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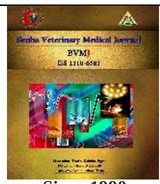

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Original Paper

The effect of reinforced marination with papain and bromelain on chilled beef meat quality.

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ABSTRACT

Beef is a very perishable food due to its higher water content and nutrient-dense matrix so, this study aimed to determine the impact of bromelain and papaya-fortified yoghurt (BY and PY) and whey-based marinade (BW and PW) mixtures on the bacteriological quality and shelf-life of chilled beef. Each treatment's seven chilling spots received one bag each, totaling seven bags. The pH, Thiobarbituric Acid, and total volatile basic nitrogen levels of chilled beef meat marinated in either whey or yoghurt, particularly yoghurt fortified with papaya (PY) or bromelain enzymes (BY), were considerably lower than those of control marinated beef ($P< 0.05$). Aerobic plate count, *Staphylococcus aureus*, psychrotrophic bacteria, and coliform count were simultaneously all negatively impacted by papaya or bromelain-fortified marinades in bacteriological counts. The PY in particular showed a stronger growth-suppressing impact than marinades enriched with bromelain ($P< 0.05$). After two days, when combined with yoghurt, this effect became noticeable, and after the tenth or twelve days, it was potent enough to totally suppress the growth of *Staphylococcus aureus*, psychrotrophic bacteria, and coliforms in beef. The sensory characteristics of the chilled beef meat were improved by all marinades, including papaya and bromelain, which were consistently rated higher than 3 overall even after 12 days. The results of the current study showed that bromelain and papain proteases offer a workable method for creating antibacterial systems for products derived from beef..

1. INTRODUCTION

Beef is a very perishable food due to its higher water content and nutrient-dense matrix (Ramanathan et al., 2022). Researchers and politicians have paid significantly less attention to food deterioration, storage, and transportation than to food security and sustainability, agricultural production (Tilman et al., 2011). Concern from stakeholders in the food system and policymakers has been raised by the amount of food loss and waste along the food supply chain (Muth et al., 2019). The food industry loses or wastes 40% of the food that is produced each year, which costs the sector $218 billion (Muth et al., 2019). Depending on their chemical makeup, different food products lose quality after harvest at varying rates (Hammond et al., 2015). Visual perception has a considerable influence on consumer impression of quality. For example, people frequently associate freshness and wholesomeness with the hue of meat.

The unavoidable realities of food production being fundamentally uneven in both time and location, as well as the fact that all food inevitably degrades, have resulted in various technical improvements in preservation, storage, and transportation (Hammond et al., 2015).

Marination is the process of soaking or injecting meat with a solution to flavor and tenderize products. Marination has been shown to reduce microbial spoilage and oxidation of meat, which can prolong the shelf life of fresh meat (Bianchi et al., 2009; Kargiotou et al., 2011). Earlier research findings have shown that the composition of the marinade, including the type and concentration of ingredients, can significantly affect the quality and safety of the marinated meat. The absorption of the marinade depends on the choice of red meat and poultry parts. Recent research (Erge et al., 2018; Nour, 2022; Rupasinghe et al., 2022; Unal et al., 2022) suggests a natural marinating of beef using fruit juices as a sour marinating process rather than synthetic chemicals. Furthermore, marinating in fruit juices may increase the diversity and range of value-added poultry products available(Augustyńska-Prejsnar and Kačániová, 2023) . Consumers are intrigued since the process is easy and quick while maintaining acceptable food sensory qualities (Gómez et al., 2020). Thus, natural materials can be used to substitute synthetic additives and additional substances that are commonly used in marinades.

