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IMPACT OF POMEGRANATE PEEL EXTRACT ،NATAMYCIN AND ACETIC ACID ON THE QUALITY AND SAFETY OF GROUND BEEF

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ABSTRACT

Efforts are ongoing to improve meat additives to increase the safety of ground meat. Hence, herbal products are often used as natural substitutes for preserving food. Therefore, the main goal of this study was to assess how pomegranate peel extract, natamycin, acetic acid, and a combination of their mixtures effectively improve the quality and shelflife of ground meat. The study examined five groups of ground meat, one being a control group, and the other groups treated with different additives. Over a two-week refrigeration period, the quality of the treated groups was evaluated, and the quality of ground meat was demonstrated for 12 days of storage. Furthermore, the stability results of pH, total volatile nitrogen (TVN), and thiobarbituric acid (TBARS) indicated the potent antioxidant properties of the additives used. Furthermore, the groups that received treatment exhibited a notable reduction in microbial growth compared to the control group. Hence, it can be concluded that pomegranate peel extract, natamycin, acetic acid, and a combination of their mixtures could be recommended to improve the shelf life and safety of ground meat.

Keywords: Ground meat, natamycin, pomegranate extract, acetic acid

INTRODUCTION

Ground meat has been recognized as an important food item deserving more attention. As a result, the safety of meat is a significant issue for consumers, food industries, government agencies, public health professionals, researchers, and the general public at different levels; local, national, and international. Nevertheless, it is widely known that ground meat has been recognized as the primary element facilitating the proliferation of different spoilage and dangerous bacteria, leading to a decline in the quality of the product (Augustyńska-Prejsnar *et al.*, 2018). Moreover, the decline in quality caused by lipid and protein oxidation during meat mincing results in changes in nutritional and sensory attributes such as flavor, texture, and color (Zareian *et al.*, 2019 and Fourati *et al.*, 2020).

New studies showed that synthetic antioxidants are used in the food industry to inhibit oxidative spoilage, and exhibit harmful properties (Smaoui *et al.*, 2019). Because of this, recent scientific research has sought out various antioxidants from natural sources (Smaoui *et al.*, 2016; Bouarab-Chibane *et al.*, 2017) to utilize them as effective preservatives in different meat

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products (Prommachart *et al.,* 2020; Alirezalu *et al.,* 2020).

The growing interest in the preservation of meat products has become a popular category in the meat industry. Precooked meats, however, are susceptible to microbial contamination, leading to food born illnesses. Thus, the control of microbial contamination has been a major concern in the meat industry. Additionally, lipid oxidation and color changes are also important factors influencing the quality and acceptability of meat and meat products (Ahn *et al.,* 2004).

Salmonella and *Shigella* remain the primary reasons for acute enteric infections and diarrhoea among bacterial pathogens. The typical way these pathogens get into the body is through consuming contaminated food and beverages. The worry regarding these bacterial pathogens leading to diarrheal illnesses. Kansakar *et al.* (2011) have outlined approaches for managing *Salmonella* and *Shigella* species.

The arils, peels, and rinds of pomegranate fruit are famous for their strong antioxidants and wide range of effects (Smaoui *et al.,* 2019), especially the potential of pomegranate peel in beef has been studied (Turgut *et al.,* 2016; Emam-Djomeh *et al.,* 2015; Forgione *et al.,* 2024).

Natamycin (NT) is derived from the fermentation of Streptomyces spp. and is a natural antifungal and antibacterial compound. Natamycin (NT) is successful in fighting fungus infections, even when they are not susceptible to antifungal medications. NT is thought to primarily prevent the growth of fungi by binding to ergosterol, a key element found in fungal membranes (Aparicio *et al.,* 2016).

Acetic acid is commonly used as a preservative to prevent food from spoiling and lengthen the shelf life of perishable items by preventing bacterial growth in meat. According to the Generally Recognized as Safe (GRAS) standard, acetic acid and its salts are deemed safe for consumption, permitting unrestricted use (Ricke, 2003). Using the provided information, the study aimed to evaluate how different additives affect the quality and storage of ground beef meat for a longer shelf-life at 4°C for 12 days.

MATERIAL AND METHODS

1. Collection of samples:

Nine kilograms of freshly ground beef was purchased directly from the butchers' shops in El-Menoufia Governorate immediately after being slaughtered. Afterwards, the sample was put into fresh plastic bags and taken to the lab in chilled containers with proper hygiene for further study.

