



Application of pomegranate pomace as a natural antibacterial and antioxidant preservative in beef

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Abstract

Although various meat and meat products may include various foodborne pathogens, meat is still an excellent source of nutrients compared to other foods. Due to bioactive materials, numerous plants and their extracts, including pomegranate, have been reported to possess antibacterial and antioxidant effects. Sixty fresh beef samples, bought from the local stores Karkh/ Baghdad, were properly divided into three similar groups; each group included 20 samples. The first group served as a control group, and it was soaked in distilled water. The second group was soaked in a 20g/L treatment dose of pomegranate pomace solution and was referred to as the treated group (T1). The third group was administered as 40g/L pomegranate pomace solution (T2). The results showed that the pomegranate pomace solution (T1 and T2) exhibited antimicrobial activity on the total bacterial count, lipid oxidation, and pH values. The current findings demonstrated that beef immersed into both concentrations of pomegranate pomace was more resistant to elevation in bacterial counting plus lower levels of peroxidation, measured using Thiobarbituric acid reactive substances plus fewer pH values compared to the control, as it was preserved for more days. The pomegranate pomace solutions might be utilized as natural preservatives of meat products, even at low concentrations.

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Introduction

Meat is an excellent source of proteins, fats, minerals, vitamins, and other essential components; as a result, it is an important aspect of the human diet (1). Despite emerging dietary patterns that advocate for a reduction and/or substitution of beef in dietary, the world's meat consumption is still rising (2). 360 million of tons were consumed annually, a rise of 58% over the preceding 20 years. Population growth, which accounted for 54% of this growth, and rising demands per person, which was influenced by shifts in customer dietary preferences and wealth, accounted for the remaining 26% of this growth (3). The use of cold chain logistics on a global scale, thermal treatments (such as ultra-chilling, sealed cooling, compression freezing or heating), and advancements in packaging (such as modified

atmospheres, vacuums, and novel materials) have all led to the expansion of beef preservation (4). Moreover, it has been applied to boost the hygiene and texture of meat and meat products to add preservatives (5). Oxidized meat is linked to its higher peptide, lipid, and carbohydrate content (6), that also causes the creation of chemical compounds with cytotoxic, mutagenic, and oxidative effects on bodily tissues, causing cancer, inflammation, and other diseases (7). Thus, after microbiological deterioration, lipid degradation in meat is among the most significant causes of meat rotting (8).

On the other hand, because of the availability of active substances, which are primarily attributed to a wide range of phenolic particles that are principally related but differ in their conformations and amounts depending on the specific source, many plants and their extracts have been confirmed to comprise antibacterial and antioxidant activities (9). One

of these plants, the pomegranate, has seen a rise in consumption in recent years due to its high health advantages and vast production of byproducts that are typically wasted or used insufficiently. However, a surprising amount of active ingredients with potential functional capabilities can be found in these substances (10). Additionally, research had been done examining the utilization of different parts of pomegranate plant as an antioxidant in animal meat products (11).

This study aimed to investigate the effect of different concentrations of pomegranate pomace as natural food preservatives, the antibacterial influences on beef at various time-points, lipid oxidation values from different treatments and measuring the pH changes of beef at different levels to enhance the safety and quality of meat.

Materials and methods

Ethical approve

The study was reviewed and approved by the scientific committee of the Department of Veterinary Public Health in its season held on September 5th 2021, and college council session of the College of Veterinary Medicine, University of Baghdad (No. 4434 P. G. 1-12-2021).

Preparation of raw beef

Sixty of fresh beef samples were purchased from the local market; the meat sample was divided randomly into 60 samples (each sample 100 gram); three groups each 20 samples. The first group was immersed with distilled water and mentioned as a control group, and the second group was immersed with 20g/L of pomegranate pomace solution and mentioned as a treated group (T1). The third group was immersed with 40g/L of pomegranate pomace solution and mentioned as treated group (T2), and then each sample was stored on Polyethylene pages and kept in a refrigerator at 4°C. Samples were harvested at zero, 1, 5, 7, 10, and 15 days to evaluate the following parameters total bacterial count, lipid oxidation changes, and pH values.

Preparation of pomegranate pomace powder (PP)

Fresh pomegranate was obtained from the local fruit market; pomegranate fruits that were mature and healthy were washed, sliced, and peeled by hand. The seeds were manually separated from the pulp. The seeds were blended by blender, the juice was then filtered via a filter paper, and only the pomace was taken and dried in the electrical oven at 40°C for 90 minutes. After that the pomace was grounded by a grinder to form pomegranate pomace powder (10).

Preparation of preservative solutions

The first solution T1 was prepared by dissolving 20 gram of pomegranate pomace powder into one liter of distilled water and then left at room temperature. Then the solution was filtered by Whatman paper. The same steps were

followed to prepare the second solution T2, but 40 g of pomegranate pomace powder was dissolved instead of 20 g of the powder; the exposure time of these groups was 30 minutes (11).

