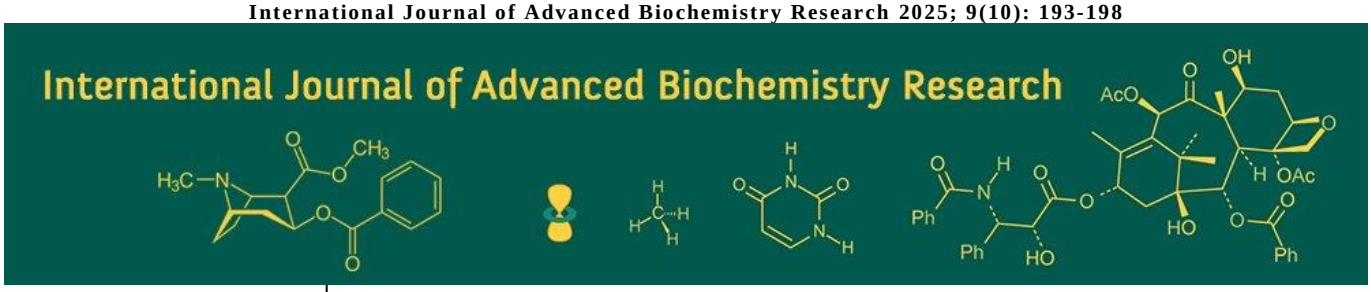

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Effect of whey based marinades on broiler meat quality

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Abstract
This research investigated how whey-based marinades influence various quality parameters of broiler chicken breast meat. Samples were subjected to four marination treatments: immersion marination with 3% salt solution (C1), vacuum tumbler marination with 3% salt solution (C2), vacuum tumbler marination with acid whey and 2.5% salt solution (T_1), and vacuum tumbler marination with sweet whey and 2.5% salt solution (T_2). All samples were analysed for physicochemical, functional, and antioxidant properties.
Marinade absorption differed significantly ($p<0.001$) among samples, with whey-marinated groups (T_1, T_2) showing higher absorption compared to controls; among controls, C2 recorded the highest value. Water-holding capacity (WHC) was significantly higher ($p<0.001$) in sweet whey-marinated samples. Tyrosine values differed significantly ($p<0.01$), with C1 showing the lowest and whey-marinated samples (T_1, T_2) recording higher values. Collagen solubility was significantly improved ($p<0.001$) in T_1 and T_2, followed by C2, compared to C1. Proximate composition revealed no significant differences in fat, whereas ash and protein content differed significantly ($p<0.001$). Whey-marinated samples and C2 exhibited significantly higher mineral content, while protein content was highest in C1. Moisture content showed a significant decline in all groups; however, between treatments, moisture was consistently higher in sweet whey-marinated samples. No significant differences were observed in cooking loss, pH, or shear force across groups.
Both acid and sweet whey marinades exhibited notable radical-scavenging activity, with values of 59.5 percent and 60.0 percent, respectively, indicating that whey-based marinades effectively enhanced antioxidant potential in chicken breast meat. Overall, incorporation of acid and sweet whey in marinades enhanced functional properties such as marinade absorption, WHC, collagen solubility, mineral enrichment, and antioxidant activity without negatively affecting texture or stability, thereby making whey-based marinades a promising strategy for improving broiler meat quality.

Keywords: Marination, whey, vacuum tumbling

Introduction
Marination is commonly applied in meat processing to enhance flavour, tenderness, juiciness, and overall acceptability of meat products (Smith and Acton, 2000) [19]. It involves the use of aqueous solutions containing salts, acids, enzymes, or functional ingredients that penetrate into muscle tissues, thereby modifying physicochemical and sensory properties. Conventional marinades commonly rely on sodium chloride and phosphates to enhance water-holding capacity, texture, and shelf life. However, increasing health concerns regarding high sodium intake and the need for clean-label products have driven the search for natural, functional alternatives (Alvarado and McKee, 2007) [1].
Whey, a major by-product of the dairy industry, is available in large volumes and contains valuable nutrients such as proteins, lactose, and minerals. Its functional properties, including buffering capacity, antioxid ant potential, and mineral richness, make it a promising candidate for use in meat processing (Smithers, 2015) [20]. Acid whey, generated during the manufacture of products like cottage cheese and Greek yogurt, and sweet whey, produced from rennet-based cheese production, differ in composition but both represent sustainable, low-cost ingredients that can add value to meat products.
Recent research has explored the use of dairy-based marinades to improve meat tenderness, juiciness, and nutritional quality while reducing reliance on synthetic additives.