2. Experimental Design:

The study consisted of 5 groups and 6 different storage periods (0, 3, 6, 9, 12, and 15 days). The experiment was repeated three times.

2.1. Treatment of samples:

The ground beef was divided into 5 portions, with each portion weighing 300 grams. Group 1 departed without receiving any treatment, acting as the control group. The remaining 4 groups received various substances for treatment: Group 2 was provided with pomegranate peel extract, Group 3 was administered natamycin, Group 4 was immersed in 2% acetic acid, and Group 5 was subjected to a combination of their mixtures. Following this, every sample was transferred to fresh polyethylene bags and stored in a 4°C setting for up to 12 days. Every 3 days during storage, beef samples were gathered to assess their physico-chemical, microbiological, and sensory attributes.

2.2. Experimental material:

Pomegranate peel extract, Natamycin, and acetic acid 2 % were obtained from Botany Department, National Research Center, Giza, Egypt.

3. Microbiological examination:

3.1. Preparation of samples (ISO 4833-1, 2013):

Ten grams of each prepared (treated) sample were homogenized with sterile 90 ml of 0.1% peptone water (HIMEDIA) using a stomacher (Lab Blender 400, Seward lab. Model No. AB 6021) for 1 minute. One ml of the clear homogenate was mixed carefully with 9 ml of buffered peptone water 0.1%, and then decimal serial dilutions (1:10) were prepared (ISO, 1999). Microbial levels were measured by tallying and documenting colonies in colony forming units per gram (cfu) of meat sample, figured out using the formula: cfu = dilution plated x colonies counted/volume plated. The average colony-forming units were also shown as these values and converted to logarithmic form with a base of 10 (log10cfu).

3.2. Determination of total aerobic plate count (APC):

As stated by ICMSF (2006), a sterile Petri dish received one ml of a prepared dilution in sterile conditions, followed by the addition of approximately 15 ml of sterile standard plate count agar at 45°C, which was mixed thoroughly in a horizontal manner. Following the solidification process, both the plates containing inoculation and the control plate were flipped and placed in an incubator at 37°C for 24 hours. The total number of colonies per gram was tallied and documented based on the plates.

3.3. Determination of *Staphylococcus* count (ISO 6888, 2018):

Precisely, 10 µl from every serial dilution that was prepared earlier was evenly distributed on two separate plates of Baird-Parker agar medium (Merck, Germany) supplied with egg yolk and a sterile curved glass spreader. The plates with and without inoculation were left to incubate for 48 hours at 37ºC. *S. aureus* colonies appeared as glossy black with a thin white border and a clear zone around them. These colonies were counted, and the overall *S. aureus* count per gram of sample was determined.

3.4. Determination of total yeasts and molds count (ISO 4833-1,2013):

Bailey and Scott (1978) introduced one ml from the serial dilutions onto plates containing Sabouraud's medium (with 0.05 mg of chloramphenicol per ml). The plates with the inoculation were set vertically and incubated at 25°C for 5 days. The dishes were first checked after 3 days of incubation to assess the yeast growth level, and in case it was substantial, a count was noted and documented on the fifth day. The number of yeast and mold colonies was tallied. The levels of yeast and mold per gram of the sample were computed and noted.

3.5. Determination of total anaerobic count:

A 0.1 ml aliquots from each group were subsequently plated onto dishes containing 5% sheep blood agar (SBA) and fortified clostridium medium (RCM) from Oxoid, USA. As previously mentioned, these plates were cultured in an anaerobic environment at 37°C for 24-36 hours. The presence of *C. perfringens* was suspected, because of double-zoned β-hemolysis colonies on SBA. The plates were counted, and the CFU per gram was recorded. The colonies were chosen and confirmed.

3.6. Isolation and identification of *Shigella* and *Salmonella (*ISO 16649-2:2001):

One gram of specimens was added to peptone broth and incubated at 37°C for 24 hours to separate *Shigella*. The specimens were later transferred to MacConkey agar and Xylose Lysine Deoxycholate agar (oxoid, P00164) for another day of aerobic incubation at 37°C. To isolate *Salmonella*, the specimens were also placed on Selenite F broth pre-enrichment broth (Mast Diagnostics, UK) and kept at 37°C for 24 hours to improve the detection of *Salmonella* species. After incubating Selenite F broth, samples were plated on MAC and XLD plates and then kept at 37°C for 24 hours. Detection of *Salmonella* and *Shigella* growth was accomplished through the examination of their distinct characteristics on MAC (smooth, colorless colonies with possible black centers that do

not ferment lactose) and XLD agar (colonies that are red with black centers).

