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Assessment of pomegranate peel powder on microbial contamination of sausage

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ABSTRACT

Pomegranate (*Punica granatum*) peel is a nutrient-rich by product whose juice and related products are directly added to foods due to their pleasant taste, palatability, and preservative effects. This review aims to using of pomegranate peels powder at concentrations of 2g, 3g, and 5 g on physicochemical and chemical quality of sausage during cold storage at (1-4˚C) for 12 days. Also, microbiological criteria of sausage samples were recorded. The pH values of all samples ranged from 7.39 to 7.30 at zero time and from 6.27 to 6.38 after 12 days of cold storage; indicating a slight decrease ($p < 0.05$) during cold storage at (1-4˚C).Values of total volatile nitrogen and Thiobarbituric acid were also reduced by adding of different concentration of powders of pomegranate during cold storage compared to control samples. Phenolic compounds in pomegranate peels could have antimicrobial properties. So, we can conclude that addition of pomegranate peel powder has an effect on lowering pH, Total volatile nitrogen (TVN), Thiobarbituric acid (TBA), Aerobic plate count (APC), total coliforms, mould, and yeast counts in sausage.

1. INTRODUCTION

Meat products were subjected to various degree of contamination through meat processing. Therefore, a concerted effort should be made to maintain sanitary condition in processing, preparation and handling. (Shaltout *et al*. 2014).

Growth of fungi on many types of foods under certain environmental conditions results in extensive spoilage of these foods due to production of off flavors, discoloration and bad tastes (Shaltout 1996).

Sausage is considered as a prepared food made from ground meat, animal fat, spices and salt, typically packed in a casing, Sausage manufacturing is a traditional food preservation technique. Traditionally, casings were made of animal intestine, but synthetic casings were recently used (Quasem- Jihad *et al*., 2009).

Exhibition of antibacterial and antifungal properties have been demonstrated by different plant extracts (Bouamama *et al*., 2006).

Punica granatum Linn. (Pomegranate) is a member of family *Punicaceae*. This plant is found all over India. Pomegranate peel is an inedible part obtained during processing of Pomegranate juice. Pomegranate peel is a rich source of tannins, flavonoids, polyphenols and some anthocyanins as delphinidins, cyanidins, etc and could act as antimicrobial agent (Abdollahzadeh *et al*., 2011; Choi *et al.*, 2011; Singh *et al*., 2014). Natural option of antimicrobial agents has been applied than synthetic one (Rosas Burgos et al., 2017). This antimicrobial activity was related to the phenolic compounds that involves precipitation of membrane proteins of microorganisms resulting in microbial cell lysis.

Our studies aim to detect the keeping quality of sausages by using different concentration of pomegranate peel powder

2. MATERIAL AND METHODS

2.1. Pomegranate peel preparation

Pomegranate fruits used were purchased and collected from local markets of Kaliobia, Egypt. After washing and peeling of the fruit, we can carefully separate their edible parts. After treated in an oven at 40˚C for 48 h, grounding occur to the air-dried peels to a powder, after that packaging in a polyethylene bags till using.

2.2. Sausage preparation

Boneless beef meat and fat were obtained fresh from a local market in Kaliobia. Fat sources are also included from inter-muscular and subcutaneous fat. Sausage samples were prepared according to Zaika *et al.* (1978). Cutting of Meat and fat tissues into pieces of egg-size and then frozen at -18 C for 24 h, then grounding occur to be like rice size, then blending to form emulsion, and stuffed manually into mutton casings befor closing and chipping of the casings Shehata (1989). The natural casings were done according to El-Deep (1987).

Using of pomegranate peel powder in different concentration (2, 3, and 5 gm) for evaluation of their antimicrobial effects on different sausage samples, control group, T1 (2 g pomegranate peel powder), T2 (3 g pomegranate peel powder, and T3 (5 g pomegranate peel

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powder, respectively) were stored at (1-4˚C) for 9 days. Samples were noticed and taken at 3, 6, and 9 days and analyzed.

