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Effect of aqueous pomelo fruit extract (*Citrus maxima*) on physico-chemical, textural, and sensorial properties of spent goat meat

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Abstract

The present work explored the efficacy of aqueous pomelo [*Citrus maxima* (Burm.) Merr.] fruit extract (PFE) as a natural tenderiser in improving the physico-chemical, textural, and sensorial properties of spent goat meat, a tough and less desirable meat type due to its high collagen content. *Longissimus thoracis et lumborum* muscles from spent goat carcasses were marinated with varying concentrations of PFE (0, 0.1, 0.3, 0.5, and 1.0% (v/v) or 15% (v/w)) for 24 h at 4 ± 1°C. Results revealed a significant ($p < 0.05$) decreases in pH, water-holding capacity (WHC), Warner-Bratzler shear force (WBSF), and a* and b* colour values with increasing PFE concentrations, while collagen solubility, cooking yield, and myofibrillar fragmentation index (MFI) showed significant ($p < 0.05$) increases. Sensorial analysis demonstrated enhanced tenderness, juiciness, flavour, and overall acceptability in PFE-treated samples, with 1% PFE achieving the most pronounced improvements. SDS-PAGE analysis confirmed substantial proteolysis in marinated samples. Compared to synthetic tenderisers and enzymatic methods like papain or bromelain, PFE exhibited milder enzymatic activity, avoiding the risk of over-tenderisation while maintaining texture and enhancing flavour. These findings suggested that PFE could be an effective natural alternative to synthetic tenderisers, offering controlled and progressive tenderisation for improving the quality of spent goat meat.

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Introduction

Meat is a key source of dietary proteins, fats, vitamins, and minerals, and plays a significant role in human nutrition. Among livestock species, small ruminants, particularly goats, contribute vitally to the Indian economy, providing livelihood and employment to millions of small and marginal farmers. Goats are among the most preferred meat animals globally. According to the Food and Agriculture Organization of the United Nations in 2021, global goat meat production reached 6.39 million tonnes, a steady increase from 5.1 million tonnes in 1970 (FAO, 2021). This growth is driven by expanding metropolitan areas, increased incomes, and population growth, particularly in developing countries, where the demand for high-quality protein from meat and animal products is rising (Thornton, 2010). Goat farming has gained popularity, especially in rural areas facing challenges such as shrinking grazing lands, harsh climates, and water scarcity, as goats are well adapted to such conditions.

Consumers prefer chevon (goat meat) over other red meats due to its leaner composition, lower cholesterol, favourable sensory qualities, superior fatty acid profiles, and specific nutritional benefits (Sen *et al*., 2004; Mazhangara *et al*., 2019). However, goat meat is often derived from spent or culled animals at the end of their productive life, resulting in tougher, more fibrous meat. The eating qualities of meat—softness, juiciness, and flavour—are crucial to consumer acceptance. Therefore, improving the quality of meat from spent goats is essential for enhancing its acceptability and market value.

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A variety of tenderisation techniques, including physical, chemical, and enzymatic methods, have been explored to improve the quality of tough meat from spent animals (Piao *et al*., 2015; Demir *et al*., 2022). However, chemical tenderisers often negatively affect the sensorial attributes of meat, thus limiting their adoption in households or restaurants. In contrast, marination with acidic ingredients, particularly citrus-based marinades, has been widely studied as an effective method to enhance meat tenderness and extend shelf life (Burke and Monahan, 2003; Serdaroglu *et al*., 2007). Citrus fruits such as lemon, lime, and orange contain natural acids and enzymes that break down muscle fibres, thus improving tenderness (Moeini *et al*., 2022). Citric acid, commonly found in citrus fruits, is especially known for reducing meat toughness through the degradation of collagen and muscle fibre solubilisation. These findings highlight the potential of citrus-based marinades in improving meat quality, though most studies have focused on common citrus fruits like lemon and orange.

Pomelo [*Citrus maxima* (Burm.) Merr.], a citrus fruit from the Rutaceae family, is widely cultivated in China, Taiwan, the Philippines, and Southeast Asia due to the region's tropical and subtropical climate. As the largest citrus fruit, pomelo has a yellowish or greenish skin with a sweet-acidic flavour, and is consumed in various forms, including as fruit, juice, or in salads. Rich in vitamin C, antioxidants (Govindaiah *et al.*, 2024), and other bioactive compounds, pomelo has been recognised for its health benefits, including its roles as an appetiser, cardiac stimulant, and stomach tonic (Kumar *et al*., 2019). Although the chemical composition, bioactive phytochemicals, and antioxidant properties of pomelo have been documented (Zacarías-García *et al*., 2021; Gupta *et al*., 2021; Saini *et al*., 2022; Govindaiah *et al.*, 2024), there is limited research on its use in meat tenderisation.

Given the promising potential of citrus-based marinades for improving meat quality, the present work was designed to evaluate the effect of marinating spent goat meat with aqueous extracts of pomelo fruit on its physico-chemical, textural, and sensorial characteristics. By exploring this novel application of pomelo extract, the present work aimed to address the gap in the literature, and contribute to the broader field of meat science.

