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# EFFECT OF POMEGRANATE PEEL POWDER ON THE HYGIENIC QUALITY OF BEEF SAUSAGE

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**ABSTRACT**

Pomegranate (*Punica granatum*) peel is a nutrient-rich byproduct whose production are extensively growing due to the exponential rise in the pomegranate juice production. Pomegranate juice and related products are directly added to foods due to their pleasant taste, palatability, and preservative effects. In this study, the effect of pomegranate peel powder was investigated at a concentration of 2.5 % and 5% on beef sausage stored at -18 ± 2°C. A significant effect on pH was detected at zero time where control, 2.5% and 5% had pH values of 6.18 ± 0.14, 5.87± 0.13 and 5.54 ± 0.17, respectively. Meanwhile, the significant effect of pomegranate peel powder on total volatile nitrogen (TVN) and thiobarbituric acid (TBA) appeared on 4th week. Total bacterial counts (TBC) and *Enterobacteriaceae* counts were reduced significantly on 1st and 3rd weeks in examined samples of different groups. Thus, in general, it can be concluded that addition of pomegranate peel powder is an effective tool to decrement pH, TVB-N, TBA and bacterial counts in oriental sausage.

**Keywords:** Beef sausage, Aerobic plate count, *Enterobacteriaceae*, TBA, TVB-N

## INTRODUCTION

Sausages are comminuted processed meat products prepared from red meat, poultry or a combination of these with water, binders and seasoning. They are commonly stuffed into a casing, and may be cured, smoked or cooked. Sausages as one of the oldest varieties of meat processing in which meats go through various modification processes to acquire desirable organoleptic and keeping properties. The Sausage manufacture is a simple procedure of allowing meat to undergo series of controlled structural and chemical changes. These are basic to all cultures but the changes rely on varied methods of preparation and seasoning to achieve desired distinctive characteristics. Even though the size and scope of operation have experienced a remarkable level of change the principles and idea behind modern day sausage manufacture in achieving products of high organoleptic value and improved shelf life remain the same **(Savić, 1985).** Pomegranate (*Punica granatum*) from the Punicaceae family is an important commercial fruit crop that is extensively cultivated in parts of Asia, North Africa, the Mediterranean and the Middle East. Some studies have reported that different parts of the pomegranate fruit such as juice, peel, and seeds may act as potential antimicrobial agents **(Duman *et al*., 2009; Abdollahzadeh *et al*., 2011; Choi *et al*., 2011; Singh *et al*., 2014)**. Recently, higher antimicrobial and antioxidant activities of pomegranate peel extracts have been reported and therefore might be suggested as a safe natural option to synthetic antimicrobial agents **(Rosas-Burgos *et al*., 2017)**. Additionally, the pomegranate peel extract showed the highest punicalagin and ellagic acid concentrations that have been identified as the principal factor behind the pomegranate antimicrobial activity. On the other hand, the antioxidant activity of pomegranate juice is higher than other fruit juices **(Seeram *et al*., 2008)**. This antioxidant activity has been correlated to the highest content of phenolic compounds, including anthocyanins (3-glucosides and 3,5-diglucosides of delphinidin, cyanidin, and pelargonidin), ellagic acid, punicalin, punicalagin, pedunculagin and different flavonoids. Pomegranate rind is an inedible part obtained during the processing of pomegranate juice. Lately, the use of pomegranate juice and rind powder as a source of natural antioxidant in chicken patties had been investigated **(Naveena *et al*., 2008)**. Furthermore, **Devatkal *et al*. (2010)** have shown a significant antioxidant effect of extracts of pomegranate rind and seed powders. Therefore, the aim of our work was to improve the hygienic quality of the sausage by the increase of different concentrations of pomegranate peel powder.