2.3. Chemical analyses

pH value was measured according to the technique recommended by Defreitas et al. (1997) as follows; Approximately 10 grams of sausage samples were chopped and homogenized in 50 ml of distilled water in bearer. The mixture was left at a room temperature for 10 minutes with frequent shaking. The pH of the homogenate was measured with a digital pH meter (Jenco 609-USSR). After adjusting its pH by placing its electrode consecutively in two solutions of previously adjusted pH at 7 and 4 then its electrode was immersed directly into the prepared meat extract samples to determine its pH value.On the other hand, detection of (TBA) was recorded according to_(E.S 63/10, 2006), However determination of (TVN) was done according to Harold et al., (1987).by using macrokjeldahl procedure and titration occur by HCl solution (0.01N) in the presence of mixed indicator (bromocrysol green / methyl red) and the result were calculated as mg nitrogen (TVN) per 100 g samples. Means of 3 replicates were reported for each treatment.

2.4. Microbiological analyses

Microbiological analysis was applied to the samples of sausage. For the Aerobic plate count (FDA, 2001), Accurately 0.1 ml from each of chosen prepared dilution was inoculated separately onto duplicate sterile plates of plate count agar. The inoculum using sterile bent glass was spread, the plates were incubated at 35°C for 48 hours. Plates containing 25-250 colonies were counted and Aerobic Plate Count (APC) per gram of the sample was recorded. However, for the enumeration of Coliform count (ICMSF, 1996), The same technique of the previous surface plating method was applied using Violet Red Bile agar medium. The plates were incubated at 37°C for 24 hours. All pink colonies measuring 0.5 mm or more in diameter on uncrowded plates were then counted and the average number of colonies were determined. By multiplying the number of colonies of the dilution to obtain the number of Coliform organisms per gram of sample was calculated.

Mycological analysis was also done for yeast and mould and all was carried out to detect the microbiological quality of sausage sample ISO (215-27-1:2008).

3. RESULTS

Change in pH values of examined samples prepared using different ratios of powder during storage at (1- 4˚C) for 12 days are given in table (1). The pH values of all samples ranged from 7.39 to 7.30 at zero time and from 6.27 to 6.38 after 12 days of cold storage: indicating a slight decrease (**P** <0.05) during cold storage. There were no significant differences **(P** <0.05) in pH values of different prepared sausage samples containing different pomegranate concentrations.

The results in table (2) indicated that, all examined samples had a closed low TBA values at zero time of storage. During the storage period, TBA values tended to increased and all samples recorded values lower than the critical limit (0.9 mg MDA/kg), except control samples which showed more than critical limit (1.002 mg MDA/ kg) TBA values after 12 days of storage, whereas the samples T1, T2, and T3 observed the lowest TBA values after 12 days of storage (0.59, 0.53, and 0.49 mg MDA/kg; respectively).

Data in table (3) indicated that, TVN content has increased (p<.05) during storage of different examined samples. The result showed that, all examined samples had closed TVN content (p<.05) at zero time of storage 8.08 to 8.52 mg TVN/100 g samples. The examined samples has a higher increasing rate in TVN content which was 8.52 mg/100 g at zero time of cold storage, and continued to increase to reach 30.48 mg / 100 g after 12 days. But sausage sample prepared with pomegranate peels powder (T3) had the lowest TVN content from the beginning of cold storage 8.08 mg / 100 g till the end of cold storage period after 12 days (21.82 mg/ 100 g).

Table 1 Experimental effect of Pomegranate extract on pH of the examined samples.