Materials and methods

Source of pomelo fruit and meat

Devanahalli pomelo fruits (protected by the Government of India's Geographical Indicators of Goods Act 1999) were obtained from retail shops of Devanahalli (Bangalore rural, Karnataka, India). The pomelo fruit extract (PFE) was obtained from pomelo fruits by aqueous extraction (Hindi and Chabuck, 2013). The longissimus thoracis et lumborum muscles from spent goats (3 – 4 years) slaughtered at Bidar district slaughterhouse were collected aseptically in low-density polyethylene bags under aerobic conditions, and transported to the laboratory for further analysis.

Experimental design

Uniform-sized meat chunks were made from longissimus thoracis et lumborum, and marinated at 15% (v/w) in different concentrations of PFE prepared with distilled water (0.1, 0.5, 1.0, 3.0, and 5.0% (v/v)) with pH of 5.0 ± 0.07, 4.4 ± 0.05, 4.1 ± 0.06, 4.1 ± 0.03, 3.9 ± 0.05, and 3.8 ± 0.05, respectively. For positive control batch, only 15% (v/w) of distilled water was added. A meat sample not subjected to any marination was considered as a negative control. After 24 h marination at 4 ± 1°C, raw and cooked meat chunks were evaluated. The experiment was conducted in five replications (n = 5).

Analytical procedures

Marination pick-up, pH, and water-holding capacity

The meat sample weights were recorded before and after marination, for calculation of marination pick up in percent. The meat sample's pH was determined after homogenising 10 g of meat with 50 mL of distilled water for 1 min in a tissue homogeniser (Model: Z742486, Benchmark, D1000 Handheld homogenizer, Malaysia), and pH of the suspension was measured using a digital pH meter (Model: SYSTRONICS pH System 361) with a glass electrode. The marination pick up was recorded as percent difference in weight of meat, before and after marination. The water-holding capacity (WHC) of the meat was estimated by centrifugal method (Wardlaw *et al*., 1973).

Protein extractability

Proteins were extracted using 0.025 M potassium phosphate buffer (pH 7.2) to extract

sarcoplasmic protein, while 0.1 M phosphate buffer (pH 7.2) with 1.1 M potassium iodide was used for total protein extraction, and protein concentration was measured against bovine serum albumin (BSA) as standard (Joo *et al*., 1999). The extractability of myofibrillar protein was estimated as difference in total and sarcoplasmic protein extractability.

Collagen content and solubility

Collagen concentration was calculated by determining the amount of hydroxyproline in the meat sample (Neuman and Logan, 1950) with some minor modification (Naveena *et al*., 2004). The amount of hydroxyproline (HP) was quantified using a standard graph, and the amount of collagen was computed by multiplying the HP amount by 7.14 (Dransfield *et al*., 1983), and represented in mg/g tissue. The solubility of collagen after cooking of the meat samples was determined by calculating the soluble HP (Mahendrakar *et al*., 1989).

Myoglobin and metmyoglobin

Myoglobin and metmyoglobin were extracted from meat sample following the method of Warris (2007), and myoglobin (mg/g) and metmyoglobin (%) were calculated using Eqs. 1 and 2, respectively:

Mb (mg/g) =
(A525 - A700) × 2.303 × dilution factor (Eq. 1)

Met-Mb (%) =
{1.395 - [(A572 - A700) / (A525 - A700)]} × 100 (Eq. 2)

where, A = Absorbance.

Muscle fibre diameter and myofibrillar fragmentation index

Muscle fibre diameter was calculated using the method described by Tuma *et al*. (1962). The diameters of at least ten muscle fibres were measured, and the average muscle fibre diameter was given in micrometre (μm). Myofibrillar fragmentation index (MFI) was calculated and reported as gram residue weight multiplied by 100 (Hawkins *et al*., 1987).

Cooking yield

The difference in weight after cooking expressed in percent was recorded as cooking yield, and measured using Eq. 3.

Cooking loss (%) = (Weight of chunks after cooking/weight of chunks before cooking) × 100 (Eq. 3)

Shear force and instrumental colour evaluation

Warner-Bratzler shear force value was measured as the force required for shearing 1 cm^2 core on texturometer (Shimadzu EZ-SX Tabletop texture analyser, Japan) with probe, and expressed in Newton (N). Colour values (CIE L* a* b*) of the muscle samples were determined using a handheld colourimeter (Model: CR10 plus Konica Minolta limited Inc, Japan) calibrated against black and white reference tiles. Measurement was performed, and results were displayed in terms of redness (a*), lightness (L*), and yellowness (b*) values, standardised by International Commission on Illumination (CIE). Hue and chroma were determined using Eqs. 4 and 5, respectively:

Hue = tan - 1 (b*/a*) (Eq. 4)

Chroma = $\sqrt{(a^{*2}+b^{*2})}$ (Eq. 5)

SDS-PAGE (Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis)

SDS PAGE was carried out using 10 μL of total protein (containing 10 μg of protein) with mini electrophoresis apparatus (Biorad, Mumbai, India) at constant voltage mode of 72 V/slab at 30 mA till termination of electrophoresis (Laemmli, 1970). Qualitative and quantitative level *viz*., peak index/profile length (Rf) and raw volume of disintegrated proteins (total proteins and sarcoplasmic proteins) from different treatments, were analysed using a gel analyser (Gel Analyser, version 19.1) (Lusiana *et al*., 2023).