The extraordinary potential of plant-derived supplements for illness prevention and therapy has long been recognized (Kumar et al., 2023). Natural enzymes such as papain and bromelain are rarely studied for their impact on meat preservation. Papain is an enzyme found in the papaya plant's secretion that protects the plant against insects (Konno et al., 2004). The pineapple plant, Ananas comosus, produces a set of enzymes called bromelain, which include sulfhydryl moieties. Bromelain has been found in recent studies to have a wide range of actions, including anti-inflammatory, anti-diabetic, anti-cancer, and anti-rheumatic

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effects. Because of these qualities, bromelain is a viable therapeutic candidate for the treatment of a variety of disorders (Kumar et al., 2023). Both papain and bromelain have been shown to increase the tenderness and flavour of fresh meat (Jančič and Gorgieva, 2021; Madhusankha and Thilakarathna, 2021; Mohd Azmi and Kumar, 2023; Praveen et al., 2014). Therefore, this study aimed to compare the preservation attributes of yoghurt and whey-based marinating mixtures enhanced with papain and bromelain enzymes on the qualitative elements of beef meat.

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 preparation.

Accurately 5-kg specimen of *Musculus longissimus thoracis* et *lumborum* (*M. longissimus*) was obtained from freshly slaughtered cattle. The muscles were cut into steaks that were 1.5 cm thick and weighed around 50±5 g each. There were two steaks in individual plastic bags. The steaks from each treatment were marinated in 2 kg of yoghurt- or whey-based marinating blends supplemented with 2% bromelain (BY and BW) or papain (PY and PW) enzymes. Over the seven chilling sites of each treatment, seven bags were allocated and deployed. Table 1 displays the various marinades' ingredients. Steak with yoghurt (CY) or whey (CW) was the control group (C). Following that, the bags were kept chilled for 2, 4, 6, 8, 10 and 12 days at 4 °C. All samples from these groups, control and treated, were evaluated for bacteriological, chemical, and sensory characteristics at the start of the experiment (within 2 hours after treatment). Trials were conducted in triplicate volunteers .

2.3. Evaluation of physico-Chemical quality

An electrical pH meter (Bye model 6020, USA) was used to determine the pH value of beef meat homogenate. Using the distillation method, the amount of total volatile basic nitrogen (TVBN) was determined ((Fishler 1995). The Thiobarbituric Acid Number (TBA) was determined by the spectrophotometric method in accordance with Egyptian organisation specifications (ES: 63-10 / 2006).

2.4. Evaluation of bacteriological quality

The method completely specified by ISO (2013) was used to determine the aerobic plate count (APC) in samples of beef meat. Decimal dilutions of the different samples were prepared, and aliquots of 0.1 ml of the appropriate dilutions were plated in duplicate on plate count agar (PCA) and cultivated aerobically for 10 days at 7°C in order to count psychrotrophic bacteria. Sterile Petri dishes containing Violet red bile agar were inoculated at 37 °C in accordance with ISO (2006)'s full instructions in order to count the coliforms. While the surface-plating approach was used to count *Staphylococcus aureus* on the Baird Parker agar plate.

2.5. Sensory evaluation

Five-member panels that have completed the required training and testing in sensory sensitivity assessed the sensory qualities of raw beef flesh samples, as earlier described by Fik and Leszczyńska-Fik (2007).

2.6. Statistical analysis

With Graph Pad Prism 8.0.2 and P< 0.01 using two-way analysis of variance (ANOVA) (Geisser-Greenhouse's epsilon), all data were statistically analyzed. Post-hoc analysis was conducted using Dunnett's multiple comparisons test. Every piece of information was expressed as the mean SD of two replicates (Greenhouse and Geisser, 1959).

3. RESULTS

The findings indicate that the pH levels of chilled beef samples varied according to the type of marinade and the length of storage (Figure 1). After two days of cooling, the pH of the beef in various papaya or bromelain-fortified marinades was lower than that of the whey and yoghurt-based control marinades (P < 0.05). The papaya-fortified marinade, particularly yoghurt, was significantly associated with acidic meat pH as compared to the bromelain (BY and BW) marinades. Additionally, longer cooling times within each condition led to higher pH values of marinated beef (P< 0.05). After 6 days, the control whey and yoghurt-based marinaded beef reached borderline pH, whereas all treatments remained within safe pH levels.

