

Effect of pomegranate peel powder on the hygienic quality of beef sausage

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Abstract:

The effect of pomegranate peel powder was investigated at a concentration of 2.5 % and 5% on beef sausage stored at $-18 \pm 2^{\circ}$ 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 appeared on 4th week. Total bacterial counts (TBC) and *Enterobacteriaceae* counts were reduced significantly on 3rd and 1st weeks in examined samples of different groups. A general as conclusion addition of pomegranate peel powder is considered as a good tool to decrement of pH, TVN , TBA and bacterial counts in oriental sausage.

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

Introduction:

Sausages are comminuted processed meat products made from red meat, poultry or a combination of these with water, binders and seasoning. They are usually stuffed into a casing and may be cured, smoked or cooked. Sausages as one of the oldest forms of meat processing in which meats go through various modification processes to acquire desirable organoleptic and keeping properties. The manufacture of sausage is a simple process 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 spicing to achieve desired distinctive characteristics. Even though the size and scope of operation have undergone 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 (FAO, 1985). Pomegranate (*Punica granatum* L.) 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. Recently, the high antioxidant activities of different parts of pomegranate fruit such as

juice, peel and seeds have been determined. The antioxidant activity of pomegranate juice is higher than other fruit juices (Seeram et al., 2008). This antioxidant activity has been correlated to the great amount of phenolic compounds, including anthocyanins (3-glucosides and 3,5-diglucosides of delphinidin, cyanidin and pelargonidin), ellagic acid, punicalin, punicalagin, pedunculagin and different flavanols. Pomegranate (*Punica granatum*) rind is an inedible part by-product obtained during processing of pomegranate juice. Recently use of pomegranate juice and rind powder as a source of natural antioxidant in chicken patties had been investigated (Naveena et al., 2008). Further, Devatkal et al. (2010) have demonstrated significant antioxidant effect of extracts of pomegranate rind and seed powders. This work was planned out to improve the hygienic quality of sausage by addition 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 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, nutmeg and ginger were obtained from local markets at Zagazig, Egypt. Beef sausage samples were prepared according to the method described by **Zaika et al. (1978)**. Meat and fat tissues were cut into pieces of about egg-size. The 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 sausage filling machine previously washed by hot water and cased in mutton casings.

Control group contain: lean meat 70%, fat 12%, sodium chloride 2.3%, water 9.3%, garlic 1%, onion 1.2% and spices mixture 1.2%.

Group 1: after mixing 2.5% removed and replaced with 2.5% dried pomegranate peel powder then mixed again.

Group 2: after mixing 5% removed and replaced with 5% dried pomegranate peel powder then mixed again

Determination of 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).

Determination of total volatile basic nitrogen (TVN) according to Conway's micro diffusion technique recommended by **(FAO 1992)**.

Determination of thiobarbituric acid (TBA) according to **(Kirk and Sawyer, 1991)**

Preparation of samples for a bacteriological examination:

Sausage samples were prepared for microbiological analysis in accordance with **ISO 6887-1 (2003)**

Aerobic plate count (Baumgrate et al., 1990):

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 ($45 \pm 1^\circ\text{C}$) plate count agar Oxoid (CM325). After solidification the inoculated plates as well as control one were inverted and incubated promptly for 48 ± 2 h at 37°C . The plates with 30-300 colonies were counted and total colony count per cm² was calculated and recorded.

Enumeration of Enterobacteriaceae (ICMSF, 1978):

Actually, 0.1ml from the original and the subsequent prepared dilutions were spread on surface of Petri dish in duplicate plates containing Violet red bile glucose agar 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 $-18 \pm 2^\circ\text{C}$

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% pomegranatum 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 on control samples. This direct effect related to the acidic pH of pomegranate peel powder. The pomegranate peel powder pH was found 3.75 **Ullah et al. (2012)**. This results in were 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 $-18 \pm 2^{\circ}\text{C}$ 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% pome granatum treated sausage. Freezing had no effect on pH value of the same

group during storage weeks, this results in agreement with **Muela et al. (2010)** postulated 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 control meat because of partial denaturation of the muscle proteins.

