different concentration of pomegranate peels powder had no significant ($P \leq 0.05$) effect on WHC values of different prepared beef burger samples at the beginning of the storage period. As the storage period increased, the WHC of different prepared beef burger samples was significantly ($P \leq 0.05$) decreased during all storage periods. It could be noticed that the WHC of all samples was progressively decreased. This could be due to secretion of water from burger samples throughout the storage period. The control sample of beef burger (T1) as well as sample T2 which contained 1% pomegranate peels powder had the lowest WHC values after 12 days of refrigerated storage compared with other samples (T3 and T4 contained 2 and 3% of pomegranate peels powder), respectively. However, beef burger samples containing dry pomegranate peels powder (T3) and (T4) showed the highest values of WHC during storage. This significant ($P \leq 0.05$) decrease in WHC was observed in all samples, possibly due to the protein denaturation or aggregation, or to the biochemical changes associated with cooling of meat products, as reported and by Naveena *et al.,* (2008); Devatkal *et al.,* (2010) and Qin *et al.,* (2013). The reduction in WHC values at the end of refrigerated storage could be ascribed to the loss of water by evaporation, rather than to any improvement of water holding capacity.

Results of change in plasticity of different raw beef burger samples prepared by addition of different ratios of pomegranate peels powder were illustrated in figure (2). Concerning the plasticity of different prepared beef burger samples, addition of different ratios of pomegranate peels powder had no significant ($P \leq 0.05$) effect with little improvement in plasticity of beef burger containing pomegranate peels powder as compared to the control sample.

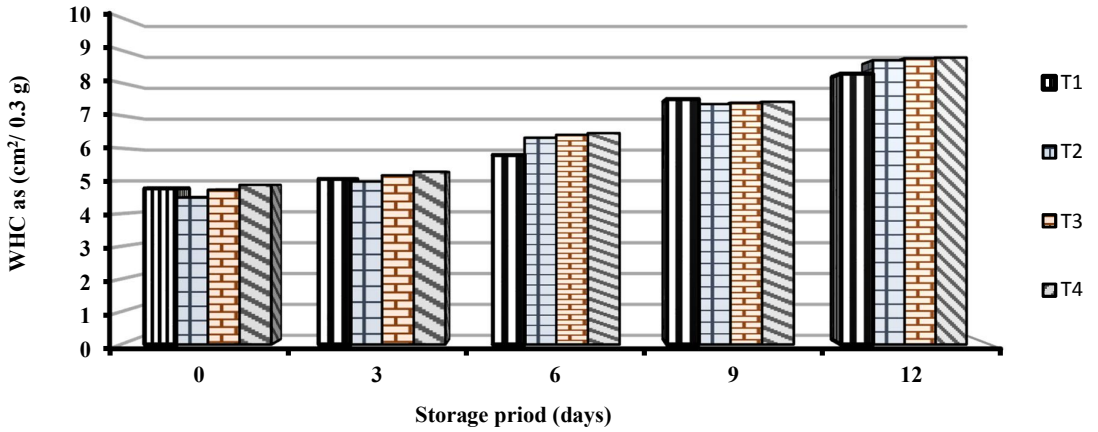

Fig. 1: Change in water holding capacity of beef burger samples prepared with different ratios of pomegranate peels powder during 12 refrigerated storage at 4±1 °C.

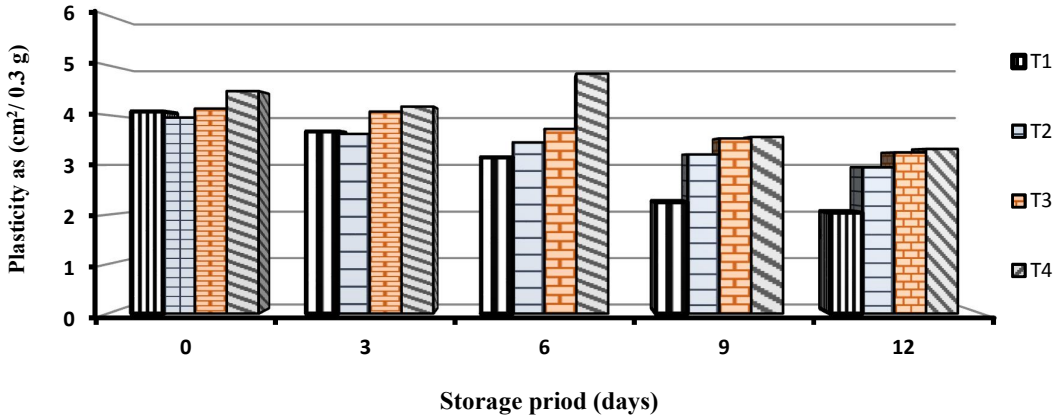

Fig. 2: Change in plasticity of raw beef burger samples prepared with different ratios of pomegranate peels powder during refrigerated storage at 4±1 °C.