4. Physicochemical analyses:

1. Measurement of Hydrogen ion concentration (pH): As stated by ISO (1999), a consistent blend of 10 grams of the specimen and 90 milliliters of distilled water was used for pH analysis with a digital pH-meter (HM-5S; TOA Electric Industrial Co. Ltd., Tokyo, Japan).
2. Measurement of total volatile basic nitrogen (TVBN): A 10g portion was blended with 100 ml of distilled water, transferred to a distillation flask with another 100 ml of distilled water, and then supplemented with 2g of magnesium oxide and an antifoaming agent. The small Kjeldahl micro distillation apparatus was used to distill the mixture. The distillate was gathered for 25 minutes in a solution comprising 25 ml of 4% boric acid and five drops of Tashero indicator. The amount of volatile basic nitrogen in the sample was determined as mg VBN/100g meat using a titration method with (0.1 M) HCl, detailed by Harold *et al.* (1981).
3. Measurement of thiobarbituric acid reactive substances (TBARs): The Vyncke (1970) technique was utilized to conduct the TBA assay. Mix 20 grams of the sample with 100 milliliters of a 7.5% trichloroacetic acid solution, and blend for 2 minutes for homogenization. The combination underwent filtration. Following filtration, 5 ml of the substance was combined with 5 ml of TBA reagent (0.02M TBA) in a test tube equipped with a screw cap. The test tubes were immersed in a water bath for 40 minutes, then the absorbance was assessed using a spectrophotometer at a wavelength of 538 nm. The value of TBARs was determined by measuring the concentration of malonaldehyde (MA) in milligrams per kilogram of meat (mg MA/kg meat).

This is the calculation for the concentration of malonaldehyde: 0.016 plus 2.872 times the absorbance in mg/kg.

Reduction percentage:

$$\text{Reduction \%} = \frac{\text{initial load} - \text{new count}}{\text{initial load}} \times 100$$

*Was calculated according to Rosowsky. (1995).

5. Sensory Evaluation:

The research was conducted based on Meilgaard *et al.* (1999) and (Patsias *et al.* (2006). Sensory evaluation of ground beef meat samples (control and treated ones) was assessed by five individuals 30 to 35 years of age, who are working in the Animal Health Research Institute. Every individual had to evaluate the hue, consistency (firmness or moisture), and taste (tartness or sugariness) levels. Participants received servings from every treatment at 75°C for 25 minutes, served in separate covered porcelain dishes in a quiet setting. The criteria used as the basis of the organoleptic assessment and the rating system were provided as scores (Excellent:9, Very very good: 8, Very good: 7, Good: 6, AccepTable 5, unacceptable:4, spoilage).

6. Statistical Analysis:

Statistical analysis was conducted using one-way ANOVA with Tukey's post hoc test in the SPSS program for Windows (Version 16) (SPSS Inc. Chicago, IL and USA) with a significance level of $p<0.05$. Microbiological data was converted into a logarithmic form for the number of colony-forming units (cfu/g) and then analyzed using analysis of variance (ANOVA). Means and standard error were determined.

RESULT

Table 1: Statistical evaluation of the impact of pomegranate peel extract, natamycin, 2% acetic acid, and a combination of their mixtures on total aerobic count.

Groups/storage period	Control	Pomegranate peel extract	Natamycin	Acetic acid 2%	A combination of their mixtures	P value
1st day	6.07±0.05	6.03±0.04	6±0.05	6.11±0.04	6.04±0.03	0.231
3rd day	7.25±0.11[a]	4.85±0.09[c]	5±0.08[b]	4.94±0.05[b]	4.81±0.04[c]	0.001
6th day	S	4.69±0.11[c]	4.94±0.07a	4.86±0.09b	4.63±0.09[c]	0.000
9th day	S	3.47±0.07[c]	3.90±0.03a	3.74±0.05b	3.39±0.04[c]	0.000
12th day	S	3.34±0.05a	**S**	S	2.10±0.07b	0.001
15th day	S	S	S	S	S	-

In the same row, varying letters show a noTable difference, with a significance level of $p < 0.05$, where S signifies spoilage.