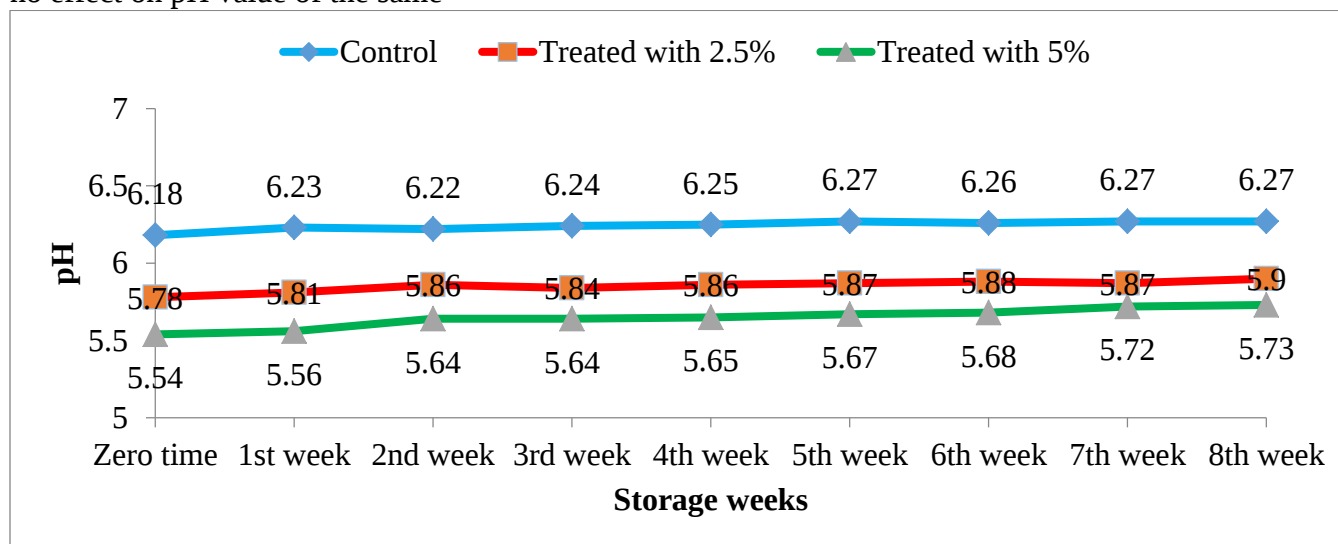


Figure (1) Effect of Pomegranate peel powder on pH during freezing weeks at $-18 \pm 2^{\circ}\text{C}$

Effect of Pomegranate peel powder on total volatile nitrogen during freezing at $-18 \pm 2^{\circ}\text{C}$

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 6.5, 6.41 and 6.39 mg/100g, 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, 11 and 9.23 mg/100g for control, 2.5% and 5% pomegranate peel powder treated groups, respectively.

By the 8 week the (TVN) values were 20.04, 16 and 14.33 mg/100g for control, 2.5% and 5% pomegranate peel powder treated groups, respectively. There were significant effects ($p < 0.05$) of pomegranate peel powder as additive at concentration of 2.5 and 5% after four and eight weeks of freezing at $-18 \pm 2^{\circ}\text{C}$. The increasing

of TVN during freezing weeks was attributed to the enzyme activity which continues (**Berry et al., 2008**).

Total volatile nitrogen values of all treatments were in the range of permissible level reported by **Egyptian Standards Specifications (2005)** which limited the content of TVN must be not over than 20 mg /100g. These results are coinciding with that obtained by **Ibrahim (2004)**.

