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Impacts of papain and bromelain fortified marinades on chilled camel meat quality

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ABSTRACT

Camel meat is a highly nutritious meat; however, there are some limitations in its acceptance due to its quality. So, the purpose of this study was to assess the impacts of marinating mixtures based on yoghurt and whey that were enhanced with papain and bromelain enzymes on the quality parameters of camel meat. Camel meat (*Musculus longissimus*) was sliced and marinated in yoghurt (y) or whey-based (w) supplemented with 2 % papain (PY and PW) or 2 % bromelain (BY and BW) enzymes. Camel meat marinated in either whey or yoghurt, particularly yoghurt supplemented with papaya or bromelain enzymes, had significantly lower pH, Thiobarbituric Acid Number, and total volatile basic nitrogen levels than camel meat marinated in unfortified whey and yoghurt ($P < 0.05$). Similar antimicrobial benefits were observed in microbiological counts. Papaya or bromelain-fortified marinades had a persistent adverse impact on aerobic plate count, *Staphylococcus aureus*, psychrotrophic bacteria, and coliforms. Compared to bromelain-fortified marinades, papaya-fortified marinades, particularly PY, had a higher growth-suppressing impact ($P < 0.05$). All marinades, including bromelain and papaya, enhanced the sensory attributes of chilled camel meat. The current research findings demonstrated that bromelain and papain proteases provide a feasible approach for developing antibacterial and antioxidant systems for camel meat-based products.

1. INTRODUCTION

It was expected that the global average per capita demand for meat will increase by 2%, from the 2020-2022 base period to 2032 (OECD and FAO, 2023). Meat quality can be described in a variety of ways depending on the industry area. A high percentage of grade A is required by the grower; nevertheless, a low microbiological count will be regarded as good quality by the fast food industry (Barbut et al., 2005). The most significant and noticeable meat characteristics that affect consumers' initial and final quality judgments before and after purchasing a meat product are appearance, texture, juiciness, wateriness, firmness, tenderness, odor, and flavor. For processors engaged in the production of value-added meat products, the measurable qualities of meat, such as water holding capacity, shear force, drip loss, cook loss, pH, shelf life, collagen content, protein solubility, cohesiveness, and fat binding ability, are essential (Mir et al., 2017).

Camel meat is nutritionally equivalent to any other conventional meat source and offers an advantage over beef or lamb due to its low intramuscular fat, low cholesterol concentration, and high iron content. Despite the increasing global acceptance of camel meat, limited commercialization is due to lesser camel quality qualities, particularly tenderness and juiciness, as well as a longer cooking time when compared to beef (Baba et al., 2021).

Overall, the marinating process improves the product's technological elements, including its efficacy and safety, as well as the sensory qualities of meat, including flavor, aroma, color, and tenderness. Limiting the proliferation of bacteria, it also prolongs the shelf life (Augustyńska-Prejsnar and Kačániová, 2023; Meneses and Teixeira, 2022) Papain is an enzyme present in the secretion of the papaya plant, Carica papaya, that defends the plant against insects (Konno et al., 2004). Because papain is a particularly heat-stable enzyme, it is difficult to deactivate, allowing for a continual change in product texture even after cooking (Dransfield and Etherington, 1981). Bromelain is a proteinase-rich extract derived from pineapple (Ananas comosus). It is a crude, aqueous extract of pineapple stems and fruits (Ananas comosus) from the Bromeliaceae family (Mamo and Assefa, 2019).

Bromelain has been reported to improve the tenderness and enhance the flavor of fresh meat (Jančič and Gorgieva, 2021; Mohd Azmi and Kumar, 2023; Praveen et al., 2014). It acts on myofibrillar proteins and collagen to provide a tenderizing effect to enhance the sensory parameters (Praveen et al., 2014). Bromelain application also increases the nutritive value of meat by increasing its essential amino acid content (Santos and Fraqueza, 2020). There are no research studies that directly answer the question of the impact of Papain and bromelain on meat preservation. Also, Research on the application of new pre-treatments to increase consumer acceptance of camel meat is quite scarce. Therefore, the goal of this study was to compare the potential impacts of yoghurt and whey-based marinating mixtures enriched with papain and bromelain enzymes on the quality aspects of camel meat.

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2. MATERIAL AND METHODS

2 .1 .Experiment management and approval

All methods used in this study were authorized by the Institutional Animal Care and Use Committee Research Ethics number (BUFVTM) at Benha University's Faculty of Veterinary Medicine under the number BUFVTM13-07-2023.