Table 2: Statistical examination of how pomegranate peel extract, natamycin powder, acetic acid, and a combination of their mixtures impact the overall Staphylococcal count.

Groups/storage period	Control	Pomegranate peel extract	Natamycin	acetic acid 2%	A combination of their mixtures	P value
1st day	3.70±0.02	3.69±0.03	3.68±0.05	3.69±0.05	3.60±0.04	0.091
3rd day	4.91±0.03[a]	3.32±0.03[c]	3.84±0.09[b]	3.39±0.07[c]	3.11±0.05[d]	0.001
6th day	S	2.39±0.04[b]	3.09±0.05[a]	2.37±0.04[b]	2.04±0.09[c]	0.000
9th day	S	2.11±0.05[b]	2.37±0.11[a]	2.22±0.09[b]	1.47±0.08[c]	0.000
12th day	S	1.56±0.03[a]	S	S	1.22±0.07[b]	0.000
15th day	S	S	S	S	S	

Significant disparities are indicated when different letters are present in the same row; significance is demonstrated with a p-value of less than 0.05. S = spoilage

Table 3: Statistical data for the impact of pomegranate peel extract, natamycin powder, 2% acetic acid, and a combination of their mixtures on total anaerobic count.

Groups/storage period	Control	Pomegranate peel extract	Natamycin	Acetic acid 2%	A combination of their mixtures	P value
1st day	4.46±0.11	4.40±0.12	4.39±0.10	4.42±0.11	4.45±0.09	0.341
3rd day	5.67±0.03[a]	3.52±0.05[c]	4.09±0.03[a]	3.51±0.04[c]	3.21±0.09[d]	0.001
6th day	S	2.95±0.03 [c]	3.86±0.04[a]	3.40±0.05[b]	2.90±0.09[c]	0.000
9th day	S	2.80±0.05[a]	S	S	2.65±0.03[b]	0.001
12th day	S	S	S	S	1.11±0.04[a]	ND
15th day	S	S	S	S	S	ND

When different letters are in the same row, it indicates a significant difference. A p-value of <0.05 is considered significant. ND stands for not detected and S denotes spoilage.

Table 4: Statistical evaluation of the impact of pomegranate peel extract, natamycin powder, acetic acid, and a combination of their mixtures on the total count of mold and yeast.

Groups/storage period	Control	Pomegranate peel extract	Acetic acid 2%	Natamycin	A combination of their mixtures	P value
1st day	5.74±0.04	5.75±0.05	5.65±0.09	5.71±0.03	5.70±0.05	0.341
3rd day	6.85±0.06[a]	4.84±0.03[b]	4.61±0.04[c]	4.37±0.04d	4.30±0.06[d]	0.001
6th day	S	4.34±0.03[a]	4.21±0.09[b]	4.17±0.03 c	4.10±0.05[c]	0.000
9th day	S	3.84±0.03[a]	3.61±0.07[b]	3.37±0.04c	3.30±0.06 [c]	0.001
12th day	S	**S**	S	3.15±0.05	2.11±0.03	0.092
15th day	S	S	S	S	S	ND

Significant difference is indicated by means in the same row with distinct letters; a p-value <0.05 is considered significant. ND stands for not detected, while S signifies spoilage.

Table 5: Assessment of pomegranate peel extract, natamycin, acetic acid, and a combination of their mixtures on samples through sensory evaluation.

Group	Days	Color	Odor	Appearance	Overall acceptability	consistency	Grade
Control	Zero	8	8	8	9	8	8
	3rd	7	7	8	8	7	7
	6th	5	6	5	6	5	5
	9th	4	4	4	4	4	4
	12th	4	4	4	4	4	4
	15th	4	4	4	4	4	4
pomegranate peel extract	Zero	8	9	8	8	9	8
	3rd	7	8	8	8	7	8
	6th	7	7	7	7	6	7
	9th	6	6	7	7	6	6
	12th	6	6	6	6	6	6
	15th	4	4	4	5	4	4
Natamycin	Zero	8	9	8	8	9	8
	3rd	8	7	8	7	8	7
	6th	7	7	7	8	8	7
	9th	7	6	6	7	6	6
	12th	5	6	5	5	5	5
	15th	4	4	4	4	4	4
Acetic acid	Zero	8	9	8	8	9	8
	3rd	8	7	8	8	7	7
	6th	7	7	7	8	7	7
	9th	7	6	6	6	7	6
	12th	5	6	6	5	5	5
	15th	4	5	4	5	4	4
A combination of the mixtures	Zero	8	9	8	9	8	8
	3rd	7	8	8	8	8	8
	6th	7	8	7	7	7	7
	9th	7	6	7	6	7	7
	12th	6	6	5	6	5	6
	15th	4	5	4	4	4	4