Effect of Pomegranate peel powder on thiobarbituric acid (TBA) during freezing at $-18 \pm 2^{\circ}\text{C}$

Thiobarbituric acid of all treatments at zero time of control, 2.5% and 5% pomegranate powder treated groups was 0.27 malonaldehyde /kg. There were no significant effects ($p > 0.05$) at zero time. Table (2)

As time of frozen storage progressed the Thiobarbituric acid values of all treatments increased gradually. The lowest Thiobarbituric acid recorded for sausage contained 5%

Pomegranate peel powder, meanwhile the highest increment of TBA value recorded for control sausage which reached 0.84 mg malonaldehyde /kg after 8 weeks from the start of freezing storage (-18°C). Generally, the increments of TBA values which observed in all sausages treatments contained pomegranate peel powder were less than that found in the control sausage. Due to their well-known abilities of

pomegranate peel powder to scavenge free radicals, i.e antioxidant power **Gill et al. (1995).**

TBA value of all samples after 8th weeks of storage within the range of permissible level limited by **Egyptian Standards Specification (2005)** which limited the value of TBA must be not more than 0.9 mg malonaldehyde /kg for frozen sausage.

Table (1): Total volatile basic nitrogen (TVB-N) mg/ 100g. In control and treated sausage during freezing at -18 ± 2°C (n= 5).

	Zero time		4 th week	8 th week
Control	Min	5.4	12	13.2
	Max	7.8	19.2	23.2
	Mean	6.5 ^{Ac}	16 ^{Ab}	20.04 ^{Aa}
	SD	1.23	1.7	4.1
Treated with 2.5%	Min	5.4	8	11.23
	Max	7.8	13.1	19.30
	Mean	6.5 ^{Ac}	11 ^{Bb}	16 ^{Ba}
	SD	1.23	1.41	3.45
Treated with 5%	Min	5.4	7.4	9.47
	Max	7.8	11.2	17.36
	Mean	6.5 ^{Ac}	9.23 ^{Bb}	14.33 ^{Ca}
	SD	1.23	1.12	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.

Table (2): Thiobarbituric acid (TBA) mg malondialdehyde/ Kg. in control and treated sausage during freezing at -18 ± 2°C (n= 5).

	Zero time		4 th week	8 th week
Control	Min	0.21	0.56	0.82
	Max	0.39	0.79	0.87
	Mean	0.27 ^{Ac}	0.62 ^{Ab}	0.84 ^{Aa}
	SD	0.11	0.15	0.09
Treated with 2.5%	Min	0.21	0.35	0.48
	Max	0.39	0.54	0.67
	Mean	0.27 ^{Ac}	0.46 ^{Bb}	0.59 ^{Ba}
	SD	0.11	0.08	0.1
Treated with 5%	Min	0.21	0.28	0.38
	Max	0.39	0.49	0.49
	Mean	0.27 ^{Ac}	0.39 ^{Bb}	0.42 ^{Ca}
	SD	0.11	0.1	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 Bacterial count (ABC) during freezing at $-18 \pm 2^\circ \text{C}$

The data presented in figure (2) showed that the mean value of TBC at zero time of control, 2.5% and 5% pomegranate peel powder treated groups was 5.65 ± 1.38 , 5.59 ± 1.92 and $5.59 \pm 1.92 \log_{10} \text{cfu/g}$, respectively.

There were no significant effects related to the addition of pomegranate peel powder of different prepared beef sausage at zero time.

Obtained data revealed that, the prepared beef sausage samples which contained different concentrations of pomegranate peels powder 2.5% and 5% had a significant reduction on TBC at 3rd and 2nd weeks, respectively.

Progressive reduction in total bacterial count over the time of storage period; where, TBC of prepared beef sausage control, 2.5% and 5% of pomegranate peels powder was 4.85 ± 0.64 , 4.39 ± 0.51 and $4.1 \pm 0.42 \log_{10} \text{cfu/g}$.

There were a significant effect 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. The observed results

seemd to be similar 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 and they found that pomegranate peels have an inhibition effect against gram positive and gram negative bacteria.