Determination of total bacterial count

The beef sample 25 g was aseptically placed into a sterile stomacher bag and diluted with 225 mL of 1.5% sterile peptone water to evaluate the total bacterial count for each sample. The sample was then homogenized in a stomacher for 2 minutes before being divided into 10-fold serial dilutions using 1.5% sterile peptone water; 0.1 mL from each dilution was plated onto standard plate count agar (PCA). After 0, 1, 5, 10, and 15 days of storage, the plates were incubated at 37°C for 24-48 hours to establish the standard plate count. For counting, plates with 25-250 colonies were used. The reciprocal of the dilution was multiplied by the average number of bacterial colonies (12).

Measurement of pH value

The pH value of the meat sample was determined with a digital pH meter, by taking 10 g of sample homogenized in 50 mL distilled water. For each treatment, the averages of the five samples were presented (13).

Preparation of Thiobarbituric acid (TBA)

Twenty grams of meat sample was minced and then mixed with 50 ml cold solution containing 20% Trichloroacetic acid (TCA) in 2M phosphoric acid. The mixture was transferred into a 100 mL volumetric flask and add 40 mL distilled water was then added and mixed by shaking strongly. A 50 mL of the mixture was taken and placed in a centrifuge for 30 minutes at 3,000 rpm, and then Whatman filter paper No. 1 is used to filter the mixture, 5 ml of filtrate was transferred to a test tube, and 5 mL of a Thiobarbituric acid (0.005 M) was added. The tube was also tightly closed at that point, and the solution was thoroughly mixed, and then heated in a water bath for 30 minutes, at 90°C. The color that resulted was then measured by a UV spectrophotometer at the wavelength 530 nm. The TBA values were calculated by multiplying the resulted measures by 5.2. The absorbance value of the samples and translated to mg malondialdehyde (MDA) per kilogram of meat were obtained according to the following formula (14). TBA value (mg MDA/kg meat) = 5.2*A530.

Statistical analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors (Concentration and Time) in study parameters. Least significant difference LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study.

Results

The total bacteria count (TBC)

The effects of different concentrations of PP compared to control, are shown in table 1 that were significantly influenced the total bacterial count at various time points including 0, 1, 5, 7, 10 and 15 days, which were stored in the refrigerator at 4 C. At the 0 and 1st days, there were no significant differences among all groups, in general. On the 5th day of storage, significant differences in TBC recorded in T1 at 6.58 and T2 at 5.91 compared to control 7.73 at log CFU/ml. On the 7th and 10th day respectively, the TBC were significantly showed variations in T2 at 7.13, 8.00 compared to T1 at 8.76, 9.34 and control at 9.85, 10.20 Log CFU/ml, although the T1 and control group did not measure a significant value at these time-points. Finally, on the 15th day, the groups demonstrated high values of TBC, especially control. However, T2 still showed significant values 10.84 compared to T1 at 10.93 and control at 11.82 at Log CFU/ml.

The lipid stability values (TBARs)

In table 2, the results illustrated the effect of various concentrations of PP, which were compared to the control at significant differences that affected the lipid stability values (thiobarbituric acid reactive substances/ TBARs) at different time-points 0, 1, 5, 7, 10 and 15 days, the samples were stored in the refrigerator at 4°C; whereas, those groups had significantly no effects on TBAR values at 0 and 1st days. Furthermore, the differences started recording on the 5th and 10th days respectively, which T1 at 1.02, 1.19 and T2 at 0.69, 0.92 had no statistical differences between them but had significantly compared to the control at 1.47, 2.38 at those periods. In contrast, on the 7th day, the T2 at 0.72 showed a significantly difference from T1 at 1.08, as well as, those were statistically different from a control that was recorded 1.81. However, all groups recorded the highest values on the 15th day, and T2 at 2.28 showed a significant measurement compared with T1 at 2.93 and control at 3.12.

Table 1: The total bacteria counts (TBC) of beef meat treated with different concentrations of PP

Concentration	Mean ± SE of TBC					LSD
	1 Day	5 Day	7 Day	10 Day	15 Day	
Control	4.91±0.05 Ac	7.73±0.13 Ab	9.85±0.12 Aa	10.20±0.21 Aa	11.82±0.02 Aa	2.11
T1	4.55±0.04 Ab	6.58±0.09 Bb	8.76±0.19 Aa	9.34±0.18 Aa	10.93±0.05 Aa	2.17
T2	4.53±0.03 Ac	5.91±0.11 Bcb	7.13±0.07 Bb	8.00±0.09 Bb	10.84±0.12 Aa	2.19
LSD	0.36	0.92	1.12	1.19	1.10	---

The values (n = 5) indicated mean±SEM. The capital letters refer to significant differences at P<0.05. While the small letters pointed out significant differences at P<0.05.