Sensorial evaluation

The meat samples were cooked and served to semi-trained panellists, and evaluated for appearance, flavour, juiciness, tenderness, and overall acceptability using an 8-point descriptive scale, with highest score for extremely desirable sample, and least score for extremely undesirable sample in a score sheet.

Statistical analysis

A completely randomised block design with longissimus thoracis et lumborum muscle as block

was used to assess the effects of PFE on the physico-chemical, textural, and sensorial properties of spent goat meat. A total of three independent trials were conducted to duplicate the experiment. For statistical purposes, we utilised an average of two independent subsamples for each of the parameters of interest. Duncan's multiple range test at $p < 0.05$ was used to determine statistical significance across groups, and SPSS (SPSS version 13.0 for windows; SPSS Inc. Chicago, IL) was employed to conduct an analysis of variance.

Results

The results of various physico-chemical characteristics, protein solubility, and textural characteristics of spent goat meat marinated at different concentrations of pomelo fruit extract (PFE) and control are presented in Tables 1, 2, and 3, and Figure 1.

Marination pickup, pH, and water-holding capacity

No significant difference in marination pickup was observed between control and treatments; however, a numerical increase in marination pickup was evident in all PFE treatments (Table 1). A significant ($p < 0.05$) decrease in pH was evident with increasing level of PFE-marinated goat meat chunks in contrast to control (Table 1). The water-holding capacity (WHC) decreased ($p < 0.05$) from 20.00 to 9.4% (Table 1) with the increase in PFE concentration.

Protein extractability

Higher ($p < 0.05$) myofibrillar protein and total protein extractability was observed in PFE-marinated samples (Table 1) compared to control up to 0.5% level with subsequent decrease. PFE treatment had no effect on sarcoplasmic protein extractability.

Collagen content and solubility

Significant difference ($p < 0.05$) was observed in collagen content of spent goat meat subjected to different concentrations of PFE (Table 1). In comparison to the control, collagen solubility increased gradually ($p < 0.05$) with an increase in PFE concentration.

Myoglobin and metmyoglobin

The percent met-Mb values decreased significantly ($p < 0.05$) in PFE-marinated samples with lowest value in 3% PFE treatment (Table 1).

Muscle fibre diameter and myofibrillar fragmentation index

The muscle fibre diameter of PFE-marinated samples was substantially smaller ($p < 0.05$) than that of control samples, whereas 1% PFE-marinated samples had lower MFD as compared to other PFE-marinated samples (Table 1). Significant ($p < 0.05$) progressive increase in MFI was observed with the increase in concentration of PFE with contrast to treatment groups.

Cooking yield

There was significant ($p < 0.05$) difference in cooking yield between control and treatments (Table 1), with highest cooking yield in 1% PFE which correlated with marination pickup with PFE treatment.

Warner-Bratzler shear force value and instrumental colour

All the PFE-marinated meat chunks had significant ($p < 0.05$) lower Warner-Bratzler shear force (WBSF) values compared to control groups (Table 1). There was reduction in shear force values of 46.17 to 34.17 N from negative control to 5% PFE-marinated samples, showing 25.99% reduction in shear force value relative to negative control due to PFE.

Compared to the unmarinated control, the colour indices of the marinated samples did not change significantly ($p > 0.05$) (Table 2). Compared to the value obtained using the negative control, the marination treatment resulted in a considerable reduction in the a* value. The a* values for all treatment groups were not significantly ($p > 0.05$) different from each other. All marinated samples had colour indices at level to satisfy majority consumers.

SDS-PAGE (Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis)

SDS-PAGE was carried out to demonstrate the effects of PFE marination on proteins from goat muscle (Figure 1). SDS PAGE revealed the degradation of several proteins in different concentrations of PFE-marinated meat samples as

Table 1. Variation in physico-chemical parameters of spent goat meat samples subjected to different concentrations of pomelo fruit extract (PFE).