Day	Control	T1 (2 g)	T2 (3 g)	T3 (5 g)
0	$7.39 \pm 0.25^{A1.}$	$7.18 \pm 0.02^{A1.}$	$7.25 \pm 0.02^{A1.}$	$7.30 \pm 0.01^{A1.}$
3rd	$6.95 \pm 0.07^{B2.}$	$6.98 \pm 0.02^{B2.}$	$6.85 \pm 0.06^{B2.}$	$6.64 \pm 0.02^{B1.}$
6th	$6.58 \pm 0.01^{C3.}$	$6.52 \pm 0.02^{C3.2.}$	$6.47 \pm 0.01^{C2.1.}$	$6.44 \pm 0.03^{C1.}$
9th	$6.41 \pm 0.01^{D2.}$	$6.40 \pm 0.02^{D2.}$	$6.37 \pm 0.01^{D2.}$	$6.31 \pm 0.01^{D1.}$
12th	$6.38 \pm 0.02^{D2.}$	$6.36 \pm 0.02^{D2.}$	$6.35 \pm 0.01^{D2.}$	$6.27 \pm 0.01^{D2.}$

Means with different letters (A, B, C) within the same column are highly significantly different ($P < 0.05$). Means with different numbers (1., 2., 3.) within the same raw are highly significantly different ($P < 0.05$).

Table 2 Statistical analysis of the experimental effect of Pomegranate extract on TBA of the examined samples

Day	Control	T1 (2 g)	T2 (3 g)	T3 (5 g)
0	$0.331 \pm 0.003^{E3.}$	$0.327 \pm 0.001^{E3.2.}$	$0.324 \pm 0.001^{E2.1.}$	$0.321 \pm 0.001^{D1.}$
3rd	$0.422 \pm 0.001^{D3.}$	$0.342 \pm 0.002^{D2.}$	$0.332 \pm 0.001^{DE1.}$	$0.334 \pm 0.002^{D1.}$
6th	$0.621 \pm 0.002^{C3.}$	$0.356 \pm 0.003^{C2.}$	$0.318 \pm 0.001^{C1.}$	$0.315 \pm 0.003^{C1.}$
9th	$0.888 \pm 0.017^{B3.}$	$0.488 \pm 0.006^{B2.}$	$0.489 \pm 0.004^{B2.}$	$0.414 \pm 0.004^{B1.}$
12th	$1.002 \pm 0.004^{A4.}$	$0.591 \pm 0.002^{A3.}$	$0.530 \pm 0.005^{A2.}$	$0.493 \pm 0.007^{A1.}$

Means with different letters (A, B, C) within the same column are highly significantly different ($P < 0.05$). Means with different numbers (1., 2., 3.) within the same raw are highly significantly different ($P < 0.05$).

Table 3 Statistical analysis of the experimental effect of Pomegranate extract on TVN of the examined samples.

Day	Control	T1 (2 g)	T2 (3 g)	T3 (5 g)
0	$8.52 \pm 0.0176^{E3.}$	$8.48 \pm 0.011^{E3.}$	$8.26 \pm 0.033^{E2.}$	$8.08 \pm 0.015^{E1.}$
3rd	$11.45 \pm 0.090^{D3.}$	$10.91 \pm 0.10^{D2.}$	$10.69 \pm 0.177^{D2.}$	$10.13 \pm 0.052^{D1.}$
6th	$19.33 \pm 0.023^{C4.}$	$18.28 \pm 0.012^{C3.}$	$17.13 \pm 0.102^{C2.}$	$15.28 \pm 0.023^{C1.}$
9th	$21.39 \pm 0.029^{B3.}$	$19.76 \pm 0.284^{B2.}$	$17.96 \pm 0.081^{B1.}$	$18.24 \pm 0.026^{B1.}$
12th	$30.48 \pm 0.310^{A4.}$	$25.06 \pm 0.044^{A3.}$	$23.12 \pm 0.040^{A2.}$	$21.82 \pm 0.031^{A1.}$

Means with different letters (A, B, C) within the same column are highly significantly different ($P < 0.05$). Means with different numbers (1., 2., 3.) within the same raw are highly significantly different ($P < 0.05$).