Table 2: The lipid stability values (TBARs) of beef meat treated with different concentrations of PP

Concentration	Mean ± SE of TBAR					LSD
	1 Day	5 Day	7 Day	10 Day	15 Day	
Control	0.589±0.03 Ad	1.47±0.20 Ac	1.81±0.13 Ac	2.38±0.22 Ab	3.12±0.06 Aa	0.447 *
T1	0.551±0.04 Ac	1.02±0.06 Bb	1.08±0.02 Bb	1.19±0.18 Bb	2.93±0.14 Aa	0.331 *
T2	0.502±0.03 Ac	0.692±0.05 Bbc	0.728±0.03 Cbc	0.921±0.03 Bb	2.28±0.21 Ba	0.294 *
LSD	0.104 NS	0.386 *	0.242 *	0.527 *	0.471 *	---

The values (n = 5) indicated mean±SEM. The capital letters refer to significant differences at P<0.05. While the small letters pointed out significant differences at P<0.05.

The pH values of beef

Table 3 showed the effect of different concentrations of PP on the pH value on various days starting at 0, 1, 5, 7, 10 and 15 days of beef samples. These samples did not record statistical differences at the 0 day. The results for the 1st and 5th days demonstrated that there was a significantly increased, which was linked to increasing at the times-points of beef. Moreover, the values of T1 at 5.46, 5.51 and T2 at 5.43, 5.39 were significantly lowest than the control at 5.58, 5.82 respectively. In contrast, the T2 group recorded significantly lower values at 5.56, 6.59 compared to T1 at 5.82, 6.76, which measured significant differences in the control at 6.31, 7.12 respectively at the 7th and 15th days.

Discussion

A previous study examined PP, which were applied as antimicrobial impacts agents against gram-positive and gram-negative bacterial strains. It has been recorded an effect of antimicrobial activities of this extract against gram-positive strain compared with gram-negative strain (15-17). Thus, the antimicrobial impact of the edible extract was also detected on meat. It was shown that its antibacterial action was mostly influenced by the kinds of microorganisms found in meat. Although a bacterial Gram-type was not assessed in the current study, our findings might significantly increase the shelf life of beef meat (18,19).

Table 3: The pH values of beef meat treated with different concentrations of PP

Concentration	Mean \pm SE of PP					LSD
	1 Day	5 Day	7 Day	10 Day	15 Day	
Control	5.58 \pm 0.06 Ad	5.82 \pm 0.06 Ad	6.31 \pm 0.01 Ac	6.64 \pm 0.22 Ab	7.12 \pm 0.06 Aa	0.326 *
T1	5.46 \pm 0.03 ABd	5.51 \pm 0.03 Bd	5.82 \pm 0.07 Bc	6.06 \pm 0.07 Bb	6.76 \pm 0.05 Ba	0.171 *
T2	5.43 \pm 0.02 Bcd	5.39 \pm 0.03 Bd	5.56 \pm 0.02 Cc	5.82 \pm 0.09 Bb	6.59 \pm 0.02 Ca	0.142 *
LSD	0.122 *	0.138 *	0.146 *	0.451 *	0.158 *	---

The values (n = 5) indicated mean \pm SEM. The capital letters refer to significant differences at P<0.05. While the small letters pointed out significant differences at P<0.05.

Furthermore, the effects of different concentrations of PP 20g/L and 40g/L showed low impacts against the general bacterial counts compared to control; it did not inhibit the bacteria counting effectively on the 15th day of storage (20,21). Also Sing *et al.* (22) reported that PP was less effective against bacteria isolated from chicken meat. Moreover, the current studies revealed that PP levels are ineffective against the bacteria during long storage; the processes of cellular damage caused by the natural plant extracts' spectrum of phenolic phytochemicals may still to be understood because it is a complicated procedure. It has been hypothesized that breakdown of the cell membrane is the most plausible mechanism behind the antibacterial action of extracts including phenolic compounds (22,23).

In the case of meat, the shelf life of treated samples was 10 days, compared to 7 days for untreated control samples to degrade after storage. Beef samples that had not been treated had high TBC levels; nevertheless, germs in samples that had been treated had been found after 15 days of storage. Table 1 shows that PP at a T1 level was able to prolong the storage life by 10 days, hence larger levels (such T2) are not necessary because the results are the same. Numerous studies have shown that phenolics from plant extracts have antibacterial properties (24).

Meat quality is significantly impacted by lipid oxidation. This study recorded that meat samples with a diverse range of possibility oxidation via differences in their compositions, which were treated with two different concentrations of PP T1 and T2, and by revealing the meat to these levels adjusted for various times. Human conceptions of lipid oxidation, as determined by a qualified taste committee, were compared to a substance's measurement of oxidation (25).