9=excellent,8=very good ,7=good,6=accepTable ,5=unaccepTable, 4=spoilage

Table 6: Reduction ratio of pomegranate peel extract, Natamycin, acetic acid and a combination of their mixtures on the microbial load %

Meat product samples	Total colony count	Total Staphylococcal count	Total mold and yeast count	Total anerobic count
Pomegranate peel extract	44.61	57.72	33.21	36.36
Natamycin	35	35.59	44.83	12.07
Acetic acid 2%	38.7	39.83	36.10	23.07
A combination of their mixtures	48.67	66.11	45.43	52.58

Table 7: Effect of varying levels of (pomegranate peel extract, natamycin, acetic acid 2% and a combination of their mixtures) on pH values of control and treated samples.

Groups	Mean PH Zero-day	Mean PH 3rd day	Mean PH 6th day	Mean PH 9th day	Mean PH 12th day	Mean PH 15th day
Control	5.73	6.64	6.89	6.95	S	S
pomegranate peel extract	5.65	5.70	5.70	5.80	5.95	S
Natamycin	5.62	6.02	6.01	6.04	6.30	S
Acetic acid 2%	5.61	6.01	6.00	5.90	6.20	S
A combination of their mixtures	5.60	5.93	5.90	5.88	5.8	S

Table 8: Effect of varying levels of (pomegranate peel extract, natamycin, acetic acid 2%, and a combination of their mixtures) on TVN values of control and treated samples.

Groups	Mean TVN Zero-day	Mean TVN 3rd day	Mean TVN 6th day	Mean TVN 9th day	Mean TVN 12th day	Mean TVN 15th day
Control	6	14	15	18.21	S	S
Pomegranate peel extract	5.6	6.5	12.3	13.10	14	S
Natamycin	5.7	6.5	9.60	17.11	S	S
Acetic acid	5.5	8.2	16.4	16.20	S	S
A combination of their mixtures	5.2	6.1	10.2	11.1	12	S

Table 9: Effect of varying levels of (pomegranate peel extract, natamycin, 2% acetic acid, and a combination of their mixtures) on TBARS values in control and treated samples.

Groups	Mean TBARS Zero-day	Mean TBARS 3rd day	Mean TBARS 6th day	Mean TBARS 9th day	Mean TBARS 12th day	Mean TBARS 15th day
Control	0.15	0.35	0.9	1.7	S	S
pomegranate peel extract	0.14	0.5	0.6	0.7	1.1	S
Natamycin	0.17	0.4	0.5	0.6	1.8	S
Acetic acid	0.19	0.6	0.7	0.7	1.9	S
A combination of their mixtures	0.11	0.3	0.5	0.6	0.9	S

DISCUSSION

In the present study, the total aerobic plate count (APC) (Table 1) on the day zero were 6.07±0.05, 6.03±0.04, 6±0.05, 6.11±0.04 and 6.04±0.03 for the control, G2, G3, G4 and G5, respectively. On the day 12, the control group spoiled. In the groups treated with pomegranate extract (group 2) and the combination group of the their mixtures, the total aerobic plate count were decreased to 3.34±0.05a and 2.10±0.07b, respectively, indicating a shelf life of the 12 day during refrigerated storage at 4ºC. This result agreed with Sharma et al. (2020), who reported that the TCC decreased with adding pomegranate extract to meat and Ibrahim-Ghada (2006), who found that the APC decreased to $1.30x10^3$ for the same treatments.

APC reduction was observed in all four treatments showing strong effectiveness (Table 6). The colony count was 44.61 % for pomegranate peel extract and 48.67 % for a combination of their mixtures. This result agreed with Agourram et al. (2013), who reported that addition of pomegranate (5 g) inhibited the bacterial count, and extends the shelf-life of refrigerated treated meat product.