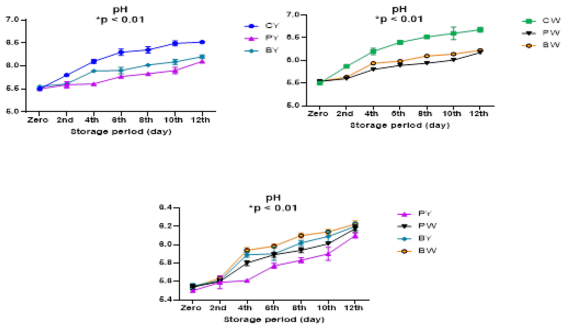

Figure 1 illustrates The impact of Bromelain and papaya-supplemented yoghurt- and whey-based marinades on the pH of chilled beef for 12 days.

The Thiobarbituric Acid Number (TBA) levels in the control chilled beef and beef marinated with bromelain (BY and BW) samples were greater after two cooling days than in the papaya (PY and PW) supplemented marinades (Figure 2). By the end of the chilling period, these significant differences remained, and chilled beef marinated in papaya—specifically, the yoghurt PY and PW—expressed less TBA than the beef marinated in bromelain (BY and BW), the control groups. All beef treatments were acceptable through the end of chilling, however, the TBA level in the control group reached the maximum level allowed after four days.

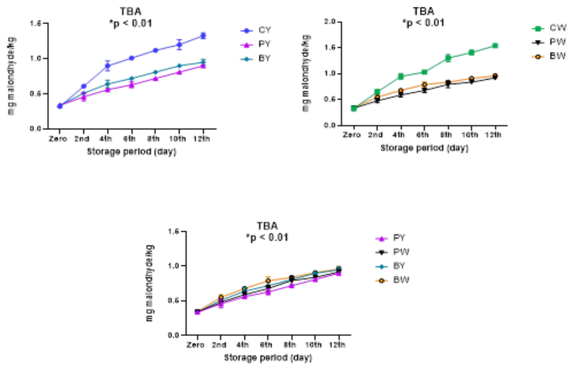

Figure 2 illustrates the effect of yoghurt- and whey-based marinades containing bromelain and papaya on the Thio barbituric acid level in chilled beef for 12 days.

After two chilling days, control chilled beef group higher TVB-N levels than beef marinated with fortified papaya and bromelain marinades groups (Figure 3). These substantial effects persisted until the final day of the chilling period, and chilled beef marinated with papaya (especially yoghurt PY) group had the strongest TVB-N reducing impact than the control chilled beef and the Bromelain marinated groups. After six days, the control group reached the maximum undesirable TVB-N level, whereas all beef treatments were acceptable until the end of chilling.

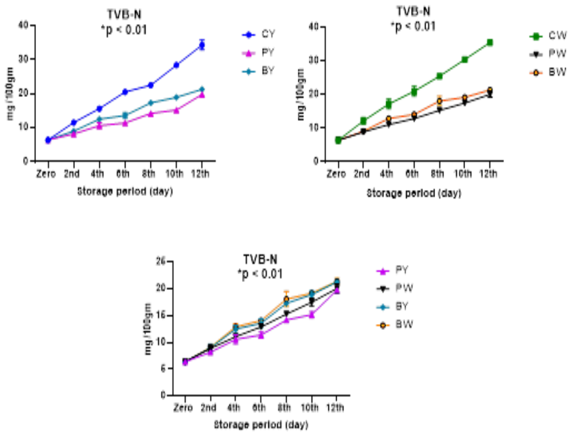

Figure 3 illustrates the effect of bromelain and papaya-supplemented yoghurt- and whey-based marinades on the total volatile basic nitrogen level of chilled beef after 12 days.

The effect of yoghurt- and whey-based marination mixes enhanced with bromelain and papaya on the total bacterial count (TBC) of chilled beef over 12 days as shown in Figure (4). All treatments had a substantial antibacterial impact on beef TBC after two cooling days, which remained until the end of the experiment (P< 0.05). Among the four treatments, papaya-fortified marinade, particularly Yoghurt (PY), outperformed bromelain-fortified marinade (P<0.05).