## MATERIALS AND METHODS

### Preparation of beef sausage

Beef meat samples including boneless neck, chuck and rounds along with associated fats were obtained from local markets at Zagazig city, Egypt, and used for preparing beef sausage samples. All sub cut fat and inter-muscular fat were included as fat sources. The beef meat and fat tissue were transported to the laboratory using an icebox. Different ingredients used in preparing beef sausage samples e.g. table salt, starch and spices mixture such as black pepper, red pepper, nutmeg and ginger were obtained from local markets at Zagazig, Egypt. Beef sausage samples were made according to the method described by **Zaika *et al*. (1978)**. Meat and fat tissues were cut into small portions (approximately 6 cm long, 5 cm wide, and 4 cm thick). These cuts were ground to particles of about a rice size (2 mm x 4 mm), then the ingredients were blended to prepare sausage mixture emulsion, which was then stuffed by sausage filling machine previously washed by hot water and cased in mutton casings. Three groups of sausage were prepared, including control group, Group 1 and 2. The Control group consists of lean meat 70%, fat 12%, sodium chloride 2.3%, water 9.3%, garlic 1%, onion 1.2% and spices mixture 1.2%). The group 1 (G1) was similar to the control but after mixing 2.5% removed and replaced with 2.5% dried pomegranate peel powder then mixed again), meanwhile group 2 (G2), after mixing 5% removed and replaced with 5% dried pomegranate peel powder then mixed again). Finally, the prepared sausage samples were stored at -18 ± 2℃ in order to simulate the normal storage temperature used in the retail sausage outlets present in Egypt.

### Chemical analyses

The determination of the pH values of different beef sausage samples were done 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). On the other hand, determination of total volatile basic nitrogen (TVB-N) was carried out according to Conway's micro diffusion technique recommended by **(FAO, 1992)**. However, determination of thiobarbituric acid (TBA) was performed according to **(Kirk and Sawyer, 1991)**.

**Bacteriological analyses**

***Preparation of samples for bacteriological examination***

Sausage samples were prepared for microbiological analysis in accordance with **ISO 6887-1(2003).** For the Aerobic plate count **(Baumgart & Firnhaber, 1986);** One ml of each previously prepared serial dilution was carefully transferred into separate, duplicate, appropriately marked Petri dishes, and thoroughly mixed with about 15 ml of previously melted and adjusted (45 ± 1°C) plate count agar (Oxiod, CM325). After solidification, the inoculated plates as well as control one were inverted and incubated promptly for 48 ± 2 h at 37°C. The countable plates with 30-300 colonies were recorded and the total colony count per $cm^2$ was calculated. However, for the enumeration of *Enterobacteriaceae* **(ICMSF, 1978);** 0.1 ml from the original and the subsequent prepared dilutions were spread on surface of Petri dish in duplicate plates containing Violet red bile glucose agar (VRBGA), and incubated at 37°C for 24 hours. All large purple colonies were counted and the average number of *Enterobacteriaceae* per gram of sample was calculated and recorded.

**RESULTS AND DISCUSSION**

**Effect of pomegranate peel powder on pH during freezing at -18 ± 2°C**

The pH value is the important physicochemical characteristic to decide the quality and shelf life of sausage. The pH value of control, 2.5 and 5% pomegranate treated sausage at zero time was 6.18 ± 0.14, 5.87 ± 0.13 and 5.54 ± 0.17, respectively. There were significant effects ($p< 0.05$) of both treatments as compared to control samples. This direct effect related to the acidic pH of pomegranate peel powder (pH=3.75) as recorded by **(Ullah *et al.*, 2012).** Our results were in disagreement with **El-Nashi *et al.* (2015)** who found that no significant differences in pH values of different prepared beef sausage samples containing 0%, 1%, 2% and 3% of pomegranate peels powder. After elapsing of 8 weeks of storage at - 18 ± 2°C the mean value of pH was 6.27 ± 0.16, 5.9 ± 0.15 and 5.73± 0.19 for control, 2.5 and 5% pomegranate treated sausage, respectively. Results shown in the figure (1) indicated that freezing had no effect on pH value of the same group during storage weeks. The obtained results were corresponded with **Muela *et al.* (2010)** who suggested that the pH of fresh meat and frozen meat did not differ significantly. On contrary, **Kim and Lee (2011)** reported that frozen meat had a higher pH than fresh meat because of partial denaturation of the muscle proteins.