The effect of pomegranate extract, natamycin powder ,acetic acid and combination of the their mixtures on total staphylococcus count was significant. The total staphylococcus count on the day zero were 3.70±0.02, 3.69±0.03, 3.68±0.05, 3.69±0.05 and 3.60±0.04 for the control, G2, G3, G4 and G5, respectively (Table 2). On the 12^{th} day, the control group was spoiled but the group treated with pomegranate extract (group 2) and the treated group with a mixture of the three materials, the Staphylococcus count decreased to 1.56±0.03a and 1.22±0.07b, respectively. This results agreed with Tayel et al. (2012), who reported that pomegranate extract was evaluated in several studies for the ability to maintain food quality. A pomegranate peel extract at 250 µg/mL was most effective at inhibiting antibiotic resistant strains of *Staphylococcus aureus* and improved sensory evaluations of quality. In another study, pomegranate peel reduced protein oxidation, inhibited microbial growth, and increased sensory acceptability for up to 12 days of refrigerated storage at 4°C (Vaithiyanathan et al.,2012).

The effectiveness of group 5 was higher than the other treatments, as evidenced by significant reductions in staphylococcal count (Table 6) with the four treatments. Pomegranate peel extract and a combination of their mixtures resulted in decreases of 57.72%, and 66.11% in staphylococcal count, respectively. This results agreed with Cai et al. (2004), who reported that natural preservatives like pomegranate extract control food spoilage and reduce Staphylococcal count.

The effect of pomegranate extract, natamycin powder, acetic acid and combination of the their mixtures on total anerobic count was significant. The total anerobic count on the day zero were 4.46±0.11, 4.40±0.12, 4.39±0.10, 4.42±0.11 and 4.45±0.09 for the control, G2, G3, G4 and G5, respectively (Table 3). On the 12^{th} day, the control group spoiled but the group treated with combination of the their mixtures the total anerobic count were decreased 1.11±0.04. Also, anaerobic bacterial count were reduced by 52.58%. This results agreed with Jaworska et al. (2021), who reported that herbal extract revealed an inhibitory effect on the anaerobe microflora of meat products after 18 days of cold storage.

The effect of pomegranate extract, natamycin powder, acetic acid and a combination of the their mixtures on mold

and yeast count was significant. the mold and yeast count on the day zero were 5.74±0.04, 5.75±0.05, 5.65±0.09, 5.71±0.03 and 5.70±0.05 for the control, G2, G3, G4 and G5, respectively (Table 4). On the 12th day, the control group spoiled but the natamycin group (G4) and group treated with combination of the three materials (G5), the total mold and yeast count were decreased in the 12th day 3.15±0.05, 2.11±0.03, respectively. This result is significant and agreed with Yuan et al. (2022), who reported that the yeast and mold count decreased by natamycin that showed high antifungal properties.

The decrease in overall mold and yeast levels was significant in the four treated groups, with the natamycin, and the combination of the three materials resulting in reduction of mold and yeast count by 44.83% and 45.43%, respectively. These results are important and confirms the research conducted by Rosas-Burgos et al. (2017) that showed natamycin's ability to reduce yeast and mold count because of its powerful antifungal properties.

Sensory Evaluation. During the storage time, all the sensory characteristics were significantly ($p<0.05$) decreased, whereas the treated samples exposed exceptional stability until 12 days. The overall acceptability of minced beef meat in the treated groups were acceptable up to 12 days ($p<0.05$) but unacceptable for untreated samples from day 6 ($p<0.05$) (Table 6). In another study, pomegranate peel reduced protein oxidation inhibited microbial growth, and enhanced sensory acceptability for 12 days when kept in a fridge at 4°C (Vaithiyanathan *et al.,* 2012). A dissimilar trend has been reported by Hawashin *et al.* (2016), Al-Juhaimi *et al.* (2020), and Muthukumar *et al.* (2020) who found an insignificant decrease or changes in sensory attributes.

pH values of control and treated groups vary considerably during refrigerated storage. There was a decrease in pH of minced meat during initial days of refrigeration. Subsequently, pH increased on the 12th day of storage. Lactic acid bacteria multiply in the beginning, which leads to disintegration of sugar into acids. Subsequently, bacterial deamination of proteins occurs which raises the pH of the product. In the present study, the control group were 5.73, 6.64, 6.89, 6.95 and decomposed through the zero day , 3rd day, 6th day, 9th day and 12th day but the treated groups the pH decreased and the shelf life of the minced meat increased (Table 7). of These results were similarly reported by Gebriel et al. (2007); Devatkal et al. (2010) and Qin et al. (2013), where the decrease of pH values usually due to lactic acid formation and glycogen breakdown.