Incorporating whey into marinades has the potential to enhance water-holding capacity, collagen solubility, and oxidative stability of meat, along with contributing to mineral enrichment and functional quality (Karageorgou *et al*., 2023; Simitzis *et al*., 2021) [10, 17]. Despite these advantages, limited studies have compared the effects of acid and sweet whey on poultry meat quality under different marination techniques.

This study aimed to assess how marinades based on acid and sweet whey influence the physicochemical, functional, and sensory quality characteristics of broiler chicken breast meat. The findings may provide insights into the sustainable utilization of dairy by-products while offering natural alternatives for improving poultry meat quality.

Materials and Methods

Broiler chicken of five weeks of age were purchased from the local market and brought to the department of Livestock Products Technology, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, India. They were slaughtered under hygienic conditions. Breast meat was then harvested, washed, and drained. The chicken breasts were divided into four equal batches.

Acid and sweet whey were prepared under hygienic conditions, and the pH was measured. pH of acid and sweet whey is 4.7 and 6.01 repectively. Marinades were prepared based on preliminary sensory trials. C1 was by immersing the chicken breast in a marinade containing 3 percent salt at 1:1 (meat: marinade) ratio for 2 hours under chiller conditions (4 ± 1 °C). Treatment C2 was by prepared by subjecting breast meat to tumbling in 3 percent salt solution. The duration, interval, and frequency of tumbling were standardized as 15 minutes of tumbling followed by 5 minutes of rest, and another 15 minutes of tumbling. Treatments 1and 2 were prepared by vacuum tumbling breast meat using acid and sweet whey-based marinades respectively which were incorporated with 2.5 percent salt in them. After marination, all samples were drained for 15 minutes.

Marinade absorption

Marinade absorption was calculated as per Yusop *et al*. (2010) [24] based on the weight difference of the meat before and after marination.

$$\text{Marinade absorption (\%)} = \frac{\text{W2-W1}}{\text{W1}} \text{ X } 100$$

W1= Initial weight of meat before marination
W2= Weight of meat after marination

Cooking loss

Cooking loss was determined according to the method of Boccard *et al*. (1981) [6], with minor modifications. Approximately 80 g of meat was sealed in high-density polyethylene (HDPE) pouches after removing entrapped air. The pouches were heated in a water bath at 75 °C for 50 minutes, then cooled under running water for 40 minutes. The cooked meat was blotted to remove surface moisture and weighed. Cooking loss was calculated using the formula:

$$\text{Cooking loss (\%)} = \frac{(X1 - X2)}{X1} \times 100$$

where
X1 = raw sample weight (g) and X2 = cooked sample weight (g).

pH

Sample pH was determined as described by AOAC (2016) [2]. Ten grams of meat was homogenized with 50 mL of distilled water using a tissue homogenizer (Kinematica, Switzerland) at 4000 rpm for one minute. The pH of the homogenate was measured with a digital pH meter equipped with a glass electrode (EUTECH Instruments pH 510, Singapore).

Water holding capacity

WHC was assessed following the method of Wardlaw *et al*. (1973) [23]. About 10 g of finely chopped meat was mixed with 16 mL of 0.6 M sodium chloride solution. The mixture was incubated at 4 °C for 30 minutes and centrifuged at 7000 rpm for 15 minutes. The supernatant was removed, and WHC was expressed as a percentage.

Tyrosine Value

The tyrosine content of meat samples was analyzed following the method of Pearson (1968) [14] with minor adjustments. Precisely 2 g of sample was homogenized with 40 mL of 5% trichloroacetic acid (TCA) for 2 minutes using a tissue homogenizer (Kinematica, Switzerland) at 4000 rpm. The homogenate was filtered, and the clear extract obtained was used for the assay. For estimation, 2.5 mL of the TCA extract was transferred into a test tube and combined with an equal volume of distilled water along with 10 mL of 0.5 N sodium hydroxide. The mixture was thoroughly shaken, after which 3 mL of diluted Folin-Ciocalteu reagent (1 mL concentrated FC reagent diluted with 2 mL distilled water) was added. The reaction mixture was kept undisturbed at room temperature for 5 minutes. Absorbance was measured at 660 nm using a UV-Visible spectrophotometer (UV/VIS Lambda 25, Perkin Elmer, Singapore). A blank prepared using TCA, distilled water, sodium hydroxide, and diluted FC reagent was run simultaneously as reference. Tyrosine concentration was derived from a standard curve and expressed as milligrams of tyrosine per 100 g of sample.