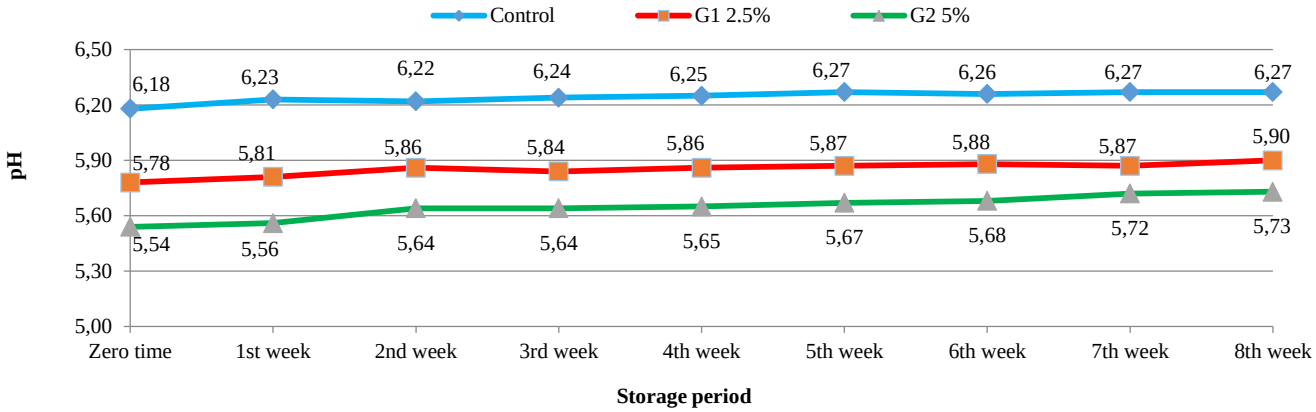


**Figure 1** pH values in control and pomegranate treated groups (G1, 2.5%) and (G2, 5%) during freezing at -18 ± 2°C

**Effect of pomegranate peel powder on total volatile nitrogen during freezing at -18 ± 2°C**

From the results achieved in table (1), it could be noticed that total volatile nitrogen (TVN) of control, 2.5% and 5% pomegranate peel powder (PGPP) treated groups was 6.50 ± 1.23, 6.41 ± 1.23 and 6.39 ± 1.23 mg/100 g, respectively at zero time. There were no significant effects of pomegranate peel powder (PGPP) at zero time. The TVN values increased gradually with increasing frozen storage period. After elapsing of four weeks the recorded values were 16.00 ± 1.70, 11.00 ± 1.41 and 9.23 ± 1.12 mg/100 g for control, 2.5% and 5% pomegranate peel powder treated groups, respectively. By the 8th week, TVN values were 20.04 ± 4.10, 16.00 ± 3.45 and 14.33 ± 4.21 mg/100 g for control, 2.5% and 5% pomegranate peel powder treated groups, respectively. There were significant effects ($p< 0.05$) of pomegranate peel powder added at concentration of 2.5 and 5% after four and eight weeks of freezing at -18 ± 2°C. The increasing of TVN during freezing weeks was attributed to the continuous enzyme activity (**Berry *et al.*, 2008**). The obtained results were concurred with that obtained by **Ibrahim (2004)**. Additionally, the total volatile nitrogen values of all treatments were in the range of permissible level (< 20 mg /100 g) established by Egyptian standard specifications **(ESS, 2005)**.

**Table 1** Total volatile basic nitrogen (TVB-N) mg/100g in control and treated sausage during freezing at -18 ± 2°C

| Period | Control group | | | | Treated group(G1) with 2.5% | | | | Treated group(G2) with 5% | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Min | Max | Mean | SD | Min | Max | Mean | SD | Min | Max | Mean | SD |
| Zero time | 5.40 | 7.80 | $6.50^{Ac}$ | 1.23 | 5.40 | 7.80 | $6.41^{Ac}$ | 1.23 | 5.40 | 7.80 | $6.39^{Ac}$ | 1.23 |
| 4th week | 12.00 | 19.20 | $16.00^{Ab}$ | 1.70 | 8.00 | 13.10 | $11.00^{Bb}$ | 1.41 | 7.40 | 11.20 | $9.23^{Bb}$ | 1.12 |
| 8th week | 13.20 | 23.20 | $20.04^{Aa}$ | 4.10 | 11.23 | 19.30 | $16.00^{Ba}$ | 3.45 | 9.47 | 17.36 | $14.33^{Ca}$ | 4.21 |