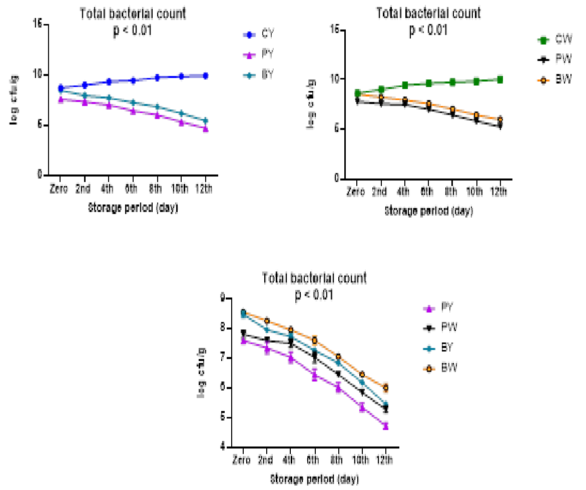

Figure 4 illustrates The effect of yoghurt- and whey-based marination mixes enhanced with bromelain and papaya on the total bacterial count (TBC) of chilled beef over 12 days.

After two days of chilling, the beef *S. aureus* count significantly decreased under all treatments, and this effect persisted through the completion of the trial (P< 0.05) (Figure 5). Papaya-fortified marinades, in particular Yoghurt (PY), surpassed the antimicrobial effect of bromelain-fortified marinades among the four treatments (P< 0.05). In PY, PW, and BY-marinated beef, *S. aureus* could not be detected after ten and twelve days, respectively.

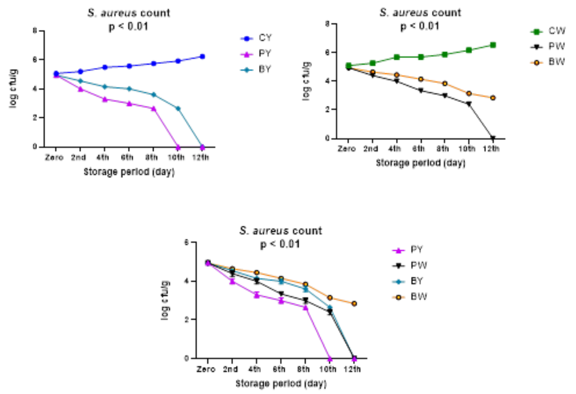

Figure 5 illustrates The impact of bromelain and papaya-fortified yoghurt and whey-based marinade mixes on the *S. aureus* count of chilled beef after 12 days.

When compared to control chilled beef after two days of cooling, the beef Psychrotrophic count reduced considerably under all marinade mixtures, and this effect persisted throughout the trial (P <0.05) (Figure 6). Among the four treatments, papaya-fortified marinades, particularly Yoghurt (PY), were consistently associated with a greater antimicrobial properties than bromelain-fortified marinades (P< 0.05). After twelve days, the Psychrotrophic count in PY and PW marinated beef could not be observed.

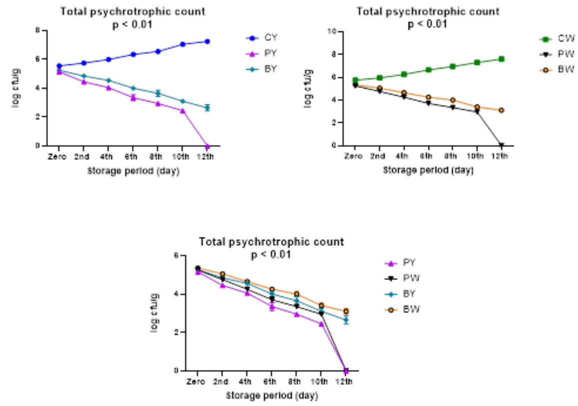

Figure 6 illustrates the effect of bromelain and papaya-fortified yoghurt and whey-based marinade mixes on the Psychrotrophic count of chilled beef after 12 days.