In table (4), during cold storage period, APC was gradually increased. It is obvious that APC of control samples prepared without pomegranate peels powder, was remarkably increased progressively over the storage time from ($1.5x10^5 \pm 0.01x10^5$) at zero time and reached ($2.5x10^7 \pm 0.03x10^7$, $5.0x10^7 \pm 0.02x10^7$, and $4.2x10^8 \pm 0.03x10^8$) after 3,6, and 9 days of cold storage, respectively).Progressive reduction in APC over the time of cold storage period has been declared where, APC of samples containing 2g,3g, and 5g of pomegranate peels powder reached $2.3x10^3 \pm 0.10x10^3$, $1.8x10^3 \pm 0.12x10^3$, and $3.9x10^2 \pm 0.13x10^2$ after 9 days of storage.

In table (5), During cold storage period, it is obvious that, total coliform counts for control sample prepared without pomegranate peels powder was increased progressively over time during storage from ($4.6x10^4 \pm 0.10x10^4$) at zero time to ($1.2x10^5 \pm 0.15x10^5$, $2.7x10^5 \pm 0.12x10^5$, and $3.5x10^6 \pm 0.13x10^6$) after 3,6, and 9 days of cold storage, respectively. Other

samples showed progressive decrease over time during cold storage.

In table (6) results revealed that, the initial mould counts at zero time of cold storage ranged from ($2.5x10^3±0.12x10^3$ and $2.1x10^3±0.10x10^3$). During cold storage period, it is obvious that, total mould count for control sample prepared without pomegranate peels powder was increased progressively over time during storage from ($4.5x10^3± 0.12x10^3$) at zero time to ($1.3x10^5±0.02x10^5$, ($2.9x10^6 ±0.20x10^6$), and ($6.7x10^7±0.03x10^7$) after 3,6, and 9 days of cold storage, respectively. Progressive decrease in mould count containing concentrations (2g, 3g, and 5g) of pomegranate over time during cold storage has been recorded.

In table (7) the results revealed that the initial yeast count at zero time of cold storage ranged from ($2.5x10^3±0.30x10^3$ and $1.1x10^3±0.02x10^3$).From the same given results, it could be noted that all samples (at zero time) exhibiting closely or similar yeast counts. During cold storage period, it is obvious that, total yeast count for control sample prepared without pomegranate peels powder was increased progressively over time during storage from ($5.2x10^4±0.10x10^4$)at zero time to ($2.1x10^5±0.01x10^5$, $3.6x10^6±0.02x10^6$, and $5.2x10^6± 0.02x10^6$) after 3,6, and 9 days of cold storage, respectively.

In table (8), Concerning the samples treated with pomegranate (2 g) at zero day the reduction rate on APC, Coliform count, mould count and yeast count were 20%,43.478%,44.444% and 95.192%, respectively. While the samples treated with pomegranate (2 g) at nine day the reduction rate on APC, Coliform count, mould count and yeast count were 99.999%, 99.957%,99.999% and 99.959%, respectively. Regarding to treated samples with pomegranate at zero day the reduction rate on APC, Coliform count, mould count and yeast count were 26.667%,45.652%,53.333% and 97.885% in case of (3 g) and were 26.667%,50.%,60.% and 97.884 in case of (5 g), respectively. Concerning the samples treated with pomegranate at nine day the reduction rate on APC. Coliform count, mould count and yeast count were 99.999%,99.993%, 99.999% and 99.996% in case of (3 g) and were 99.999%,99.998%, 100% and100% in case of (5 g), respectively

Table 4 Statistical analysis of the experimental anti-microbial effect of Pomegranate extract on APC (cfu/g) in the examined samples.