Means carrying different superscript capital letters on the same column are significantly different ($P< 0.05$) on different group.
Means carrying different superscript small letters on the same row are significantly different ($P< 0.05$) on same group.

**Effect of pomegranate peel powder on thiobarbituric acid (TBA) during freezing at -18 ± 2°C**

As shown in table (2), the level of thiobarbituric acid of all treatments at zero time of control, 2.5% and 5% pomegranate powder treated groups was 0.27 ± 0.11 mg malonaldhyde/kg. There were no significant effects ($p> 0.05$) at zero time. As the frozen storage time progressed, the thiobarbituric acid values of all treatments increased gradually. However, the lowest TBA value was recorded for sausage contained 5% pomegranate peel powder, meanwhile the highest increment of TBA value was noted for control sausage which reached 0.84 mg malonaldhyde/kg after 8 weeks from the start of freezing storage (-18°C). Generally, the increases of TBA values that observed in all sausages treatments contained pomegranate peel powder were less than that found in the control sausage that might be explained by the ability of pomegranate peel powder to scavenge free radicals, and its antioxidant power (**Gil *et al.*, 2000).** TBA values of all samples after the eight weeks of storage were within the range of permissible level (< 0.9 mg malonaldhyde/kg for frozen sausage) set by Egyptian standard specifications **(ESS, 2005)**.

**Table 2** Thiobarbituric acid (TBA) mg malondialdehyde/kg in control and treated sausage during freezing at -18 ± 2°C

| Period | Control group | | | | Treated group(G1) with 2.5% | | | | Treated group(G2) with 5% | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Min | Max | Mean | SD | Min | Max | Mean | SD | Min | Max | Mean | SD |
| Zero time | 0.21 | 0.39 | $0.27^{Ac}$ | 0.11 | 0.21 | 0.39 | $0.27^{Ac}$ | 0.11 | 0.21 | 0.39 | $0.27^{Ac}$ | 0.11 |
| 4th week | 0.56 | 0.79 | $0.62^{Ab}$ | 0.15 | 0.35 | 0.54 | $0.46^{Bb}$ | 0.08 | 0.28 | 0.49 | $0.39^{Bb}$ | 0.10 |
| 8th week | 0.82 | 0.87 | $0.84^{Aa}$ | 0.09 | 0.48 | 0.67 | $0.59^{Ba}$ | 0.10 | 0.38 | 0.49 | $0.42^{Ca}$ | 0.13 |

Means carrying different superscript capital letters on the same column are significantly different (P< 0.05) on different group.
Means carrying different superscript small letters on the same row are significantly different (P< 0.05) on same group.