Shear Force

Shear force was determined according to the procedure of Sams (1990) [16] with slight modifications. Meat samples were sealed in HDPE (High-Density Polyethylene) pouches, cooked until the internal temperature reached 80 °C (monitored using a probe thermometer) for 50 minutes, and then chilled at 2-3 °C overnight. The cooked meat was cut into pieces measuring 1.5 × 1.5 × 0.5 cm and subjected to shear testing using a wedge-shaped blade attached to a texture analyser (Model EZ-SX, Shimadzu Corporation, Japan) operated at a crosshead speed of 200 mm/min. Shear force values were recorded and expressed in Newtons (N).

Collagen Solubility

Collagen solubility in muscle samples was estimated following the method described by Hill (1966). The

calculation was based on the hydroxyproline content present in the sample.

2,2-Diphenyl-1-picryl hydrazyl (DPPH) Radical Scavenging assay of marinades

The antioxidant activity of the marinade solution was evaluated using the DPPH free radical scavenging assay as outlined by Zare *et al*. (2019) [25], with slight modifications. A methanolic solution of DPPH was prepared by dissolving 4 mg of the reagent (Sisco Research Laboratories Pvt. Ltd., Mumbai, India) in 1000 mL of methanol. For the assay, 100 mg of sample was mixed with 5 mL of the DPPH solution and incubated in the dark for six hours. The absorbance was then measured at 517 nm. A control solution without the sample was included for comparison. Antioxidant activity (%) was calculated using the formula:

$$\text{Antioxidant activity (\%)} = \frac{A_0 - A_T}{A_0} \times 100$$

Where

A_0= absorbance of the control and A_T= absorbance of the test sample.

Chemical Composition

Moisture Content

Moisture content was estimated according to AOAC (2016) [2]. Approximately 20 g of finely minced sample was weighed into a clean, dry evaporating dish and dried in a hot-air oven (Rotek, Mumbai) at 100±2 °C for 18 hours. The dried sample was cooled in a desiccator and weighed. Moisture percentage was calculated based on weight loss during drying using the formula:

$$\text{Moisture (\%)} = \frac{\text{W2} - \text{W3}}{\text{W2} - \text{W1}} \times 100$$

where:

- $W1$= weight of empty dish
- $W2$= weight of dish + fresh sample
- $W3$= weight of dish + dried sample

Fat Content

Fat percentage was determined according to AOAC (2016) [2]. Approximately 3 g of accurately weighed, moisture-free sample was extracted with petroleum ether (boiling point 60-80 °C) using a Soxhlet extraction unit (Socs Plus SCS 06E, Pelican Equipment, Chennai) for 2.5 hours. The recovered ether extract was dried in a hot air oven at 100 °C until a constant weight was obtained, cooled in a desiccator, and then weighed. Fat percentage was calculated on a dry matter basis, converted to wet matter basis, and expressed as percentage of sample weight using the formula:

$$\text{Fat (\%)} = \frac{\text{W2} - \text{W1}}{\text{W3}} \times 100$$

where:

- W1= weight of empty oil flask
- W2= weight of oil flask + fat
- W3= weight of sample taken

Protein

Protein content was analysed as per AOAC (2016) [2] using the Micro-Kjeldahl method. About 2 g of accurately weighed sample was digested in a Micro-Kjeldahl digester (Kelplus-KES06LE, Pelican Equipment, Chennai) with a catalyst mixture (90 parts sodium sulphate + 10 parts copper sulphate) and 25 mL concentrated sulphuric acid (36 N). Digestion was carried out initially at low heat until frothing subsided, and then continued at higher temperature until the solution became clear. The digest was cooled, transferred into a 200 mL volumetric flask, and diluted to volume with distilled water.

From this solution, 10 mL aliquot was distilled with 20 mL of 40% sodium hydroxide in a Micro-Kjeldahl distillation unit (Kelplus Distyl-EMBA, Pelican Equipment, Chennai). Steam distillation was performed into 5 mL of 2% boric acid solution containing a mixed indicator (0.2% methyl red: 0.2% bromocresol green = 1:2). A total of 35 mL distillate was collected and titrated against 0.1 N sulphuric acid. Nitrogen percentage was calculated using the following formula:

$$\text{Nitrogen (\%)} = \frac{(\text{A} - \text{B}) \times 0.0014 \times \text{Total Volume Made}}{\text{Weight of Sample Taken} \times \text{Volume Distilled}} \times 100$$

where:

- A= titration value of sample
- B= titration value of blank

Protein percentage was obtained by multiplying nitrogen content with the conversion factor (6.25), assuming that all nitrogen originated from protein.