Day	Control	T1(2g)	T2(3g)	T3(5g)
0	$1.5x10^5±0.01x10^{5C1.}$	$1.2x10^5±0.10x10^{5A2.}$	$1.1x10^5±0.10x10^{5A2}$	$1.1x10^5±0.11x10^{5a2.}$
3	$2.5x10^7±0.03x10^{7B1}$	$4.4x10^4±0.10x10^{4B2}$	$1.4x10^4±0.14x10^{4B2}$	$7.2x10^3±1.00x10^{3B3}$
6	$5.0x10^7±0.02x10^{7BC1}$	$6.2x10^3±0.10x10^{3BC2}$	$4.5x10^3±0.13x10^{3BC2}$	$2.3x10^3±0.20x10^{3BC3}$
9	$4.2x10^8±0.03x10^{8A1}$	$2.3x10^3±0.10x10^{3B2}$	$1.8x10^3±0.12x10^{3B2}$	$3.9x10^2±0.13x10^{2C3}$

Means with different letters (A, B, C) within the same column are highly significantly different ($P < 0.05$). Means with different numbers (1., 2., 3.) within the same raw are highly significantly different ($P < 0.05$).

Table 5 Statistical analysis of the experimental anti-microbial effect of Pomegranate extract on coliform count (cfu/g) in the examined samples

Day	Control	T1(2g)	T2(3g)	T3(5g)
0	$4.6x10^4±0.10x10^{4C1}$	$2.6x10^4±0.01x10^{3A2}$	$2.5x10^4±0.13x10^{4A2}$	$2.3x10^4±0.19x10^{4A2}$
3	$1.2x10^5±0.15x10^{5B1}$	$4.5x10^3±0.12x10^{3B2}$	$2.6x10^3±0.12x10^{3B2,3}$	$6.5x10^2±0.13x10^{2B3}$
6	$2.7x10^5±0.12x10^{5B1}$	$2.4x10^3±0.1x10^{3BC2}$	$8.1x10^2±0.14x10^{2BC2,3}$	$2.1x10^2±0.11x10^{2B3}$
9	$3.5x10^6±0.13x10^{6A1}$	$1.5x10^3±0.1x10^{3BC2}$	$2.3x10^2±0.13x10^{2C2,3}$	$7.4x10±0.12x10^{C3}$

Means with different letters (A, B, C) within the same column are highly significantly different ($P < 0.05$). Means with different numbers (1., 2., 3.) within the same raw are highly significantly different ($P < 0.05$).

Table 6 Statistical analysis of the experimental anti-microbial effect of Pomegranate extract on MOULD count (cfu/g) in the examined samples.

Day	Control	T1(2g)	T2(3g)	T3(5g)
0	$4.5x10^3±0.12x10^{3D1}$	$2.5x10^3±0.12x10^{3A1}$	$2.1x10^3±0.10x10^{3A1}$	$1.8x10^3±0.12x10^{3A1}$
3	$1.3x10^5±0.02x10^{5C1}$	$1.4x10^3±0.11x10^{3A2}$	$4.5x10^2±0.11x10^{2B3}$	$1.5x10^2±0.1x10^{2B3}$
6	$2.9x10^6±0.20x10^{6B1}$	$1.2x10^3±0.10x10^{3BC2}$	$1.3x10^2±0.11x10^{2B3}$	$7.4x10±0.11x10^{BC4}$
9	$6.7x10^7±0.03x10^{7A1}$	$5.4x10^2±0.37x10^{2C2}$	$6.4x10±0.31x10^{C2,3}$	$<10^{C3}$

Means with different letters (A, B, C) within the same column are highly significantly different ($P < 0.05$). Means with different numbers (1., 2., 3.) within the same raw are highly significantly different ($P < 0.05$).

Table 7 Statistical analysis of the experimental anti-microbial effect of Pomegranate extract on YEAST count (cfu/g) in the examined samples.

Day	Control	T1(2g)	T2(3g)	T3(5g)
0	$5.2x10^4±0.10x10^{4C1}$	$2.5x10^3±0.30x10^{3A1}$	$1.1x10^3±0.02x10^{3A1}$	$1.1x10^3±0.01x10^{3A1}$
3	$2.1x10^5±0.01x10^{5B1}$	$2.3x10^3±0.2x10^{3A2}$	$9.0x10^2±0.12x10^{2AB2}$	$1.6x10^2±0.1x10^{2B3}$
6	$3.6x10^6±0.02x10^{6A1}$	$2.2x10^3±0.12x10^{3A2}$	$2.7x10^2±0.30x10^{2B3}$	$1.4x10^2±0.1x10^{2B3}$
9	$5.2x10^6±0.02x10^{6A1}$	$2.1x10^3±0.11x10^{3AB2}$	$2.0x10^2±0.10x10^{2B3}$	$<10^{C4}$

Means with different letters (A, B, C) within the same column are highly significantly different ($P < 0.05$). Means with different numbers (1., 2., 3.) within the same raw are highly significantly different ($P < 0.05$).