Plasticity of all beef burger samples under investigation tended progressively to decrease during refrigerated storage at 4±1 °C until the end of studied storage period (12 day). This might be explained on the basis of denaturation and / or aggregation of protein during refrigerated storage, as well as the decrease in WHC and this reduction might be due to the tightening of beef burger structure because of evaporation of water. The aforementioned results may be explained by the small amount of pomegranate peels powder added during the preparing of beef burger (1, 2 and 3%) and the aim of addition was primarily targeting their effect as natural preservatives. The decrease of plasticity was clearly pronounced in the control sample T1 at the end of storage period. These results agree with the results of Georgantelis *et al.,* (2007); Gibriel *et al.,* (2007); Naveena *et al.,* (2008) and Devatkal *et al.,* (2010).

Cooking quality criteria of prepared beef burger:

Cooking characteristics of different prepared beef burger samples contained different ratios of pomegranate peels powder were presented in table (4). Addition of pomegranate powder led to reduction in cooking loss of prepared beef burger sample, especially to level 2 and 3%. However the cooking loss was significantly ($P \leq 0.05$) increased as refrigerated storage period progressed. Such increase reached 27.34 and 19.73% for samples T1 and T2, respectively after refrigerated storage at 4±1 °C for 12 days. However, the beef burger samples T4 and T3, which prepared with 2 and 3% pomegranate peels powder showed the lowest initial cooking loss (18.52 and 16.84%, respectively). These results are in agreement with those obtained by Madkour *et al.,* (2000); and Gibriel *et al.,* (2007). They reported that, the cooking

loss was progressively increased as the period of storage increased. Such hypothesis could be confirmed by the results of Berry, (1997), who reported that more additional water was lost during cooking with low fat beef patties if the ratio of water to starch was changed in the formula.

Table 4: Cooking quality criteria of beef burger samples prepared with different ratios of pomegranate peels powder during refrigerated storage at 4±1 °C for 12 days.

Storage period (days)	Treatments			
	T1	**T2**	**T3**	**T4**
		Cooking loss %		
Zero	17.21 ^{dA} ±0.41	12.01 ^{cdB} ±0.47	9.94 ^{dC} ±0.42	8.87 ^{dD} ±0.28
3	18.29 ^{cdA} ±0.28	13.73 ^{cB} ±0.21	11.93 ^{cC} ±0.18	11.18 ^{cC} ±0.32
6	19.82 ^{cA} ±0.31	15.39 ^{bcB} ±0.29	14.21 ^{bBC} ±0.27	13.23 ^{bC} ±0.41
9	21.61 ^{bA} ±0.19	16.77 ^{bB} ±0.34	15.65 ^{bBC} ±0.34	14.93 ^{bC} ±0.30
12	27.34 ^{aA} ±0.35	19.73 ^{aB} ±0.16	18.52 ^{aBC} ±0.51	16.84 ^{aC} ±0.27
		Cooking yield %		
Zero	82.78 ^{aC} ±0.41	87.99 ^{aB} ±0.47	90.05 ^{aA} ±0.42	91.11 ^{aA} ±0.28
3	81.71 ^{aC} ±0.28	86.27 ^{aB} ±0.21	88.06 ^{bA} ±0.18	88.81 ^{bA} ±0.32
6	80.18 ^{bC} ±0.31	84.61 ^{bB} ±0.29	85.79 ^{cAB} ±0.27	86.77 ^{cA} ±0.41
9	78.42 ^{cC} ±0.19	83.22 ^{cB} ±0.34	84.31 ^{cA} ±0.34	85.09 ^{dA} ±0.30
12	72.66 ^{dC} ±0.35	80.27 ^{dB} ±0.16	81.48 ^{dAB} ±0.51	83.16 ^{eA} ±0.27
		Change in diameter %		
Zero	10.55 ^{dA} ±0.37	8.84 ^{dB} ±0.22	6.34 ^{eC} ±0.19	5.60 ^{dD} ±0.23
3	12.68 ^{cA} ±0.18	10.53 ^{cB} ±0.31	9.56 ^{dBC} ±0.22	8.48 ^{cC} ±0.41
6	14.08 ^{bA} ±0.22	11.44 ^{cB} ±0.46	10.91 ^{cBC} ±0.35	9.31 ^{cC} ±0.17
9	17.24 ^{aA} ±0.29	13.83 ^{bB} ±0.18	12.51 ^{bC} ±0.27	10.61 ^{bD} ±0.21
12	18.45 ^{aA} ±0.31	15.11 ^{aB} ±0.27	14.08 ^{aC} ±0.13	12.32 ^{aD} ±0.34