Parameter	-C	+C	T1	T2	T3	T4	T5	SEM
Marination pickup (%)	97.54 ± 1.04^{a}	102.86 ± 0.38^{b}	103.23 ± 0.88^{b}	103.87 ± 0.97^{b}	102.67 ± 0.95^{b}	103.90 ± 0.37^{b}	103.39 ± 1.13^{b}	0.54
pH	5.92 ± 0.05^{d}	5.78 ± 0.04^{c}	5.69 ± 0.04^{bc}	5.56 ± 0.04^{ab}	5.75 ± 0.03^{c}	5.66 ± 0.06^{abc}	5.53 ± 0.04^{a}	0.03
WHC (%)	20.00 ± 0.713^{c}	15.80 ± 0.73^{b}	18.20 ± 1.07^{c}	15.40 ± 0.87^{b}	14.80 ± 0.97^{b}	10.40 ± 0.68^{a}	9.40 ± 0.60^{a}	0.67
SPE (mg/g)	164.90 ± 9.31^{a}	153.40 ± 4.03^{a}	161.70 ± 2.92^{a}	163.06 ± 1.97^{a}	158.60 ± 5.56^{a}	165.75 ± 5.53^{a}	157.35 ± 2.38^{a}	1.86
MFPE (mg/g)	143.33 ± 14.95^{abc}	168.09 ± 11.87^{cd}	120.81 ± 3.04^{a}	174.27 ± 3.79^{d}	139.36 ± 3.86^{ab}	134.20 ± 5.42^{a}	160.62 ± 4.29^{bcd}	4.76
TPE (mg/g)	308.24 ± 5.91^{bc}	321.49 ± 9.34^{c}	282.51 ± 5.17^{a}	337.32 ± 2.39^{d}	297.96 ± 1.73^{b}	299.96 ± 2.00^{b}	317.96 ± 2.36^{c}	4.03
Collagen content (mg/g)	0.60 ± 0.02^{b}	0.61 ± 0.01^{b}	0.61 ± 0.01^{b}	0.53 ± 0.01^{a}	0.52 ± 0.01^{a}	0.75 ± 0.02^{c}	0.93 ± 0.03^{d}	0.03
Collagen solubility (%)	12.14 ± 2.07^{a}	8.51 ± 0.57^{a}	16.82 ± 1.05^{b}	19.03 ± 0.93^{bc}	22.31 ± 1.77^{c}	11.73 ± 1.30^{a}	11.12 ± 0.78^{a}	1.11
Myoglobin (mg/g)	3.40 ± 0.08^{b}	3.16 ± 0.09^{b}	3.26 ± 0.08^{b}	3.16 ± 0.08^{b}	2.82 ± 0.08^{a}	2.81 ± 0.08^{a}	2.61 ± 0.08^{a}	0.06
Met-myoglobin (%)	46.94 ± 1.23^{c}	47.69 ± 0.81^{c}	46.51 ± 1.43^{c}	46.37 ± 1.04^{c}	43.28 ± 0.64^{c}	33.07 ± 2.49^{b}	37.87 ± 2.25^{a}	1.12
MFD (μ)	47.59 ± 1.37^{b}	49.45 ± 3.42^{b}	43.74 ± 1.09^{ab}	41.67 ± 1.17^{a}	45.79 ± 1.96^{ab}	45.85 ± 1.06^{ab}	44.06 ± 0.78^{ab}	0.75
MFI (%)	49.50 ± 0.65^{b}	47.75 ± 0.85^{ab}	48.00 ± 1.41^{ab}	46.00 ± 0.82^{a}	47.25 ± 0.48^{ab}	46.50 ± 0.50^{a}	46.00 ± 0.82^{a}	0.36
Cooking yield (%)	54.69 ± 0.86^{a}	58.04 ± 0.42^{ab}	61.26 ± 1.15^{bc}	61.31 ± 1.27^{bc}	62.90 ± 1.01^{c}	57.93 ± 1.30^{ab}	59.60 ± 2.35^{bc}	0.71
WBSF (N)	46.17 ± 0.95^{d}	40.83 ± 1.35^{c}	36.50 ± 0.99^{ab}	38.33 ± 1.41^{bc}	37.67 ± 1.38^{abc}	37.50 ± 1.48^{abc}	34.17 ± 1.42^{a}	0.72

Mean ± SE values having different lowercase superscripts within similar row differ significantly ($p < 0.05$) with different concentrations of pomelo extract. -C: Negative control; +C: Positive control; T1: 0.1% PFE; T2: 0.3% PFE; T3: 0.5% PFE; T4: 1% PFE; and T5: 5% PFE.

Table 2. Instrumental colour of spent goat meat samples subjected to different concentrations of pomelo fruit extract (PFE).

Parameter	-C	+C	T1	T2	T3	T4	T5	SEM
L* value	43.42 ± 0.98^{a}	44.57 ± 1.97^{a}	44.22 ± 0.63^{a}	44.17 ± 1.12^{a}	43.50 ± 1.31^{a}	43.90 ± 0.60^{a}	44.40 ± 0.88^{a}	0.41
a* value	13.15 ± 0.71^{b}	8.15 ± 0.85^{a}	7.75 ± 0.43^{a}	7.90 ± 0.60^{a}	6.18 ± 0.79^{a}	8.03 ± 0.43^{a}	7.63 ± 0.69^{a}	0.39
b* value	14.95 ± 0.37^{c}	11.77 ± 1.10^{b}	12.58 ± 0.51^{b}	12.40 ± 0.65^{b}	9.78 ± 0.48^{a}	10.87 ± 0.63^{ab}	11.13 ± 0.21^{ab}	0.32
Hue	48.67 ± 0.68^{a}	55.29 ± 0.97^{b}	58.37 ± 0.52^{d}	57.50 ± 0.79^{c}	57.71 ± 0.86^{c}	53.53 ± 0.53^{ab}	55.56 ± 0.59^{b}	NA
Chroma	19.91 ± 0.60^{d}	14.31 ± 0.84^{bc}	14.78 ± 0.41^{bc}	14.70 ± 0.68^{bc}	11.57 ± 0.80^{a}	13.51 ± 0.61^{b}	13.50 ± 0.57^{b}	NA