**Effect of pomegranate peel powder on aerobic plate count (APC) during freezing at -18 ± 2°C**

The data presented in figure (2) showed that the mean value of APC at zero time of control, 2.5% and 5% pomegranate treated groups was 5.65± 1.38, 5.59 ± 1.92 and 5.59 ± 1.92 $\log_{10}$ CFU/g, respectively. There were no significant effects related to the addition of pomegranate peel powder of different prepared beef sausage at zero time. The obtained data revealed that beef sausage samples treated with 2.5% and 5% concentrations of pomegranate peels powder, had a significant reduction on APC at 2nd and 3rd week, respectively. APC of prepared beef sausage (control, 2.5% and 5% pomegranate treated groups) was progressively reduced to 4.85 ± 0.64, 4.39 ± 0.51 and 4.10 ± 0.42 $\log_{10}$ CFU/g, respectively over the time of storage period. Moreover, the results showed a significant reduction at the 8th week ($p<0.05$) in both 2.5 and 5% pomegranate powder treated sausage. These results could be due to the antimicrobial effect of pomegranate peels powder especially when the concentration of pomegranate peels powder was increased. According to **Rosas-Burgos *et al.* (2017)**, the peel of the pomegranate fruits is a rich source of antifungal and antibacterial compounds such as ellagic acid and punicalagins (α and β), and this might be employed as a natural alternative to synthetic antimicrobial agents. The observed results seemed to be similar to the results of **El-Nashi *et al.* (2015)**, **Agourram *et al.* (2013)**, **Kanatt *et al.* (2010)** and **Al-Zoreky (2009)** who evaluated the antimicrobial characteristics of pomegranate peels. They found that pomegranate peels have an inhibition effect against gram positive and gram-negative bacteria. A gradual decrease was observed in APC of control beef sausage samples during the storage period. The count became 4.85 ± 0.64 $\log_{10}$ CFU/g at the end of storage period. On the other hand, the pomegranate 2.5 % and 5% treated groups had the same pattern of the gradual decrease and became 4.39 ± 0.51 and 4.10 ± 0.42 $\log_{10}$ CFU/g, respectively. The significant effect of freezing on reduction of APC appeared on control and pomegranate treated sausage in the 3rd and 5th week, respectively. Our results accorded with **(Ray and Bhunia, 2007)** who found that maximum lethality is seen with slow freezing where exposure to high concentrations is prolonged. Survival is greater with rapid freezing where exposure to these conditions is minimized. However, food-freezing processes are not designed to maximize microbial lethality but to minimize loss of product quality.

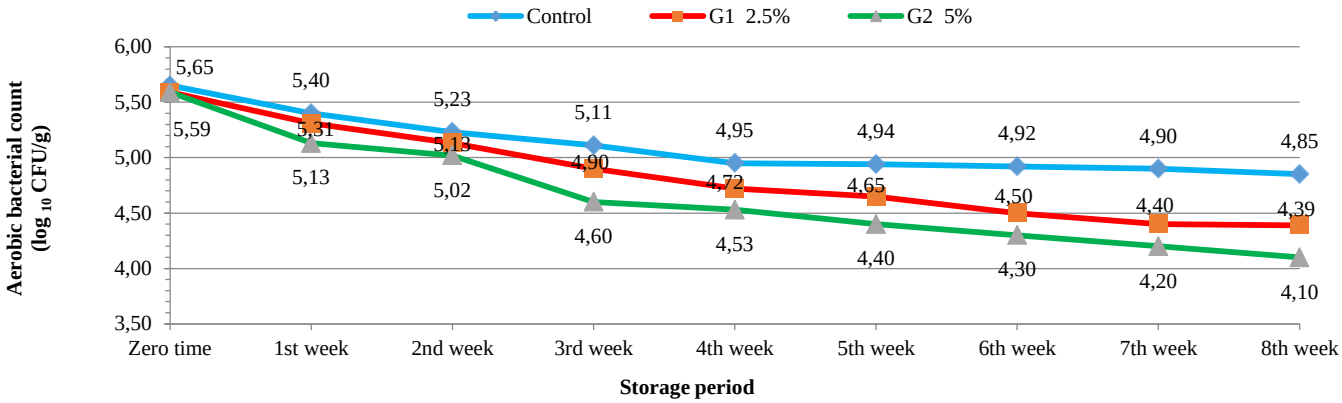


**Figure 2** Aerobic bacterial counts ($\log_{10}$ CFU/g) in control and pomegranate treated groups (G1, 2.5%) and (G2, 5%) frozen at -18 ± 2°C