- 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≤0.05).
- Means followed by different capital letters in the same raw (effect of treatments) are significantly by Duncan´s multiple test (p≤0.05).

Concerning the cooking yield of different prepared beef burger samples, it could be noticed that beef burger samples T1 and T2 prepared without addition of pomegranate peels powder and with 1.0% pomegranate peels powder, respectively showed the lowest initial cooking yield and achieved a values of 82.78 and 87.99%, respectively. On other side, the beef burger samples T4 and T3 prepared with 3.0 and 2.0% pomegranate peels powder showed highest initial cooking yield 90.05 and 91.11 %, respectively.

In the same time, all prepared beef burger samples showed a remarkable significantly (P≤0.05) decrease in cooking yield during refrigerated storage at (4±1 °C for 12 days). Such a behavior could be explained by the damage in dietary fiber granules present in burger and starch in different concentration of pomegranate peels powder upon cold and cooking processing, reducing capability of starch and dietary fibers in pomegranate peels powder to absorb high amount of water and swelling during cooking causing slightly losses of moisture to the heating media. These results are in agreement with those obtained by (Madkour *et al*., 2000; Gibriel *et al.,* 2007; Naveena *et al.,* 2008; Devatkal *et al.,* 2010 and Qin *et al.,* 2013).

Diameter of the cooked beef burger samples are important parameter for consumer acceptability. Therefore, addition of 2.0% starch with different ratios of pomegranate peels powder in beef burger Processing was recommended to keep these reductions at the minimum levels especially during refrigerated storage. Similar trend of aforementioned results of cooking loss and yield was observed for results of change in diameter of different prepared beef burger samples. The reduction in diameter was observed as a result of cooking of different beef burger samples. The samples T1 and T2 showed the highest reduction in diameter (10.55 and 8.84%, respectively).

On the other hand, beef burger samples T3 and T4 contained 2 and 3% dry pomegranate peels powder, respectively showed the lowest diameter reduction at zero time (6.34 and 5.60%, respectively) and after 12 days of refrigerated storage (14.08 and 12.32%, respectively).

Reduction in diameter is an ultimate result of the losses in cooking yield and moisture loss. As expected, beef burger samples with low cooking yield and high moisture losses showed the highest reduction in diameter after 12 days of refrigerated storage. The positive effect of addition of pomegranate peels powder in improving the cooking characteristics of prepared beef burger 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.

Microbiological criteria of prepared beef burger:

Results of the effect of addition of different concentration of pomegranate peels powder as natural preservative in preparing beef burger samples and during stored at 4 ±1 ℃ for 12 days are presented in table (5). It should be mentioned that *E. coli, Staphylococcus aureus and Salmonella sp*. (as pathogenic criteria) were not detected in all prepared beef burger samples containing 0, 1, 2 and 3% of pomegranate peels powder (Data are not given).

Table 5: Microbiological criteria of beef burger samples prepared with different ratios of pomegranate peels powder during refrigerated storage at 4±1°C for 12 days.

Storage period (days)	Treatments			
	T1	T2	T3	T4
Total Bacterial Counts (log cfu/g)				
Zero	3.32	3.45	3.66	3.50
3	3.79	3.40	3.59	3.28
6	4.23	3.27	3.45	3.23
9	5.17	3.04	2.48	2.74
12	5.32	2.87	2.41	2.18
Yeast and Mold counts (log cfu/g)				
Zero	2.40	2.48	2.65	2.70
3	2.90	2.42	1.98	1.93
6	3.23	2.40	1.93	1.86
9	3.30	2.26	1.86	1.74
12	4.41	2.11	1.81	1.60
Spore Forming Bacteria counts (log cfu/g)				
Zero	1.30	1.18	1.17	1.25
3	1.81	1.12	≤ 1	≤ 1
6	2.11	≤ 1	≤ 1	≤ 1
9	2.77	≤ 1	≤ 1	≤ 1
12	3.28	≤ 1	≤ 1	≤ 1
Coliform counts (log cfu/g)				
Zero	1.78	1.87	1.74	1.81
3	1.90	1.82	1.72	1.65
6	2.36	1.77	1.68	1.48
9	3.32	1.75	1.58	1.40
12	3.51	1.72	1.54	1.32