Mean ± SE values having different lowercase superscripts within similar row differ significantly ($p < 0.05$) with different concentrations of pomelo extract. -C: Negative control; +C: Positive control; T1: 0.1% PFE; T2: 0.3% PFE; T3: 0.5% PFE; T4: 1% PFE; and T5: 5% PFE.

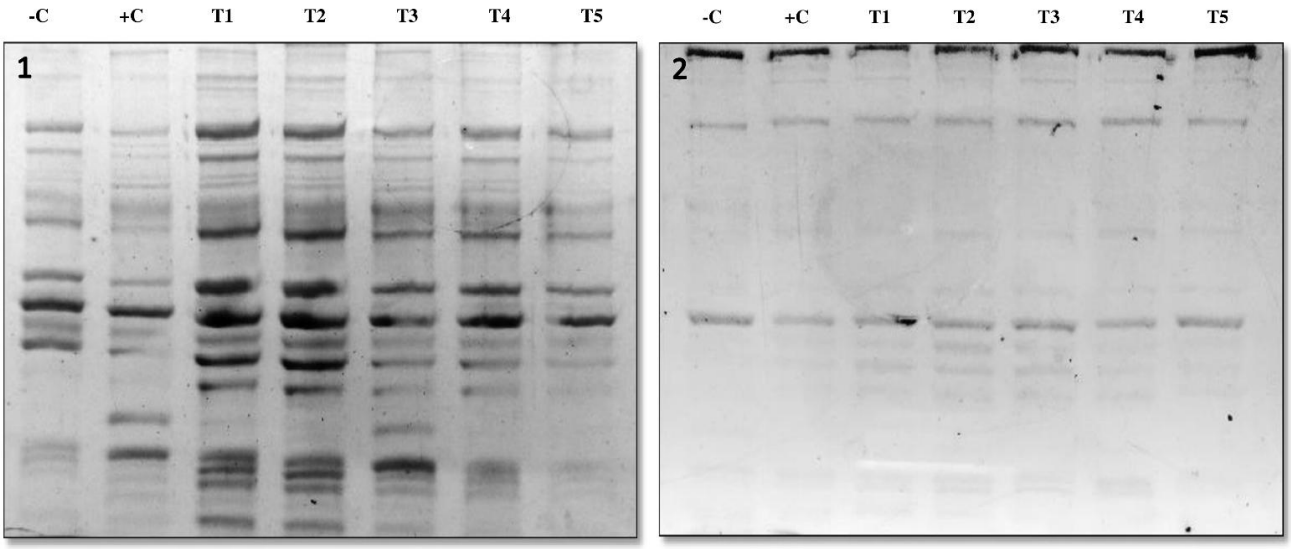

Figure 1. Variation in total proteins (1) and sarcoplasmic proteins (2) of spent goat meat samples subjected to different concentrations of pomelo fruit extract (PFE) (T1: 0.1% PFE; T2: 0.3% PFE; T3: 0.5 % PFE; T4: 1% PFE; and T5: 5% PFE) and control groups (-C: Negative control; and +C: Positive control).

compared to negative and positive control samples. Gel analyser results showed several degraded proteins with a greater number of peaks in PFE-marinated samples compared to negative and positive control groups, and increased peak index/profile length (Rf) and raw volume in total and sarcoplasmic protein bands among PFE-marinated samples were observed compared to control. The higher Rf values and raw volume in both total protein and sarcoplasmic were found in T1 and T2 groups.

Sensorial evaluation

The effect of different concentration of PFE treatments on the sensorial attributes of goat meat samples are presented in Table 3. Tenderisation of spent goat meat showed that using different concentrations of PFE marination caused significant ($p < 0.05$) increase in the mean sensory analysis scores for tenderness, juiciness, flavour, and overall acceptability.

Discussion

Marination pickup, pH, and water-holding capacity

Tissue disintegration owing to increased acidic pH and enzymatic activity may account for the enhanced marination uptake with varying levels of PFE (Yogesh *et al*., 2015). It has been reported that marinating meat in marinades with pH below 5.0 improved the water uptake during marination (Burke and Monahan, 2003; Fencioglu *et al*., 2022).