During the storage period a gradual decrease was observed in APC of control beef sausage samples. The count became $4.85 \pm 0.64 \log_{10} \text{cfu} / \text{g}$ 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 $4.1 \pm 0.42 \log_{10} \text{cfu} / \text{g}$, respectively.

The significant effect of freezing on reduction of APC appeared on control and pomegranate treated sausage in the 5th and 3rd week, respectively. Our results coincided with (Ray and Bhunia, 2008) 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.

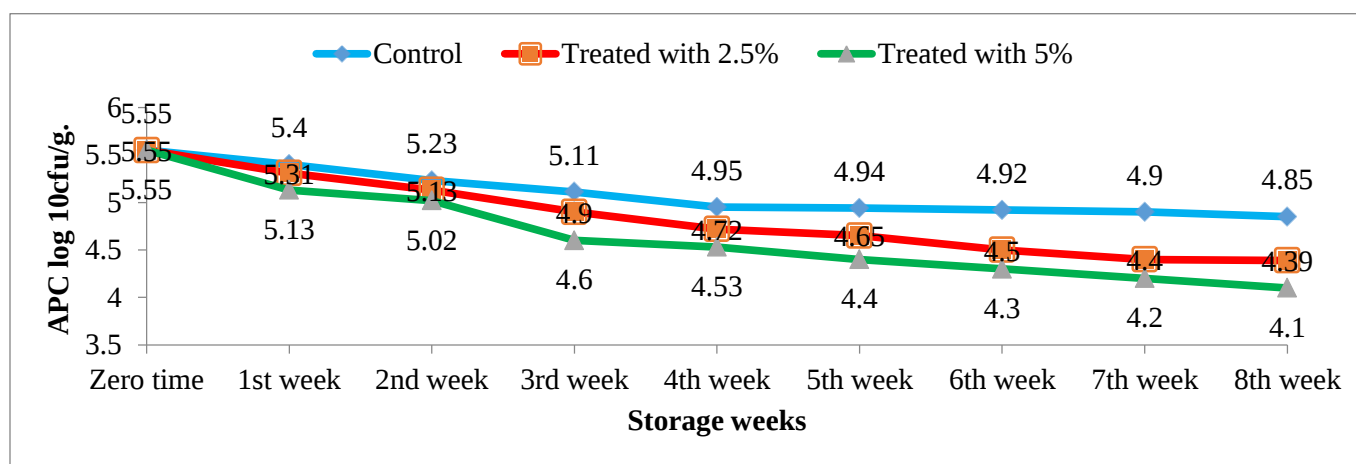


Figure (2) Effect of Pomegranate peel powder on APC during freezing weeks at $18 \pm 2^\circ \text{C}$.

Effect of Pomegranate peel powder on *Enterobacteriaceae* count during freezing at $-18 \pm 2^\circ \text{C}$

The data presented in figure (3) showed that the mean value of *Enterobacteriaceae* count at zero time of control, 2.5% and 5% pomegranate peel powder treated groups was 3.18 ± 1.13 , 3.12 ± 1.19 and $3.09 \pm 0.98 \log_{10} \text{cfu/g}$, respectively.

There were no significant effects related to the addition of pomegranate peel powder of different prepared beef sausage at zero time.

Obtained data revealed that, the prepared beef sausage samples which contained 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; where, in 8th week the *Enterobacteriaceae* count of prepared beef sausage control, 2.5% and 5% of pomegranate peels powder reached to 2.95 ± 0.65 , 2.28 ± 0.87 and $2.2 \pm 0.984 \log_{10} \text{cfu/g}$.

There were a significant effect at the 8th week ($p < 0.05$) in both 2.5 and 5% pomegranate powder treated sausage.