Ash Content

Ash percentage was estimated by the gravimetric method as per AOAC (2016). Between 5-10 g of ground meat sample was placed into a pre-weighed crucible, charred on a hot plate at 100 °C for 30 minutes, and then incinerated in a muffle furnace (Rotek, Mumbai) at 550 °C for 5 hours. After that, the crucible was cooled in a desiccator containing fused calcium chloride and reweighed after one hour. Ash content was calculated using the formula:

$$\text{Ash (\%)} = \frac{\text{Weight of Ash}}{\text{Weight of Sample Taken}} \times 100$$

Results and Discussion

Marinade absorption differed significantly among groups (p<0.001), with acid whey (T_1) and sweet whey (T_2) marinated samples exhibiting higher uptake than the controls. Among controls C2 showed highest marinade absorption. This finding agrees with previous reports demonstrating that dairy-based marinades enhance absorption efficiency in meat. Augustyńska-Prejsnar *et al*. (2019b) [4] observed that acid whey improved absorption in chicken breast compared to lemon juice, while Karageorgou *et al*. (2023) [10] noted greater uptake in pork and chicken marinated with yoghurt acid whey, particularly with longer marination periods. The role of vacuum tumbling was also evident, as Singh *et al*. (2020) [18] reported that tumbling significantly improved absorption in chicken, indicating that both marinade composition and application method play a crucial role in uptake and subsequent meat quality.

Water holding capacity values also differed significantly among groups (p<0.001), with sweet whey-marinated samples maintaining relatively higher values compared to

controls. This effect could be attributed to whey proteins interacting with muscle proteins to improve water retention through gel formation and protein-protein interactions. Similar improvements in WHC, tenderness, and juiciness were reported by Augustyńska-Prejsnar *et al*. (2019b) [4] in chicken breast marinated with acid whey relative to lemon juice.

Proteolytic changes, as indicated by tyrosine values, showed a significant difference (p<0.01) across all groups. The control group (C1) consistently exhibited the lowest values, whereas T_1 and T_2 recorded significantly higher values. Tyrosine levels increased progressively during storage, indicating enhanced protease activity in whey-marinated samples. The presence of organic acids and bioactive compounds in whey, coupled with vacuum tumbling, likely facilitated proteolytic activity, a trend consistent with observations by Ge *et al*. (2022) [8] in chicken marinated with organic acid solutions.

Collagen solubility differed significantly (p<0.001) among samples, with both whey-based groups (T_1 and T_2) showed higher values than controls, and C2 exhibiting higher values than C1. This enhancement is associated with improved tenderness, as soluble collagen is less resistant to shear force than heat-stable, cross-linked insoluble collagen. Rahman *et al*. (2023) [15] reported that acidic components such as vinegar, lemon juice, and soy sauce enhanced collagen solubility by breaking down connective tissues, while Kim *et al*. (2014) [13] similarly found that acidic marinades increased collagen solubility in tumbled chicken breast. The organic acids and bioactive compounds present in whey therefore appear to contribute significantly to collagen breakdown and tenderness.

Both acid and sweet whey marinades also demonstrated notable radical-scavenging activity, with values of 59.5% and 60.0%, respectively. This suggests that whey can enhance oxidative stability in meat, consistent with previous findings. For instance, Kim and chin, (2021) [12] reported improved antioxidant activity when whey-based marinades were supplemented with *Cudrania tricuspidate* fruit powder, and Febrianta *et al*. (2021) [7] showed that turmeric extract in whey marinades increased DPPH radical-scavenging capacity during storage. Keska *et al*. (2019) [11] further observed superior antioxidant activity in bovine muscle peptide extracts marinated in acid whey. These observations confirm that whey, besides its tenderizing properties, contributes to oxidative stability through bioactive peptides and antioxidant compounds.

No significant differences (p>0.05) were observed among treatments in cooking loss, pH, or shear force. This indicates that while whey-based marinades substantially improved parameters such as absorption, WHC, collagen solubility, and oxidative stability, they did not adversely affect fundamental technological properties of meat during cooking or alter its acidity. The absence of variation in shear force further suggests that improvements in tenderness were more likely driven by biochemical changes such as enhanced collagen solubility and proteolytic activity.