The significant reduction in pH might be attributed to the low pH of PFE extract, mainly due to the presence of acids in pomelo fruit. The reduction in pH may improve the tenderness of meat due to the disruption of lysosomal membranes and release of cathepsins (Dutson and Pearson, 1985). In addition, reduced pH has a huge impact on water-holding capacity, meat colour, and stability by influencing other proteins and enzymatic systems causing proteolysis. PFE had significantly accelerated the post-mortem pH decline in marinated samples compared to control. Based on findings by other researchers, the pH level of the meat significantly decreased throughout the process of marinating with organic acids (Jinap *et al*., 2018; ben Braïek and Smaoui, 2021; Sengun *et al*., 2021; Moeini *et al*., 2022).

The WHC is vital since this affects yield, quality, and other qualities including juiciness, flavour, and colour, all of which contribute to the meat's economic value. Low WHC in meat has been reported at reduced pH, and could be attributed to the net charge effect (Serdaroglu *et al*., 2007). In addition, acidic marinade diffuses into muscle tissues causing the hydration of proteins with swelling and weakening of the muscle, thereby resulting in water loss (Kim *et al*., 2013).

Protein extractability

Higher myofibrillar protein extractability of PFE-marinated samples are attributed to myofibrillar

Table 3. Sensory evaluation scores of spent goat meat samples subjected to different concentrations of pomelo fruit extract (PFE).

Parameter	-C	+C	T1	T2	T3	T4	T5
Appearance	5.33 ± 0.17^{a}	6.17 ± 0.11^{b}	6.50 ± 0.13^{bc}	6.75 ± 0.17^{cd}	7.08 ± 0.24^{d}	7.17 ± 0.17^{d}	7.17 ± 0.17^{d}
Juiciness	5.17 ± 0.11^{a}	5.75 ± 0.11^{b}	5.83 ± 0.11^{b}	5.83 ± 0.28^{b}	6.50 ± 0.22^{c}	6.58 ± 0.15^{c}	6.58 ± 0.24^{c}
Tenderness	5.50 ± 0.26^{a}	5.92 ± 0.08^{ab}	6.33 ± 0.17^{ab}	6.00 ± 0.29^{bc}	6.83 ± 0.11^{c}	6.83 ± 0.33^{c}	7.00 ± 0.18^{c}
Flavour	5.08 ± 0.08^{a}	5.83 ± 0.17^{b}	6.25 ± 0.17^{bc}	6.50 ± 0.22^{cd}	6.83 ± 0.21^{de}	6.92 ± 0.27^{de}	7.25 ± 0.11^{e}
Overall acceptability	5.42 ± 0.20^{a}	6.25 ± 0.21^{b}	6.50 ± 0.18^{bc}	6.83 ± 0.17^{bc}	7.00 ± 0.22^{c}	6.92 ± 0.27^{c}	7.17 ± 0.21^{c}

Mean ± SE values having different lowercase superscripts within similar row differ significantly ($p < 0.05$) with different concentrations of pomelo extract. -C: Negative control; +C: Positive control; T1: 0.1% PFE; T2: 0.3% PFE; T3: 0.5% PFE; T4: 1% PFE; and T5: 5% PFE.

disintegration with PFE treatment. Protein extractability describes the quantity of protein that is released from an ordered myofibrillar structure when it is processed. Protein extractability is measured as the amount of protein that can be extracted from the structure. The extractability of a protein is essentially determined by the thermodynamics of the protein-water interaction, and the distribution of ionic amino acids on its surface. The reason for reduction in extractability above 0.5% level may be due to salting in-salting out property of proteins. Reduced pH is known to accelerate tenderisation by enhanced proteolysis with physical disruption of muscle fibre. Increased meat tenderness may be achieved by using plant-based exogenous enzymes (Ha *et al*., 2012). This is because these enzymes have the capacity to promote myofibril protein disintegration, thus altering the muscle microstructure, leading to greater meat tenderness (Naveena *et al*., 2004).

The well-known proteolytic enzymes papain and bromelain are capable of greatly increasing protein extractability due to their strong ability to hydrolyse proteins. For example, due to its broad proteolytic specificity, bromelain has been found to hydrolyse myofibrillar proteins more effectively than papain (Madhusankha and Thilakarathna, 2021). Both papain and bromelain tend to break down proteins more quickly and aggressively than PFE, which if not controlled carefully could result in over-tenderisation or a mushy texture (Bekhit *et al.,* 2014; Jun-Hui *et al.,* 2020). PFE appeared to offer a more moderate tenderising effect, possibly due to its lower proteolytic activity compared to these enzymes, making it potentially more suitable for achieving controlled tenderisation without compromising the sensory properties of the meat. Therefore, while PFE was effective in improving protein extractability and tenderness, the enzymatic activity was milder compared to papain and bromelain, providing a distinct advantage in terms of avoiding undesirable textural changes.