These results may be attributed to the antimicrobial effect of pomegranate peels and this illustrated in several studies have shown that this byproduct is an important source of bioactive compounds such as phenolic compounds, which are secondary plant metabolites and possess antibacterial, or antiviral activities, its use remained very limited and traditional (Cai *et al.*, 2004 and Li *et al.*, 2006).

During the storage period a gradual decrease in TBC of control beef sausage samples was observed and became $2.95 \pm 0.65 \log_{10} \text{cfu/g}$ at the end of storage period. On the other hand, the pomegranate 2.5 % and 5% treated groups had the same pattern of gradual decrease and became 2.28 ± 0.87 and $2.1 \pm 0.98 \log_{10} \text{cfu/g}$, respectively.

The significant effect of freezing on reduction of *Enterobacteriaceae* appeared in control and pomegranate treated sausage in the 5th and 4th weeks, respectively.

Freezing effect pronounced on *Enterobacteriaceae* count, where cold shock affect gram negative bacteria than gram positive (Dodd *et al.*, 2007).

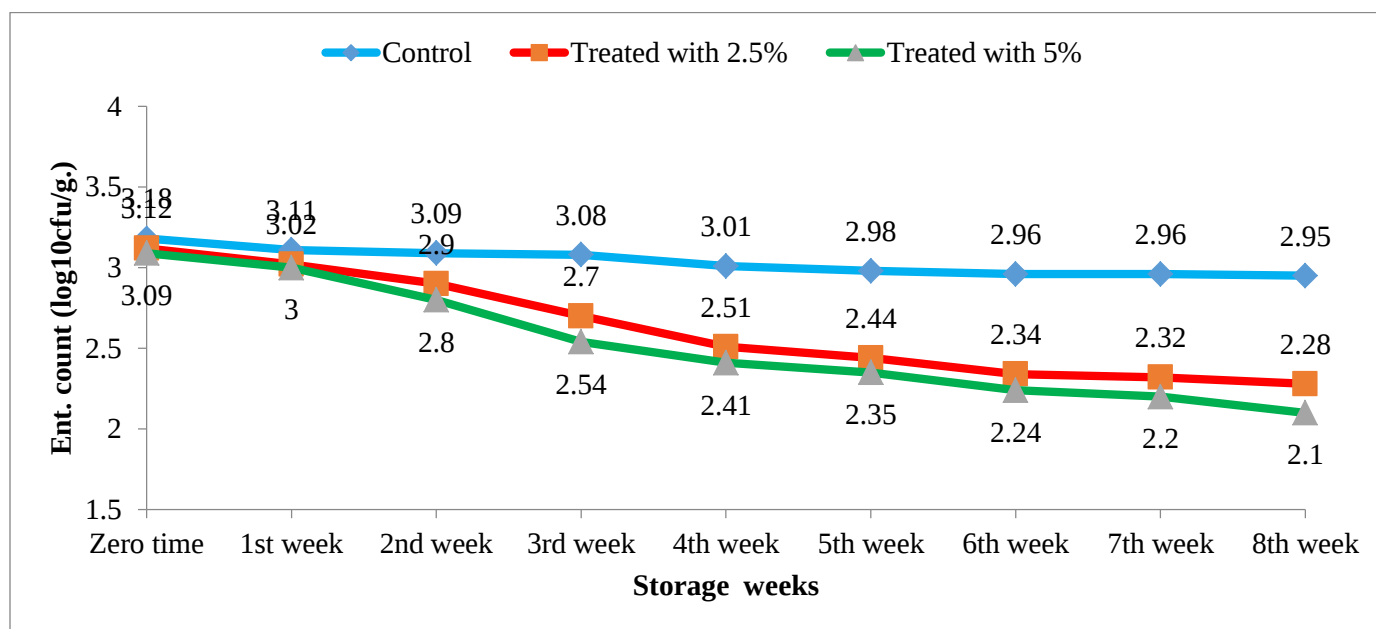


Figure (3) Effect of pomegranate peel powder on *Enterobacteriaceae* count during freezing weeks at $-18 \pm 2^\circ$