Different beef burger samples were analyzed for total bacterial counts, yeast and mold counts, spore forming bacterial counts and coliform counts. Total bacterial count of different prepared beef burger samples was in the range from 3.32 to 3.66 log cfu/g at the beginning of refrigerated storage period. It could be noted that all samples (at zero time) exhibited closely or similar total bacterial count. This may be related to maintaining the sanitary conditions during beef burger preparation.

Total bacterial count of prepared beef burger sample without addition pomegranate peels powder (T1) was remarkably increased progressively over the storage period from 3.32 log cfu/g at zero time and reached 3.79, 4.23, 5.17 and 5.32 log cfu/g after 3, 6, 9 and 12 days of refrigerated storage, respectively. On the other hand, the other prepared beef burger, which contained different ratios of pomegranate peels powder (1, 2 and 3%) showed a progressive reduction in total bacterial counts over the time of refrigerated storage period; while, total viable bacteria counts of prepared samples contained 1, 2 and 3% of pomegranate peels powder reached to 2.87, 2.41 and 2.18 log cfu/g after 12 days of storage, respectively.

The aforementioned results of total bacterial count 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 burger samples containing different ratios of pomegranate peels powder in comparison to control beef sample prepared without addition of pomegranate peels powder.

These results could be due to the antimicrobial effect of pomegranate peels powder, especially when the concentration of pomegranate peels powder was increased. The observed results are in agreement with the results of Al-Zoreky, (2009); Kanatt *et al.,* (2010) and Agourram *et al.,* (2013), 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.

Sensory quality criteria of beef burger samples:

Sensory evaluation is an important indicator of potential consumer preferences. Sensory characteristics, appearance, color, tenderness, juiciness, flavor and overall acceptability of prepared beef burger samples containing different concentration of pomegranate peels powder were evaluated and the results are presented in table (6).

It could be noticed that, addition of pomegranate peels powder had a significant effect ($p \leq 0.05$) on improving the sensory characteristics of prepared beef burger samples and increased their acceptability during storage. In general, as concentration of pomegranate peels powder increased the acceptability of prepared beef burger samples also increased.

Conclusion

It could be concluded that, the utilization of pomegranate peels powder at ratios of 1, 2 and 3% has proved to be effective as a natural preservative in producing high quality beef burger samples. WHC, plasticity, pH, TBARS, TVN and total bacterial counts, yeast and mold, spore forming bacteria and coliform of prepared beef burger samples were evaluated and it could be concluded that, addition of different concentration of pomegranate peels powder improved the aforementioned quality criteria. The application of different ratios of pomegranate peels powder has improved the cooking characteristics e.g. cooking loss, cooking yield and change in diameter. In the same time, utilization of the tested ratios of pomegranate peels powder could be useful to achieve high stability of beef burger during refrigerated storage with positive effects on the sensory characteristics of the product.

Table 6: Sensory quality criteria of beef burger samples prepared with different ratios of pomegranate peels powder during refrigerated storage at 4±1°C for 12 days.