Table 8 Reduction % of microbial counts after addition of pomegranate.

Group	Day	APC	Coliform count	Mould count	Yeast count
T1 (2 g)	0	20.000	43.478	44.444	95.192
	3	99.824	96.250	98.923	98.905
	6	99.988	99.111	99.958	99.938
	9	99.999	99.957	99.999	99.959
T2 (3 g)	0	26.667	45.652	53.333	97.885
	3	99.946	97.833	99.654	99.571
	6	99.991	99.700	99.995	99.993
	9	99.999	99.993	99.999	99.996
T3 (5 g)	0	26.667	50.000	60.000	97.884
	3	99.971	99.458	99.885	99.924
	6	99.995	99.922	99.996	99.997
	9	99.999	99.998	100.0	100.0

4. DISUCSSION

Pomegranate peels are characterized by an interior network of membranes comprising almost 26–30% of total fruit weight and contain phenolic compounds

The pH values of all samples ranged from 7.39 to 7.30 at zero time and from 6.27 to 6.38 after 12 days of cold storage: indicating a slight decrease ($p < 0.05$) during cold storage. storage.

In table 1, There were no significant differences ($p < 0.05$) in pH values of different prepared sausage samples containing 0, 2, 3, and 5 gm of pomegranate peels powder. During storage period, the pH values of prepared samples were decreased with little significant effect ($p<0.05$) for examined samples containing different concentrations of pomegranate peels powder. On the other contrary, pH values of the control samples which prepared without pomegranate peels powder (0 g) was decreased during storage period.

The decrease of pH values usually due to lactic acid formation and glycogen breakdown. Similarity of these results occurred with (Gebriel *et al.,* 2007); (Devatkal *et al.,* 2010) and (Qin *et al.,* 2013).

By TBARS acid test, we can estimate the extent of lipid oxidation in meat and meat products, Wu *et al*. (2000). The condensation between TBA and MDA, a product of fatty acid oxidation, is the core of TBA test. The test measures the secondary products starting to occur in the reaction mixture after formation of hydroperoxides. This compound is responsible for deterioration of foods as a result of the oxidative rancidity and formation of off-flavors and unpleasant taste, and damage of human tissues, where it may be a cause of cancer, inflammatory disease, mutagenicity, atherosclerosis, etc. Murray *et al. (*1993).

Greene and Cumuze, (1982) mentioned that TBA value may be taken as a good chemical parameter for quality assurance and measuring the extent of the secondary oxidant of edible lipids.

TBA values (expressed as mg malonaldehyde /kg) of examined samples with pomegranate powder were measured during storage at (1-4˚ C) of different samples.

The obtained data in table 2 revealed the positive effect in TBA on samples containing 2,3, and 5g pomegranate powder.

The results indicated that, all examined samples had a closed low TBA values at zero time of storage. During the storage period, TBA values tended to increased and all samples recorded values lower than the critical limit (0.9 mg malonaldhyde/kg) reported by Greene and Cumuze, (1982), except control samples which showed more than critical limit (1.002 mg malonaldhyde/ kg) TBA values after 12 days of storage, whereas the samples T1, T2, and T3 observed the lowest TBA values after 12 days of storage (0.59, 0.53, and 0.49 mg malonaldhyde/kg; respectively).

From the previous results, it can be seen that TBA values of sausage treated by pomegranate peels powder exhibited their beneficial effect on the deterioration reactions happened in samples lipids during storage.