References:

- Agourram, A., Ghirardello, D., Rantsiou, K., Zeppa, G., Belviso, S., Romane, A., ... & Giordano, M. (2013):** Phenolic content, antioxidant potential, and antimicrobial activities of fruit and vegetable by-product extracts. *International Journal of Food Properties*, 16(5), 1092-1104.
- Al-Zoreky, N. S. (2009):** Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. *International journal of food microbiology*, 134(3), 244-248.
- Baumgrate J., Firhaber J., Spicherr C., Timm, F. and Zchaler R. (1990) :** Mikrobiologische Untersuchung Von Lebensmitteln. Behr's Verlag .Hamburg.
- Berry, M., Fletcher, J., McClure, P. and Wilkinson, J.(2008):** Effects of freezing on nutritional and microbiological properties of foods. In: Evans, J.A. (Ed.). 2008. Frozen Foods Science and Technology. Blackwell Publishing. Ames (IA).
- Cai, Y., Luo, Q., Sun, M. and Corke, H. (2004):** Antioxidant Activity and Phenolic Compounds of 112 Traditional Chinese Medicinal Plants Associated with Anticancer. *Life Sci.*, 74: 2157–2184.
- Defreitas, Z., Sebranek, J.G., Olson, D.G. and Carr, J.M., (1997):** Freeze/thaw stability of cooked pork sausage as affected by salt, phosphate, pH and Cartageenan. *J. Food Sci.* 62, 551–554.
- Devatkal, SK, Narsaiah, K. and Borah, A. (2010):** Anti-oxidant effect of extracts of kinnow rind, pomegranate rind and seed powders in cooked goat meat patties. *Meat Sci* 85:155–159.
- Dodd, C.E.R., Richards, P.J. and Aldsworth, T.G. (2007):** Suicide through stress: A bacterial response to sub-lethal injury in the food environment. *Int. J. Food Microbiol.* 120, 46-50.
- Egyptian Standards Specifications (2005):** Frozen sausage No. 1972. Egyptian Organization for Standardization and Quality Control, Ministry of Industry., Egypt.
- El-Nashi, H. B., Fattah, A. F. A. K. A., Rahman, N. R. A., & El-Razik, M. A. (2015):** Quality characteristics of beef sausage containing pomegranate peels during refrigerated storage. *Annals of Agricultural Sciences*, 60(2), 403-412.
- FAO (1985):** ‘Small-Scale Sausage Production’, FAO Animal Production and Health Paper 52.
- Ibrahim, M.M.M., (2004):** Quality changes occurring during frozen storage of chicken sausage containing buffalo spleen. *Egypt, J. Agric. Res.*, 82 (3):327 – 342.
- International Commission on Microbiological Specifications for Foods "ICMSF" (1978):** Microorganisms in foods, 2. Sampling for microbiological analysis: principles and specifications for foods Univeristy: Toronto Toronto, Canada.
- ISO 4833, (2003):** Microbiology of Food and Animal Feeding Stuffs –Horizontal Method for the Enumeration of Microorganisms Colony-count Technique at 30 degrees C.
- Kanatt, S. R., Chander, R., & Sharma, A. (2010):** Antioxidant and antimicrobial activity of pomegranate peel extract improves the shelf life of chicken products. *International journal of food science & technology*, 45(2), 216-222.
- Kim BS and Lee YE (2011):** Effect of antioxidant on quality of ground beef during the

refrigeration storage. Korean J. Food Nutr. 24(3):422- 433.

Kirk, S., & Sawyer, R. (1991): *Pearson's composition and analysis of foods* (No. Ed. 9). Longman Group Ltd.

Li, Y. F., Guo, C. J., Yang, J. J., Wei, J. Y., Xu, J. and Cheng S. (2006): Evaluation of Antioxidant Properties of Pomegranate Peel Extract in Comparison with Pomegranate Pulp Extract. Food Chem., 96: 254–260.