Collagen content and solubility

The findings for the muscle collagen content observed in the present work agreed with previously reported data (Burke and Monahan, 2003), emphasising that collagen is a major connective tissue protein significantly influencing meat toughness, particularly in spent animals with high collagen content. The solubility of collagen is a crucial factor in determining meat tenderness; greater solubility leads to softer and more tender meat after cooking. The observed changes in collagen solubility could be attributed to the hydrolysis of collagen and elastin by proteolytic enzymes present in the fruit extract (Wada *et al*., 2002), which tenderises the connective tissue of meat. Furthermore, marination in acidic solutions, such as those found in PFE, has been suggested to lower the thermal stability of connective tissue, promoting swelling of muscle fibres and/or connective tissue. This process results in a weakened muscular structure due to accelerated proteolytic changes, ultimately leading to increased collagen solubilisation during cooking (Żochowska-Kujawska *et al*., 2012).

PFE's acidic pH, primarily due to organic acids like ascorbic and citric acids, aids in breaking down collagen fibres by weakening the hydrogen bonds within the collagen triple-helix structure, enhancing its solubility during heating. Additionally, the flavonoids in PFE may further facilitate collagen breakdown through hydrolysis, converting insoluble collagen to soluble gelatine, thereby improving tenderness. In mature meats, such as spent goat, this effect can substantially enhance the sensory perception of tenderness. Various mechanisms have been proposed to explain the effects of acidic marinades, including the swelling of meat, enhanced proteolysis by cathepsins, and the accelerated conversion of collagen to gelatine at low pH during heat treatment (Berge *et al*., 2001). However, the effect of PFE was milder operating over time, wherein the proteolytic effect is coupled with organic acids and the strength of those acids in how they are contained in the PFE compared to papain and bromelain. Such slow action may leave room for controlling level of collagen breakdown, obviating the risk of over-tenderisation associated with the use of either bromelain or papain (Bekhit *et al.,* 2014; Jun-Hui *et al.,* 2020). Thus, PFE was much more progressive in the way it helped in collagen solubilisation coating and recovering from the unhealthy condition of enhancing the sweetness of meats. Controlled solubilisation of this nature is particularly desirable in meat made from older animals, which usually has high collagen content, where the excess breakdown of collagen caused by the enzymatic actions would be detrimental to the meat texture.

Myoglobin and metmyoglobin

Colour of the muscle is determined primarily by the concentration and chemical state of myoglobin. Colour and its stability in fresh meat has been mainly related to the inter-conversion of three myoglobin forms *i.e*., deoxy-myoglobin (deoxy-Mb), oxy myoglobin (oxy-Mb), and met-myoglobin (met-Mb) affected by various ante- and post-mortem factors. The decreased Met-Mb% leads to lower colour intensity which might be attributed to lower pH of acid marinade (Arganosa and Marriott, 1989).

Muscle fibre diameter and myofibrillar fragmentation index

Muscles with small fibres have been reported to be more tender in cattle and pigs, confirming correlation between muscle fibre diameter and tenderness (Renand *et al*., 2001). It has been shown that the MFI is a useful measure of the level of proteolysis, since it reflects the breakdown of structural proteins inside the I-band of the sarcomere as well as inter-myofibril linkages (Kim *et al*., 2013). The myofibril fragmentation involves the shortening of the myofibril length due to the destruction of the Z-lines. The acidic marination and proteolytic enzymes present in PFE caused breakdowns of Z-lines of muscle fibre leading to enzymatic tenderisation, accompanied by shortened myofibril length, and reduced sarcomere number with increased MFI (Zhao *et al*., 2012).

Cooking yield

The observation contradicted the results of WHC, indicating weak force of water binding in fresh meat which were lost during cooking. There are several structural changes that occur in myofibrillar and connective tissue proteins as a function of cooking temperature and length of time that have a direct bearing on the final product's yield, texture, and overall palatability. Citric acid marination of beef resulted in a similar improvement in cooking yield (Ke *et al*., 2009).

Warner-Bratzler shear force value and instrumental colour

The observed results of WBSF values in the present work were in agreement with Burke and Monahan (2003), who proposed that lowered meat pH during marination may increase proteolysis by cathepsins with optimal pH in range of 3.5 - 5.0. Marination with acidic marinade is known to cause swelling of meat due to weakened muscle structure, increased proteolysis, and increase gelatine formation during cooking, leading to improved tenderness (He *et al*., 2015).

Colour, along with tenderness, is commonly acknowledged as a major factor in determining whether people would buy a certain cut of meat. Customers reported a high degree of satisfaction in especially for meat with a bright red bloom (Howes *et al*., 2015). To be regarded acceptable, the a* and L* values in fresh lamb meat must be greater than 9.5, and the values must increase to 14.5 and 44, respectively, to please 95% of the customers (Khliji *et al*., 2010). Meat that had been acid-treated seemed lighter because the acid seemed to increase the conversion of myoglobin to metmyoglobin, which has a lower colour intensity (Arganosa and Marriott, 1989). The higher yellowness value in PFE-marinated samples might have been due to the presence of flavonoids, carotenoids, and phenolic compounds in the pomelo fruit.