The beef coliform count significantly decreased under all marinade mixes as compared to control beef after two days of refrigeration, and this effect persisted over the entire experiment (P< 0.05) (Figure 7). Papaya-fortified marinades, particularly PY, were consistently linked to stronger antimicrobial capabilities than bromelain-fortified marinades among the four treatments (P< 0.05). The coliform count in the marinated beef from PY and PW could not be seen after twelve days.

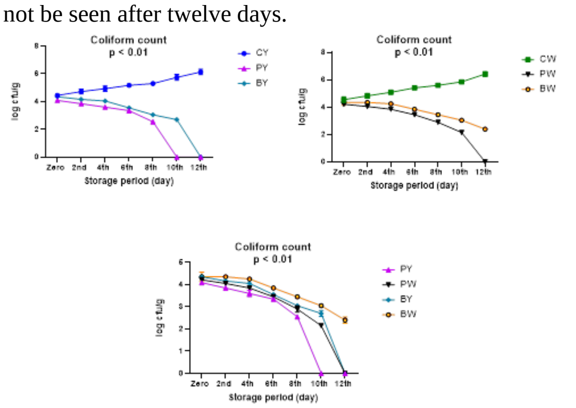

Figure 7 illustrates The effect of bromelain and papaya-enriched yoghurt and whey-based marinade mixes on the coliforms count of chilled beef after 12 days.

Based on the heat map, Control beef's color, aroma, consistency, and overall acceptability deteriorated over twelve days, reaching poor grade zero and one after six to eight chilling days. All marinades, including bromelain and papaya, improved chilled beef sensory attributes, which were always rated higher than 3 even after 12 days (Figure 8).

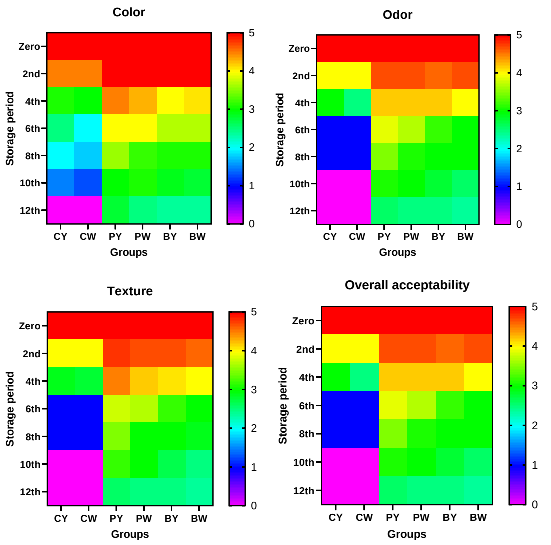

Figure 8 illustrates the impact of 2% marination combinations on the sensory quality of chilled beef over 12 days. These combinations include papaya-fortified yoghurt (PY), papaya-fortified whey marinade (PW), bromelain-fortified yoghurt (BY), and bromelain-fortified whey (BW).

4. DISCUSSION

The primary objective of this research was to assess the practical preservation properties of yoghurt and whey-based marinating remedies enhanced with papain and bromelain enzymes on chilled beef meat. The pH, Thiobarbituric Acid Number, and total volatile basic nitrogen levels of beef marinated in papaya- and bromelain-supplemented yoghurt- or whey-based marinades groups were substantially lower than those of control chilled beef. Fresh beef has a pH between 5.5 and 6.2, with 5.6 and 5.8 being the best range for meat quality A healthy pH level helps to preserve meat for a longer time while also guaranteeing that it is fresh, tender, and juicy (Hopkins et al., 2014; Shange et al., 2018). The pH of chilled beef samples in different papaya or bromelain-fortified marinades was lower than that of the whey and yoghurt-based control marinades ones after two days of chilling. In comparison to the bromelain (BY and BW) marinades, the papaya-fortified marinade, especially yoghurt, was strongly related to acidic meat pH.