**Effect of pomegranate peel powder on *Enterobacteriaceae* count during freezing at -18 ± 2℃**

The data presented in figure (3) showed that the mean values of *Enterobacteriaceae* count at zero time of control, 2.5% and 5% treated groups were 3.18 ± 1.13, 3.12 ± 1.19 and 3.09 ± 0.98 $\log_{10}$ CFU/g, respectively. No significant effects related to the addition of pomegranate peel powder to the prepared beef sausage were detected at zero time. The obtained data revealed that, the beef sausage samples treated with different concentrations of pomegranate peels powder (2.5% and 5%) led to a significant reduction of *Enterobacteriaceae* count at 3rd week. Progressive reduction of the *Enterobacteriaceae* count over the time of storage period; whereas, in 8th week, the *Enterobacteriaceae* count of the control group, 2.5% and 5% of treated groups reached to 2.95 ± 0.65, 2.28 ± 0.87 and 2.20 ± 0.98 $\log_{10}$ CFU/g, respectively. Moreover, there were a significant effect at the 8th week ($p<0.05$) in both 2.5 and 5% pomegranate powder treated groups. The obtained results may be attributed to the antimicrobial effect of pomegranate peels. According to **Li *et al.* (2006)**, pomegranate peel powder is an important source of bioactive compounds such as phenolic compounds, which are secondary plant metabolites and possess antibacterial or antiviral activities (**Cai *et al.*, 2004**). The significant effect of freezing on reduction of *Enterobacteriaceae* appeared in control and pomegranate treated sausage in the 4th and 5th weeks, respectively. Furthermore, the freezing effect pronounced on *Enterobacteriacea*e count, where cold shock affect gram-negative bacteria than gram positive (**Dodd *et al.*, 2007**).

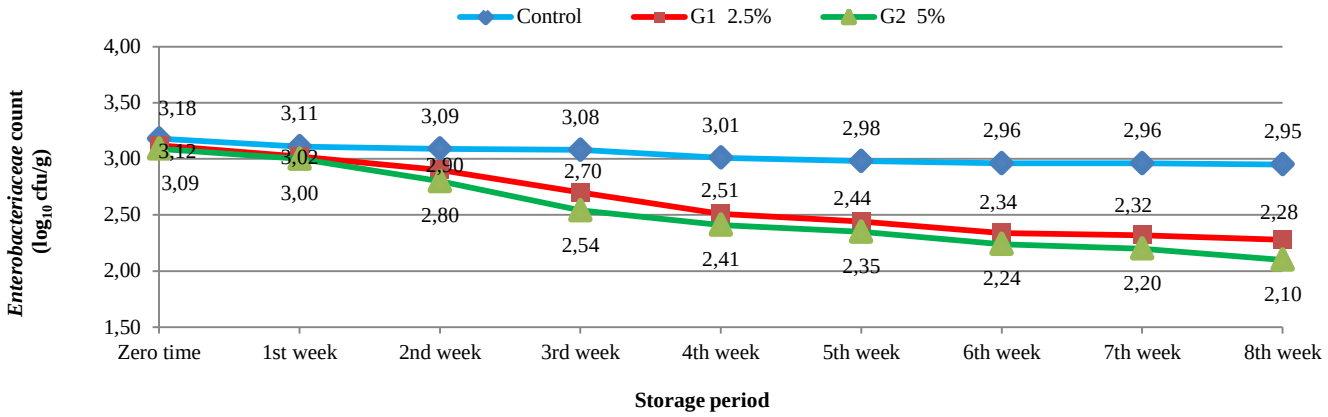


**Figure 3** Enterobacteriaceae count (log $_{10}$ CFU/g) in control and treated groups (G1, 2.5%) and (G2, 5%) frozen at -18 ± 2℃

## CONCLUSION

The aim of the presented study was to evaluate the impact of pomegranate peel powder (2.5 % and 5%) on beef sausage stored at -18 ± 2℃ . It was found that pomegranate peel powder had a substantial effect on pH, total volatile nitrogen (TVN) and thiobarbituric acid (TBA) over the storage period compared to the control group. Moreover, the total bacterial counts (TBC) and *Enterobacteriaceae* counts were reduced significantly in the pomegranate treated group. Therefore, the use of pomegranate peel powder is considered as effective food additive to decrement pH, TVB-N, TBA and bacterial counts in sausage. Further future studies are necessary to measure the color and organoleptic attributes of the sausages, in addition, the application of pomegranate peel powder alone or in combination with other antibacterial agents such as essential oils, organic acid salts to control foodborne pathogens in different food products.

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