Muela, E., Sañudo, C., Campo, M. M., Medel, I., & Beltrán, J. A. (2010): Effect of freezing method and frozen storage duration on instrumental quality of lamb throughout display. *Meat science*, 84(4), 662-669.

Naveena, BM, Sen AR, Vaithiyanathan, S, Babji Y. and Kondaiah, N. (2008): Comparative efficacy of pomegranate juice, pomegranate rind powder and BHT in cooked chicken patties. *Meat Sci* 80:1304–1308.

Ray, B. and Bhunia, A. (2008): *Fundamental Food Microbiology*. 4 ed. CRC Press, Boca Raton (FL).

Seeram, N. P., Aviram, M., Zhang, Y., Henning, S. M., Feng, L., Dreher, M., & Heber, D. (2008): Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. *Journal of agricultural and food chemistry*, 56(4), 1415-1422.

Ullah, N., Ali, J., Khan, F. A., Khurram, M., Hussain, A., & Rahman, I. U. (2012): Proximate composition, minerals content, antibacterial and antifungal activity evaluation of pomegranate (*Punica granatum* L.) peels powder. *Middle-East Journal of Scientific Research*, 11(3), 396-401.

Zaika, L.L., Zell, T.E., Palumbo, S.A. and Smith, J.L., (1978): Effect of spices and salt on fermentation of Lebanon bologna-type sausage. *J. Food Sci.* 43, 186–189.

تأثير إضافة مسحوق قشر الرمان على الجودة الصحية للسجق البقري

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تم دراسة تأثير استخدام مستخلص قشر الرمان بعد إضافته أثناء تصنيع السجق بتركيزات ٢,٥% و ٥% مقارنة بالمجموعة الضابطة. تم ملاحظة التأثير المباشر لمسحوق قشر الرمان على الأس الهيدروجيني لعينات السجق والتي أدى ذلك إلى خفضها حيث تحولت من 6.18 ± 0.14 في المجموعة الضابطة إلى 5.87 ± 0.13 و 5.54 ± 0.17 في المجموعتين الأولى والثانية والتي أضيف لهما مسحوق قشر الرمان بتركيزات ٢,٥% و ٥% على التوالي.

وكان لمسحوق قشر الرمان تأثير على المواد النيتروجينية المتطايرة بعد مرور أربعة أسابيع من الحفظ عند $18 \pm 2^\circ \text{C}$ حيث كانت متوسطات المجموعات الضابطة، المجموعات ٢,٥% والمجموعات ٥% من مسحوق قشر الرمان ١٦، ١١ و ٩.٢٣ مجم/ ١٠٠ جم على الترتيب. أيضا أوضحت النتائج التأثير الجيد لمسحوق قشر الرمان كمضاد للأكسدة بعد مرور أربعة أسابيع وازداد معدل خفض تكوين المألون داي ألدهيد في الأسبوع الثامن من التجميد حيث كانت النتائج ٠.٨٤، ٠.٥٩ و ٠.٤٢ مجم مألون داي ألدهيد / كجم في المجموعات الضابطة، المجموعات ٢,٥% والمجموعات ٥% من مسحوق قشر الرمان على الترتيب.

أثبتت نتائج الفحص البكتيري للعدد الكلي للمكروبات الهوائية والأمعانيات أن إضافة مسحوق قشر الرمان تؤدي إلى خفض معنوي في تلك الأعداد بعد ثلاثة أسابيع في المجموعات التي تحتوي على ٢,٥% من مسحوق قشر الرمان. بينما تنخفض تلك الأعداد الميكروبية معنويا بعد أسبوع من استخدام تركيز ٥% من مسحوق قشر الرمان.

توصى الدراسة بإمكانية استخدام مسحوق قشر الرمان في السجق لتقليل درجة الحموضة وكذلك خفض تكون المواد النيتروجينية المتطايرة و معدل التزنخ وتقليل العدد البكتيري.