2.2. Marinade content and preparation

Camel meat (*Camelus dromedarius*) *Musculus longissimus thoracis et lumborum* (*M. longissimus*, rib-eye loin) was collected 2 hours after slaughter from the butcher. The meat was sliced into 1.5 cm thick steaks weighing approximately 50±5 g each. The steaks were marinated with yoghurt- or whey-based marinating blends supplemented with cooking oil 3%, salt (3%), and vinger (1%) for preparation of base marinades, each of the main base marinades was sub-grouped based on supplementation of bromelain (2%) or papain (2%) enzymes. A total of 6 sub-groups (3 from each based marinades) were prepared as follows; CY: control yoghurt-based marinade, PY: control yoghurt-based marinade + papain enzyme (2%), BY: control yogurt-based marinade + bromelain enzyme (2%), CW: control whey-based marinades, PW: control whey-based marinades + papain enzyme (2%), and BW: control whey-based marinades + bromelain enzyme (2%). Marinated camel steaks from each group were packed in poly-ethylene bags and kept chilled for 4 °C. Steaks samples from each group were examined periodically at 0, 2, 4, 6, 8, 10, and 12 day of storage for microbiological, chemical, and sensory characteristics. Trials were applied in a separately triplicated manner.

2.3. Evaluation of physico-chemical quality

Values of pH were determined using an electrical pH meter (Bye model 6020, USA) of camel meat homogenate. The amount of total volatile basic nitrogen (TVBN) was measured by the distillation approach (Pearson, 1968). Moreover, the Thiobarbituric Acid Number was evaluated according to Egyptian organization requirements, the spectrophotometric method was used to calculate the Thiobarbituric Acid Number (TBA) (EOS, 2006) .

2.4. Evaluation of microbiological quality

The aerobic plate count (APC) of camel meat samples was assessed in the same manner as fully described by ISO (2013).

To account psychrotrophic bacteria, decimal dilutions of the various samples were made, and aliquots of 0.1 ml of the suitable dilutions were plated in duplicate on plate count agar (PCA) and cultured aerobically for 10 days at 7°C (FDA, 2001).

Coliforms number was evaluated using sterile Petri dishes of Violet red bile agar at 37 °C as fully described by ISO (2006). While *Staphylococcus aureus* count was counted on the Baird Parker agar plate using the surface-plating method (FDA, 2001).

2.5. Sensory evaluation

The sensory attributes of raw camel meat samples were evaluated by six-member panels that had undergone the necessary training and testing in sensory sensitivity, as earlier described by Fik and Leszczyńska-Fik (2007).

2.6. Statistical analysis

All data were statistically examined using two-way analysis of variance (ANOVA) (Geisser-Greenhouse's epsilon), with Graph Pad Prism (8.0.2). Dunnett's multiple comparisons test was used for post-hoc analysis, with significant differences regarded when $p<0.05$. All values were expressed as the mean ± SD of three replicates (Greenhouse and Geisser, 1959).

3. RESULTS

Results in (Figure 1) revealed that camel meat marinated with papaya and bromelain blended with whey or yoghurt had a considerably lower pH level than camel meat marinated with unfortified whey and yoghurt ($P< 0.05$). The acidic pH of the camel meat and the papaya-fortified marinade, notably yoghurt, had a notable interaction. Furthermore, higher pH values were obtained with a longer chilling duration of marinated camel meat within each treatment ($P < 0.05$). This pH increase was slower in camel meat marinated with papaya than in marinades enriched with bromelain ($P< 0.05$).

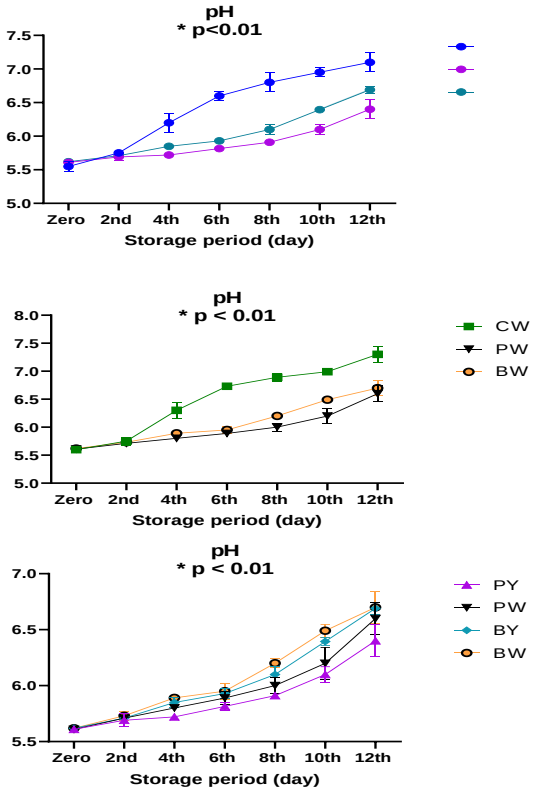

Figure 1 Impacts of papain and/ or bromelain enzymes fortified in yogurt or whey-based marinades on PH values of chilled camel meat all over 12 days storage period.