The total volatile basic nitrogen (TVB-N) content is a physicochemical measure used to assess meat freshness (Ma et al., 2013). TBA is used to gauge the degree of lipid oxidation in beef and may be the primary non-microbial element influencing the safety and quality of meat and meat products (Lorenzo and Gómez, 2012). By the second chilling day, beef marinated in papaya and bromelain samples had significantly lower TBA and TVB-N levels than control chilled beef marinades ones those persisted through the chilling process. Additionally, compared to the chilled beef marinated in bromelain and the control group, the beef marinated in papaya—more specifically, the yoghurt PY—expressed less TBA and TVB-N. Through the end of chilling, all chilled beef treatments were acceptable; however, after four days and six days, the TBA and TVB-N levels in the control group reached the upper acceptable limits.

Beef TBC is an essential indicator of beef quality and safety since high TBC readings might suggest bacterial contamination and meat deterioration (Dirpan and Hidayat, 2023). Staphylococcal food-borne disease is one of the most frequent food-borne issues globally, caused by preformed *S. aureus* enterotoxins in food. The presence of microorganisms in food products poses a possible risk to customers and causes significant economic and human productivity loss due to food-borne sickness (Byrd-Bredbenner et al., 2013). Psychrotrophic bacteria are frequently found in chilled meat, and their proliferation can significantly lower the meat's shelf life and cause spoiling (Marquezini et al., 2016). Although the vast majority of coliforms have recently been linked to the environment, they remain indicators of fecal contamination (Martin et al., 2016).

In terms of bacteriological indices, the antimicrobial effects of papaya or bromelain enzymes were especially apparent in the bacteriological indices TBC, *S. aureus*, Psychrotrophic count, and coliform count of marinated beef boosted with these enzymes. Bromelain and papain have been shown in several investigations to exhibit antimicrobial activity against a variety of bacteria, including *Escherichia coli*, *Proteus* species, *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *L. monocytogenes* (Abdulrahman, 2015; Eshamah, 2013; Hidayat et al., 2018; Mamo and Assefa, 2019; Praveen et al., 2014). In the past, researchers observed that bromelain was effective against all isolated strains of both aerobic and anaerobic bacteria (Praveen et al., 2014) and that it suppressed Gram-positive bacteria's growth more potently than Gram-negative bacteria (Abdulrahman, 2015). The antibacterial effects of bromelain and papain extracts were also evident in *S. aureus* biofilms, where bromelain and papain considerably reduced biomass more than lysostaphin, causing EPS matrix breakdown and bacterial detachment (Dawkins et al., 2003; Watters et al., 2016).

The precise mechanism by which bromelain and papain act to prevent bacteria from growing is unknown, however, it is believed that it either hydrolyzes certain peptide bonds in the bacterial cell wall or breaks down proteins and peptides necessary for bacterial survival and growth (Amini et al., 2018; Eshamah, 2013; Jančič and Gorgieva, 2021). To completely comprehend the mechanism underlying bromelain's antimicrobial activity and to identify its potential uses in biomedicine and food preservation, additional study is required (Kumar et al., 2023).

The most common application for papain and bromelain is to tenderize chilled beef. High doses of papain, on the other hand, are not recommended because they might create pastiness in meat (Istrati, 2008). Over twelve days, the control beef's color, flavor, consistency, and general acceptability all decreased, reaching poor grades zero and one after six to eight chilling days. All marinades, including those with papaya and bromelain, enhanced the sensory qualities of cold beef, which were consistently rated better than 3 even after 12 days of chilled beef storage.

5. CONCLUSIONS

Besides, improved sensory chilled beef properties, papaya and bromelain-fortified marinades suppressed meat spoilage indices; PY in particular shown a higher growth-suppressive effect than bromelain. According to the current study's findings, bromelain and papain proteases provide a practical way to build antibacterial systems for beef-derived products.

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