Meat spoilage is often evaluated by measuring total volatile basic nitrogen using the traditional chemical method. EOS (2008) states that meat could turn to bad if the overall volatile basic nitrogen surpasses 20 mg N/100g, which is the highest advised threshold. Based on Table (8), control samples had initial TVBN values of 6, 14, 15, and 18 on day zero. The values consistently rose and hit 21 on day 12, signifying that spoilage started for the control samples at this time. Agunbiade *et al.* (2010) also discovered that fresh beef had a TVBN value of 12.6±0.1mg N/100g when stored at 1-2°C for zero days. Our research also found a notable drop in TVBN levels ($P<0.05$) in all treated group samples, similar to the findings of Sharma *et al.* (2020).

The TBARS value in both the control and treated groups showed a significant increase throughout the refrigerated storage period. (Table 9) Nevertheless, the TBARS values in the treated groups were notably lower than the control group throughout the entire storage period. This outcome was consistent with El-Gharably and Ashoush. (2011), who claimed that bioactive substances such as phenols and flavonoids

found in pomegranate peel extract had antioxidant properties and prevented lipid oxidation in the samples, leading to a reduced increase in TBARS values in those treatments.

A previous study reported that TBARS values of meat product treated by pomegranate peels exhibited their beneficial effect on the deterioration reactions happened in samples lipids during storage (Jia *et al.*, 2012). The inhibitory effect of pomegranate peels 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 .

Salmonella and *Shigella* species are respectively the most important pathogens affecting human health. In the present study, both microorganisms were not detected. These results underscore the importance of maintaining a vigilant surveillance program and enhancing infection control measures in order to decrease infection rates. This results agreed with McGuinness. (2009), who reported that no *Salmonella* was found on fresh meat.

It can be concluded that pomegranate peel extract and these additives have the preservatives potential in food to control food spoilage.

Conclusions

The minced beef meat susceptible to spoilage and pathogenic bacteria ,In this regard, we demonstrated the big effect on microbiological, physico-chemical, and sensory analyses in minced beef meat carried by the pomegranate peel extract, natamycin, acetic acid, and a combination of their mixtures Hence, it can be concluded that the combination of their mixtures improve the shelf life and safety of ground meat.Also could be effectively as a promising tool and secured method, used soon in meat products preservation

Conflict of interest:

The authors declare that they have no conflict of interest.

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تأثير مستخلص قشر الرمان والنتاميسين وحمض الاسيتيك على جودة وسلامة لحم البقر المفروم

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لا تزال الجهود مستمرة لتحسين إضافات اللحوم لزيادة سلامة اللحوم والموافقة عليها. ومن ثم، غالبا ما تستخدم المنتجات العشبية كبدائل طبيعية لحفظ المواد الغذائية، والمعروفة باسم "الأطعمة الخضراء". ولذلك، كان الهدف الرئيسي من هذه الدراسة هو تقييم مدى فعالية مستخلص قشر الرمان، والناتاميسين، وحمض الخليك، ومزيج من هذه المركبات في تحسين جودة وطول عمر اللحم المفروم. تناولت الدراسة خمس مجموعات من اللحم المفروم، إحداها مجموعة ضابطة. على مدى فترة التبريد لمدة أسبوعين، تم تقييم نوعية المجموعات المعالجة. وأظهرت النتائج وجود تحسن كبير **($P \geq 0.05$)** بشكل عام في مجموعات الاضافات محل الدراسة, وخاصة في المجموعة التي أعطيت مزيجًا من الثلاث مواد، حيث أظهروا استمرارية الجودة لمدة تصل إلى 12 يومًا من التخزين. علاوة على ذلك، أشارت نتائج ثبات الرقم الهيدروجيني والنيتروجين المتطاير الكلي **(TVN)** وحمض الثيوباربيتوريك **(TBARS)** إلى خصائص مضادات الأكسدة القوية للمواد المضافة المستخدمة. علاوة على ذلك، أظهرت المجموعات التي تلقت المعاملات انخفاضًا ملحوظًا في نمو الميكروبات مقارنة بالمجموعة الضابطة. ومن ثم، يمكن استنتاج أنه يمكن التوصية بمزيج من مستخلص قشر الرمان، والناتاميسين، وحمض الأسيتيك، وخليط من المركبات الثلاثة لتحسين مدة صلاحية اللحوم المفرومة وسلامتها.