Results of TBA in Figure (2), and those of TVBN in Figure (3) matches greatly to those of pH in Figure (1), where, the camel meat marinated with papaya or bromelain-fortified mixes, particularly the papaya one ($P < 0.05$), showed a slower rate of rise in stored camel meat TBA and TVBN than the camel meat marinated with the other treatments ($P < 0.05$).

Concerning bacterial quality of marinated camel meat, When compared to control yoghurt (CY) and control whey (CW) groups, the lowering effect of marination combinations on TBC was statistically significant from the second day of storage ($P < 0.05$) (Table 2). While control yoghurt (CY) and control whey (CW) groups showed an increase in TBC. Papaya and bromelain fortified marinade

produced a lowering impact that lasted until the end of the experiment, and on the fourth day, papaya-fortified marinade, especially Yoghurt (PY), produced a greater reduction effect than bromelain fortified marinade ($P < 0.05$).

Moreover, the lowering effect of Papaya or Bromelain marination mixtures on *S. aureus* count was visible on the second day of storage ($P < 0.05$) and continued until *S. aureus* could not be retrieved on the sixth chilling day of the treated camel meat ($P < 0.05$). From the eighth day of the experiment till its end, *S. aureus* was not identified in the camel meat marinated with Papaya or Bromelain combinations ($P < 0.05$). On the other hand, the control yoghurt (CY) and control whey (CW) had no effect on *S. aureus* count and the count rose until the end of the trial (Table 3).

The average number of psychrotrophic bacteria in control yoghurt (CY) and control whey (CW) marinated camel meat increased over twelve days, reaching a maximum count of 7.45 ($P < 0.05$), (Table 4). While papaya or bromelain-marinated camel meat generated a reducing growth curve until no psychrotrophic bacteria could be detected at the eighth storage day in papaya-marinated camel meat ($P < 0.05$). However, no psychrotrophic bacteria could be found at the tenth day of storage in bromelain-marinated camel meat, especially when combined with yoghurt ($P < 0.05$). Comparing to control yoghurt (CY) and control whey (CW), papaya-marinated camel meat had much fewer psychrotrophic bacteria than bromelain-marinated camel meat, and both had a reducing effect on psychrotrophic bacteria ($P < 0.05$).

The results showed that all papaya and bromelain-fortified marinades, particularly papaya when mixed with yoghurt, had an antimicrobial impact on chilled camel meat coliforms (Table 5). This impact was noticeable after two days of chilling in papaya-fortified marinades, particularly when associated with yoghurt, which was powerful enough to diminish after eight days. In contrast, over twelve days, the typical number of coliforms in CY and CW marinated camel meat generated an increasing growth curve, reaching a maximum count of 5.37 ($P < 0.05$).

The impact of enzymatic marinades on sensory attributes was evaluated, the color, odor, consistency, and general acceptability of camel meat marinated in CY and CW declined over twelve days, reaching poor grade one after 10 days (Figure 4). While all marinades, including bromelain and papaya, especially when mixed with yoghurt, improved chilled camel meat sensory qualities, which were always graded higher than 3 even after 12 days.

4. DISCUSSION

Camel meat is a popular ethnic meal in the desert regions of the Middle East and North-East Africa. It has the potential to be a viable red meat substitute for human consumption worldwide. Camel meat quality varies according to age, breed, and muscle type ingested. Various procedures, including aging, low-temperature storage, and antioxidant pre-treatment, improve the quality and shelf life of camel meat (Baba et al., 2021).

Camel meat marinated in either whey or yoghurt, particularly yoghurt supplemented with papaya or bromelain enzymes, had significantly lower pH, Thiobarbituric Acid Number, and total volatile basic nitrogen levels than camel meat marinated in unfortified whey and yoghurt ($P < 0.05$). Similar antimicrobial benefits were observed in microbiological counts, where papaya or bromelain-fortified marinades had a persistent adverse impact on aerobic plate count (APC), *Staphylococcus aureus*, psychrotrophic bacteria, and coliforms. One of the most important methods for determining the chemical freshness of meat in long-term preservation is the TVB-N concentration (Cheng et al., 2016). Up to the end of the experiment, both papaya and bromelain-fortified marinades had a reducing effect on camel meat TVBN.