SDS-PAGE

Several key findings in the present work highlighted the influence of PFE on protein structure and tenderisation mechanisms in spent goat meat. For instance, the analysis of DNA polymorphism using gel analyser software revealed a high degree of precision in detecting proteins that were otherwise difficult to distinguish through SDS-PAGE alone. The Rf (peak index/profile length) values allowed for a more detailed identification of protein bands, further supporting the occurrence of proteolysis during tenderisation (Skosyrev *et al*., 2013; Lusiana *et al*., 2023). This evidence corroborated the observed structural changes caused by PFE treatment.

In PFE-treated samples, the reduced intensity and number of protein bands observed through SDS-PAGE suggested enhanced proteolysis. Degradation of higher molecular weight proteins, accompanied by an increase in lower molecular weight proteins, indicates a more advanced tenderisation process (Naveena *et al*., 2011). This was consistent with studies on acidic marinades, where the breakdown of proteins has been linked to tenderness improvements in various meats, such as beef (Berge *et al*., 2001). Enhanced proteolysis plays a crucial role in the solubilisation of key muscle proteins like myosin, the conversion of titin isomers, and the depolymerisation of actin, all of which contribute to a softer meat texture (Sikes and Warner, 2016).

Furthermore, PFE's low pH, mainly due to organic acids like citric acid, induced partial protein denaturation, unfolding protein molecules, and enhancing their ability to retain water, which improved both the juiciness and texture of the meat (Ke *et al*., 2009). Additionally, PFE's phenolic compounds may promote protein cross-linking through oxidative mechanisms, forming covalent bonds between protein molecules that contribute to a firmer texture. In spent goat meat, moderate cross-linking, combined with collagen solubilisation, can enhance tenderness (Isaschar-Ovdat and Fishman, 2018).

Sensory evaluation

The primary factor determining the quality of meat is the consumer's assessment of its eating quality, with tenderness, juiciness, and flavour being the most significant components (Alahakoon *et al*., 2014). These sensorial attributes are critical for consumer acceptance, and PFE could influence them through multiple pathways involving both chemical reactions and direct effects on meat proteins and lipids.

PFE contained various volatile compounds and flavonoids that enhance the flavour profile of meat. Ascorbic acid, a potent antioxidant present in PFE, could reduce lipid oxidation which is a major cause of off-flavours in meat, especially in spent animals with higher fat content. By inhibiting oxidative rancidity, PFE could help preserve desirable flavours. Moreover, flavonoids could interact with Maillard reaction intermediates during cooking, contributing to the formation of complex flavour compounds (Alahakoon *et al*., 2014). When comparing PFE to other tenderisers like papain and bromelain, key differences in sensory effects emerge. While all improve tenderness, papain and bromelain focus mainly on breaking down proteins through strong proteolytic activity, lacking PFE's volatile compounds and antioxidants that enhance flavour. This limits their contribution to flavour development, and excessive use risks over-tenderisation, leading to a mushy texture (Bekhit *et al.,* 2014; Jun-Hui *et al.,* 2020). PFE also better retained moisture due to its flavonoids and acids, improving juiciness, whereas papain and bromelain may cause dryness from excessive water loss during cooking. PFE's controlled tenderising action could avoid the over-softening often seen with papain and bromelain (Bekhit *et al.,* 2014; Jun-Hui *et al.,* 2020), thus preserving structure and enhancing palatability in tougher meats like spent goat.

In addition to enhancing flavour, the softening effect of PFE on collagen and its influence on protein structures significantly contributed to the textural properties of meat. Increased collagen solubility and partial protein denaturation resulted in more tender meat, while moisture retention enhanced overall mouthfeel. These textural modifications are particularly beneficial for spent goat meat, which is typically tougher due to its high collagen content, making it more palatable and appealing to consumers.

Conclusion

The marination of spent goat meat with varying levels of pomelo fruit extract (PFE) significantly influenced its physico-chemical, mechanical, and sensorial properties. Increasing concentrations of PFE (up to 1%) resulted in a significant ($p < 0.05$) decrease in pH, water-holding capacity (WHC), myofibrillar protein extractability, collagen content, muscle fibre diameter, and Warner-Bratzler shear force (WBSF). These changes were accompanied by notable ($p < 0.05$) increases in collagen solubility, cooking yield, and myofibrillar fragmentation index (MFI), all of which contributed to improved meat tenderness. Furthermore, SDS-PAGE analysis revealed pronounced proteolytic degradation of myofibrillar proteins, confirming the enzyme-like activity of PFE in breaking down muscle proteins. Sensorial analysis corroborated these findings, demonstrating that marination with PFE enhanced the tenderness, juiciness, and flavour of the meat, with 1% PFE delivering the most favourable results. The present work also highlighted that PFE-treated meat exhibited a natural, balanced tenderisation effect without the risk of over-softening, as seen with synthetic or more aggressive enzymatic tenderisers like papain or bromelain.

In conclusion, the results clearly indicated that 1% PFE marination could be an effective natural tenderisation method, offering substantial improvements in the quality of spent goat meat. Given its efficacy, simplicity, and natural origin, PFE can be recommended as a viable tenderisation solution both at the household and industrial levels for improving the palatability of tougher meat cuts.

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