Analysis of proximate composition revealed no significant differences in fat, while protein and ash contents varied significantly. Protein content was highest in the control group (C1), whereas whey-marinated groups exhibited lower values, likely due to the dilution effect of water uptake during tumbling, as reported by Bishnoi *et al*. (2017) [5]. In contrast, ash content was significantly higher in whey-marinated samples, which can be attributed to the mineral-rich nature of whey being absorbed into the muscle during marination. Similar increases in mineral content were noted by Augustyńska-Prejsnar *et al*. (2021) [3] in poultry and by Wójciak *et al*. (2015) [23] in beef marinated with acid whey and set milk. Moisture content declined overall during storage, but sweet whey-marinated samples consistently retained higher levels than controls, supporting the observed dilution effect on protein content. Previous studies by Ge *et al*. (2022) [8] and U-Chupaj *et al*. (2021) [21] linked such higher water retention in dairy-marinated samples to improved juiciness and tenderness.

Overall, the findings indicate that whey-based marinades not only altered the proximate composition of chicken breast by reducing protein content while enhancing ash and moisture levels, but also significantly improved functional properties including marinade absorption, water retention, collagen solubility, and antioxidant capacity. While the proteolytic and hydrating effects of whey enhanced tenderness, juiciness, and mineral enrichment, the associated dilution effect slightly lowered protein concentration, reflecting a trade-off between sensory improvements and nutritional density. These results are consistent with previous reports highlighting the multifaceted role of whey in meat processing (Simitzis *et al*., 2021; Karageorgou *et al*., 2023) [17, 10]. Collectively, acid and sweet whey-based marinades, particularly when applied with vacuum tumbling, offer a promising approach for improving the functional, nutritional, and oxidative stability characteristics of broiler chicken breast meat.

Table 1: pH, Marinade absorption (%), Cooking loss (%), Water holding capacity (mL), Tyrosine value (mg/100g), Shear force (N) and Collagen solubility (%) of C1, C2, T_1 and T_2

Parameter	C1	C2	T_1	T_2	T_3
pH	5.55±0.05	5.45±0.25	5.54±0.06	5.38±0.10	0.354^{ns} (0.787)
Marinade absorption (%)	2.58d±0.14	6.98c±0.09	11.78a±1.02	11.68b±2.29	634.3** (<0.001)
Cooking loss (%)	23.25±0.56	23.21±0.57	23.23±0.12	23.20±0.37	0.003^{ns} (1.00)
Water holding capacity (mL)	15.04^{a}±0.004	14.56^{c}±0.005	14.85^{b}±0.09	14.33^{d}±0.01	42.456** (<0.001)
Tyrosine value (mg/100g)	10.98^{c}±0.62	14.36^{b}±1.78	16.72^{a}±0.98	17.21^{a}±0.94	5.999** (0.010)
Shear force (N)	139.2±18.4	113.46±12.78	99.44±8.26	99.68±6.55	2.279^{ns} (0.132)
Collagen solubility (%)	30.25^{c}±0.068	32.15^{b}±0.262	34.95^{a}±0.138	35.11^{a}±0.268	133.862** (<0.001)

Table 2: Moisture (%), Ash (%) and Protein (%) of C1, C2, T_1 and T_2

Moisture (%)	77.17^{a}±0.06	77.22^{a}±0.03	77.15^{a}±0.01	77.54^{b}±0.02	886.426** (<0.001)
Ash (%)	1.34^{c}±0.01	1.42^{b}±0.01	1.46^{a}±0.01	1.46^{a}±0.01	30.782** (<0.001)
Protein (%)	21.46^{a}±0.10	19.38^{b}±0.10	19.16^{b}±0.06	19.14^{b}±0.05	201.58** (<0.001)
Fat (%)	1.83±0.03	1.83±0.02	1.83±0.05	1.83±0.02	0.004^{ns} (1.00)

Conclusion

Incorporation of acid and sweet whey into marinades was found to substantially improve the functional characteristics and nutritional value of broiler chicken breast meat. Whey-based marinades improved marinade absorption, water-holding capacity, collagen solubility, mineral enrichment, and antioxidant activity, while promoting proteolytic changes that contributed to improved tenderness and juiciness. Although protein content decreased slightly due to dilution from higher water uptake, ash content increased, reflecting the mineral-rich composition of whey. Importantly, no adverse effects were observed on key technological properties such as cooking loss, pH, and shear force, indicating that whey marinades did not compromise processing or textural stability.

Overall, the findings highlight whey, a sustainable dairy by-product, as a natural and functional alternative to conventional marinades. Its application, particularly in combination with vacuum tumbling, offers an effective strategy to improve the quality, oxidative stability, and nutritional value of poultry meat, while supporting the valoriation of dairy industry by-products for meat processing applications.

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