Papain and bromelain have been shown in several investigations to exhibit antimicrobial activity against a variety of bacteria, including *Escherichia* coli, *Proteus* species, *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Streptococcus pyogenes* (Praveen et al., 2014; Abdulrahman, 2015; Anjos et al., 2015; Hidayat et al., 2018; Mamo and Assefa, 2019). This may explain the persistently negative influence of papaya or bromelain-fortified marinades currently observed on pre-treated camel meat microbiological counts, TBC, *S. aureus*, psychrotrophic bacteria, and *coliforms*.

Staphylococcus aureus can be effectively eliminated by heat treatment and virtually all sanitizing agents. In light of this, the presence of this bacterium or its enterotoxins in prepared foods or on food processing machinery is an indication of poor hygiene (Hait et al., 2018). The fortified marination with papaya and bromelain showed a lowering impact on *S. aureus* count in the treated camel meat, which was observed from the second day of storage and continued to the end of the experiment (12^{nd} day of storage). Antibacterial activity of papaya and bromelain against *S. aureus* and other bacteria has been observed in various investigations. Carica papaya seeds have antibacterial properties that prevent the growth of both gram-positive and gram-negative bacteria. The observed activity was irrespective of fruit maturity stage (Dawkins et al., 2003).

Bacteria developing on meat at chill temperatures are regarded as psychrotrophic, and they belong to microbial genera of both gram-positive, such as lactic acid bacteria, and gram-negative bacteria, such as Pseudomonas (Ercolini et al., 2009; Atika et al., 2023). Papaya marinated camel meat had a larger suppressing effect on psychrotrophic bacteria than bromelain marinated camel meat, and both had a decreasing effect on psychrotrophic bacteria when compared to control yoghurt (CY) and control whey (CW).

Coliform bacteria are regarded as indicator organisms because their presence in food suggests that conditions are favorable for enteric pathogens to exist and can indicate inadequate sanitary conditions (Eden, 2014). The current findings demonstrated that all marinades including bromelain and papaya, especially papaya when combined with yoghurt, had an antibacterial effect on chilled camel meat coliforms. Bromelain was effective in vitro against *E. coli* and *L. monocytogenes*, respectively, at 25 and 35 °C (Eshamah, 2013).

The mechanism underlying bromelain's antibacterial effect is unknown (Jančič and Gorgieva, 2021), but it is thought that bromelain may inhibit bacterial growth by hydrolyzing certain peptide bonds in the bacterial cell wall (George et al., 2014). Papain and bromelain, as proteolytic enzymes, have an inherent property of digesting proteins, which may disintegrate bacterial membranes. The cell wall is destroyed when bromelain digests the surface proteins, allowing the cell to leak, expand, and collapse (Nguyen et al., 2010; Anisha et al., 2012). As a result, the preceding finding is supported by the way that Papain and bromelain inhibit both Gram-positive and Gram-negative bacteria.

The improved antibacterial properties of papaya-fortified marinades in comparison to bromelain-fortified marinades, especially those based on yoghurt (PY), were not previously

explored and may call for further, more in-depth research to demonstrate these distinctions.

According to past and current research findings, Bromelain and papain proteases have been found to improve meat safety and shelf life when stored at the proper temperatures, in addition to enhancing the sensory qualities of camel meat, color, odor, consistency, and general acceptability. Higher pathogen reductions might be attained if these enzymes are paired with current antibacterial methods (Eshamah, 2013).

5. CONCLUSIONS

In conclusion, enzymatic marinades enhanced the chemical quality of camel meat. AS, the chemical quality parameters of camel meat marinated in either whey or yoghurt, particularly yoghurt fortified with papaya or bromelain enzymes, were considerably lower than those of camel meat marinated in unfortified whey and yoghurt. Moreover, positive impacts on the bacterial quality of camel meat (APC, *Staphylococcus aureus*, psychrotrophic bacteria, and coliforms) were recorded. Particularly those based on yoghurt (PY) exhibited a greater growth-suppressing effect than bromelain-fortified marinades. The current study found that bromelain and papain proteases are an effective approach for improving camel meat acceptability and establishing chemical and antimicrobial systems for camel meat-based products.

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