The beef samples were obtained from local shops Karkh/ Baghdad that had been applied to two different concentrations of PP. Table 2 showed the TBARS values of beef. No oxidation was observed on the 0 and 1st days of the display, a significant observation since the meat had been kept sealed and packed until was used. On the 5th, 7th and 10th days of the display, all samples reached TBAR values under 1.5 except those at the end of the experimental time at 15th day, which showed an average value over 3.0 in the control and T1 groups, however, TBARS values in the T2 stayed under 2.0. The concentrated PP played an important role in

lipid oxidation in beef, which would have different content, especially polyphenols making these samples oxidatively unstable. TBARS also increases in meat that has been previously chilled, due to damages in some cellular structures thus encouraging oxidation and acting as a natural antioxidant (26). On the 15th day days of display in treated groups, only samples applied to the control had TBARS values upper than 3.0, probably due to the higher proportion of fatty acids in the beef meat, which suggested by Femandez *et al.* (27) that PP demonstrated high phenolic content and antioxidant activities. In treated samples, the TBARS findings were significantly lower compared to the control after a long time of storage.

The TBAR values have a relationship with pH values in beef and meat that were determined by Emam *et al.* (14) who measured TBAR levels during storage of samples from individual beef forequarters and the values for those different samples were recorded significantly different, which is same to current findings. Variation between samples from various beef samples may be important concerning the use of TBARS as an index of quality; although all values for these samples were of low magnitude. As pointed out for beef, pH values of individual beef meat may also influence by TBAR values (28). In the present study, each treated group showed decreasing pH values that resulted related to levels of PP. Moreover, polyphenols contained in the PP used in the preparation could affect bacterial inhibition through the above-mentioned mechanism (29). Many studies have shown the antimicrobial activity of pomegranate extracts and their concentrations against several microorganisms due to their content in phenolic compounds (30,31).

Furthermore, the polyphenols in the PP utilized in the planning may have an impact on bacterial prevention via the mentioned method (29). Due to their richness in phenolic compounds including phenolic contents, several studies have demonstrated the antibacterial action of pomegranate extracts and their concentrations against a variety of microorganisms (32,33). However, fewer studies have been conducted on the antibacterial properties of pomegranate peel pomace in treated beef.

Conclusion

From the findings of the present study, we concluded that the use of pomegranate pomace increases the storage period of beef until 10 days compared to samples without any treatment, also using pomegranate pomace in high concentration influence the storage period represented by the total bacterial counts and the pH changes, and lipid oxidation values.

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Conflict of interest

The authors announced that there are no conflicts of interest concerning the publication of this research paper.

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استخدام ثفل الرمان كمادة طبيعية مضادة للبكتيريا ومضادة للأكسدة في حفظ اللحم البقري

نور صفاء هذب و محمد مؤنس دخيل

فرع الصحة العامة، كلية الطب البيطري، جامعة بغداد، بغداد، العراق

الخلاصة

على الرغم من احتواء مجموعة متنوعة من اللحوم ومنتجاتها على ممرضات مختلفة تنقلها الأغذية، إلا أن اللحوم تعد مصدرا أساسيا للعناصر الغذائية المستحصلة من الأطعمة، ونظرا لاحتواء النباتات على مواد نشطة بيولوجيا، فقد تم تسجيل العديد من هذه النباتات ومستخلصاتها على أن لها تأثيرا مضادا للجراثيم ومضادا للأكسدة، والرمان أحد هذه النباتات. تم شراء ستون من لحوم البقر الطازجة من المتاجر المحلي الكرخ/ بغداد، ثم تم تقسيمها الى ستين عينة لحم، وقسمت عشوائيا إلى ثلاث مجموعات متساوية، تحتوي كل مجموعة على ٢٠ عينة. عملت المجموعة الأولى كمجموعة تحكم وتم نقعها في الماء المقطر. تلقت المجموعة الثانية نقعت في محلول ثفل الرمان بتركيز ٢٠غم/لتر وتمت الإشارة إليها ١. أما المجموعة الثالثة كانت فقد نقعت بمحلول ثفل الرمان بتركيز ٤٠ غرام/لتر وتر وسميت المجموعة ت٢. أظهرت العينات التي تم نقعها بمحلول ثفل الرمان نتائج إيجابية، حيث أظهرت النتائج فعاليتها في خفض معدل النمو البكتيري الكلي بمجموعة السيطرة. إضافة الى تأثيرها الواضح في خفض معدل أكسده الدهون في العينات المعاملة والتي يتم قياسها باستخدام المواد المتفاعلة لحمض الثايوباروجيرات، فضلا عن لقيم الأس الهيدروجيني. إذ أظهرت النتائج الحالية أن لحم البقر الذي تم غمره في محلول ثفل الرمان كان أكثر مقاومة لزيادة العد البكتيري مقارنة بمجموعة السيطرة.