The inhibitory effect of pomegranate peels powder on lipid oxidation might be related to its phenolic constituents and other biochemical compounds. Pomegranate peels might inhibit lipid oxidation by blocking radical chain reaction in the oxidation process (Jia *et al.,* 2012).

Results in table (3) revealed that, the examined samples have a higher increasing rate in TVN content which was 8.52 mg/100 g at zero time of cold storage, and continued to increase to reach 30.48 mg / 100 g after 12 days. On other side, the corresponding value for the sausage sample prepared with pomegranate peels powder (T3) had the lowest TVN content from the beginning of cold storage 8.08 mg / 100 g until the end of cold storage period after 12 days (21.82 mg/ 100 g).

Our results match with Naveena *et al.*, 2008; Devatkal *et al.*, 2010 and El-Gharably and Ashoush (2011) who declared that by decreasing the rate of lipid oxidation of the samples by pomegranate peels, we can improve the storage stability of sausage at cold storage. Break-down of nitrogenous substances by microbial activity lead to increase of TVN. Madkour *et al.,* (2000) and Gibriel *et al.;* (2007) had the similar results.

Positive effect of addition of pomegranate peels powder may be due to inhibition of microorganism and preventing of protein breakdown resulting in volatile nitrogen compounds. These results declared especially by high concentrations of pomegranate powder.

The antimicrobial mechanisms of phenolic compounds involve the reaction of phenolics with microbial cell membrane proteins and/or protein sulfhydryl groups that yield bacterial death due to membrane protein precipitation and inhibition of enzymes such as glycosyltransferases (Naz et al., 2007).

The obtained data in table (4) revealed that, the prepared samples, which contained different ratios of pomegranate peels powder (2g,3g, and 5g) showed a progressive reduction in APC over the time of cold storage period;

These results could be due to the antimicrobial effect of pomegranate peels powder, especially when the concentration of pomegranate peels powder was increased. This result agrees with Agourram *et al.* (2013).

The coliform group of bacteria includes all the aerobic and facultative anaerobic, gram negative and non-sporulating bacilli that produce acid and gas during utilization of lactose. The observed results in table (5) on total coliform count ($\times 10^3$ CFU/g) are in agreeing with El-Nashi et al. (2015) and Agourram *et al.,* (2013), who recorded the inhibitory effect of pomegranate powder against bacteria.

Progressive decrease in mould and yeast count with concentrations of (2g, 3g, and 5g) of pomegranate over time during cold storage in comparison to control samples contained no pomegranate. Similarity of this result come with El-Nashi et al. (2015) who discussed the antifungal action of pomegranate peel powder.

The peel of pomegranate contains antifungal and antibacterial compounds Rosas-Burgos et al. (2017). Ppomegranate peel powder contain phenolic compounds (Li *et al*., 2006), that have antimicrobial properties (Cai *et al.*, 2004). It is very important to consider the antimicrobial effect of pomegranate peel powder.

The information given by the achieved results proved that treatment of sausage by addition of pomegranate (5 g) inhibited the bacterial count, Coliform count, mould and yeast count, and extends the shelf-life of refrigerated treated sausage. It can be concluded that pomegranate peel powders these plants have the potential to be used in food as flavouring and natural preservatives to control food spoilage.

5. CONCULOSION

Concentrations of (2, 3 and 5 g) of pomegranate peels powder exhibited a natural preservative in producing good quality sausage.

pH, TBA, and TVN as quality criteria are presented and evaluated during storage at (1-4˚C) for 12 days and has been founded to be affected substantially by various

concentrations of Pomegranate peel powder. Microbiological quality criteria (APC, Coliform, mould and yeast) of examined samples have been discussed and evaluated during storage at (1-4˚C) for 9 days and were decreased in the pomegranate treated group. So, pomegranate peel powder exhibit antimicrobial activity (APC, Coliforms, mould and yeast counts) and could reduce pH, TBA, and TVN in the examined samples

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