Storage period (days)	Treatments			
	T1	T2	T3	T4
Appearance				
Zero	8.50 ^{aC} ±0.54	9.70 ^{aA} ±0.43	8.50 ^{aC} ±0.71	9.50 ^{aB} ±0.42
3	8.50 ^{aB} ±0.61	8.80 ^{bAB} ±0.70	8.40 ^{aC} ±0.42	9.10 ^{aA} ±0.47
6	7.30 ^{bC} ±0.64	8.90 ^{bA} ±0.48	7.70 ^{bBC} ±0.53	8.90 ^{aA} ±0.51
9	7.10 ^{bC} ±0.43	8.50 ^{bcA} ±0.45	7.50 ^{bcB} ±0.66	8.30 ^{bAB} ±0.46
12	6.40 ^{cC} ±0.41	8.10 ^{cA} ±0.32	7.10 ^{cB} ±0.42	7.90 ^{bAB} ±0.42
Color				
Zero	8.24 ^{aD} ±0.47	9.80 ^{aA} ±0.63	9.10 ^{aC} ±0.71	9.70 ^{aB} ±0.52
3	8.20 ^{aC} ±0.57	9.70 ^{aA} ±0.52	8.60 ^{ab} ±0.67	9.50 ^{abAB} ±0.63
6	7.60 ^{abC} ±0.67	9.50 ^{aA} ±0.42	8.40 ^{bB} ±0.67	9.40 ^{abA} ±0.57
9	7.50 ^{bD} ±0.42	9.10 ^{bA} ±0.48	8.10 ^{bBC} ±0.63	8.70 ^{bA} ±0.70
12	6.80 ^{cD} ±0.45	8.70 ^{bA} ±0.49	7.60 ^{cBC} ±0.51	8.10 ^{cB} ±0.64
Tenderness				
Zero	8.50 ^{aC} ±0.62	9.80 ^{aA} ±0.42	9.80 ^{aB} ±0.47	9.60 ^{aAB} ±0.51
3	8.30 ^{abC} ±0.47	9.70 ^{abA} ±0.52	8.90 ^{aB} ±0.53	9.20 ^{aAB} ±0.79
6	7.70 ^{bC} ±0.56	9.40 ^{abA} ±0.42	8.50 ^{abB} ±0.32	8.90 ^{aA} ±0.57
9	7.30 ^{cD} ±0.44	9.20 ^{bA} ±0.57	8.10 ^{bcBC} ±0.63	8.20 ^{bB} ±0.63
12	6.50 ^{dC} ±0.61	8.70 ^{cA} ±0.61	7.30 ^{cB} ±0.52	7.20 ^{bAB} ±0.37
Juiciness				
Zero	8.80 ^{aC} ±0.71	9.90 ^{aA} ±0.42	9.60 ^{aB} ±0.76	9.40 ^{aAB} ±0.64
3	8.40 ^{abC} ±0.67	9.70 ^{abA} ±0.48	9.20 ^{aB} ±0.67	9.20 ^{aA} ±0.53
6	7.90 ^{bD} ±0.97	9.60 ^{bA} ±0.48	8.70 ^{abBC} ±0.67	9.10 ^{aA} ±0.42
9	7.60 ^{bcC} ±0.79	9.30 ^{cA} ±0.52	8.50 ^{bcB} ±0.57	8.90 ^{bA} ±0.53
12	7.40 ^{cC} ±0.54	8.70 ^{cA} ±0.57	8.10 ^{cB} ±0.38	8.40 ^{bAB} ±0.75
Flavor				
Zero	8.90 ^{aB} ±0.62	9.80 ^{abA} ±0.58	8.90 ^{abB} ±0.63	9.40 ^{aAB} ±0.81
3	8.40 ^{aC} ±0.57	9.70 ^{aA} ±0.52	8.80 ^{aB} ±0.48	9.30 ^{aAB} ±0.63
6	7.80 ^{bC} ±0.52	9.50 ^{bA} ±0.57	8.50 ^{bcB} ±0.32	8.90 ^{abA} ±0.48
9	7.40 ^{bB} ±0.47	8.90 ^{cA} ±0.53	8.20 ^{cA} ±0.32	8.40 ^{bcA} ±0.99
12	6.50 ^{cC} ±0.41	8.30 ^{dA} ±0.45	7.40 ^{dB} ±0.55	7.90 ^{cA} ±0.54
Over acceptability				
Zero	8.90 ^{aB} ±0.36	9.80 ^{aA} ±0.54	9.10 ^{aB} ±0.61	9.80 ^{aA} ±0.37
3	8.70 ^{aD} ±0.52	9.70 ^{abA} ±0.71	8.70 ^{aBC} ±0.82	9.40 ^{aAB} ±0.79
6	8.40 ^{aC} ±0.57	9.20 ^{bcA} ±0.74	8.20 ^{abC} ±0.63	8.60 ^{bBC} ±0.70
9	7.40 ^{bD} ±0.57	8.80 ^{cdA} ±0.48	7.80 ^{bcC} ±0.67	8.40 ^{bBC} ±0.57
12	6.60 ^{cD} ±0.41	8.40 ^{dA} ±0.33	7.50 ^{cC} ±0.57	8.20 ^{bAB} ±0.44

- 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≤0.05).
- Means followed by different capital letters in the same raw (effect of treatments) are significantly by Duncan´s multiple test (p≤0.05).

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