JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

EFFECT OF PAPAIN EXTRACTED FROM PAPAYA PEEL USING AQUEOUS TWO-PHASE SYSTEM ON TENDERIZATION OF CHICKEN AND MUTTON

Mohit Singla[1, 2], *Bhaskar Jyoti Kalita*[1], *Nandan Sit*[1]*

Address(es): Prof. Nandan Sit,
[1] Department of Food Engineering and Technology, Tezpur University, Assam-784028, India.
[2] Department of Biotechnology Engineering and Food Technology, Chandigarh University, Punjab – 140413, India.

*Corresponding author: nandansit@yahoo.co.in

https://doi.org/10.55251/jmbfs.11501

ARTICLE INFO

Received 3. 7. 2024
Revised 24. 4. 2025
Accepted 19. 5. 2025
Published 1. 8. 2025

Regular article

OPEN ACCESS

ABSTRACT

The aim of this study was to investigate the enzymatic tenderization of mutton and chicken meat using papain, an enzyme derived from the peel of papaya, through the utilization of aqueous two-phase extraction. The extracted papain enzyme was then employed in a dipping method to assess its influence on the tenderness of the meat. Different concentrations of papain (ranging from 0.025% to 0.2%) were added to a solution, and the meat samples were dipped in specific concentrations of the enzyme solution. Texture profile analysis, cooking profile evaluation, and sensory assessment were conducted to determine the tenderness of the meat. The results demonstrated that as the concentration of papain increased, the tenderization of the meat improved. For chicken meat, a 0.2% papain concentration resulted in a hardness of 1.53N, compared to 29.83N for the control sample. Similarly, for mutton, a 0.2% papain concentration led to a hardness of 7.97N, while the control sample exhibited a hardness of 77.9N. Based on sensory evaluation, the 0.05% papain concentration was chosen for chicken meat, and a 0.125% concentration was selected for mutton to achieve optimal tenderization. These findings provide confirmation that papain, as a proteolytic enzyme, effectively enhances meat quality by improving tenderness.

Keywords: Papaya peel, papain, tenderization, chicken, mutton

INTRODUCTION

For many people, meat is their preferred source of animal protein as it's the most valuable livestock product **(Jayawardena *et al*., 2022)**. Meat is either eaten as processed meat or as a component of home-style meal preparations. Rigor mortis, a natural process that occurs after animal slaughter, causes the muscle protein fibres to shorten and the tissue to become tough **(Simpson *et al*., 2012)**. Tenderness, juiciness, and taste are the three factors that matter most when evaluating meat quality. In animal meat, tenderness is preferably a significant organoleptic property **(Lawrie and Ledward 2014)**. It varies by animal species, breed, age, sex, and specific skeletal muscle tissue. Myofibrils and intermediate filaments in muscle fibres as well as the collagen-rich endomysium and perimysium of the intramuscular connective tissue are responsible for tenderness **(Elzalaky, 2024; Abdalla *et al*., 2013)**.
Meat can be tenderized in several ways, including chemically or physically. The well-known tenderization techniques, such as aging and electrical stimulation, have their own limitations on use in the industrial sector **(Khanna and Panda 2007)**. Several tenderizing compounds, including enzymes, chlorides, phosphates, and others, have reportedly been used effectively to tenderize old and tough meat **(Fayaz *et al*., 2024; Shi *et al*., 2021; Sachdev and Verma 1990)**. Focus has now switched to creating products with little to no synthetic chemicals due to growing consumer knowledge of and desire for natural and minimally processed animal products **(Weiss *et al*., 2010)**. But nowadays, by-products from the fruit processing sector create enormous amounts of waste each year, and a significant fraction of this waste is not appropriately utilized.
Papaya (*Carica papaya* L.) is well popular for its medicinal and health benefits and being an ordinary fruit is grown across the tropical region of the globe **(Islam and Molinar-Toribio 2013)**. The chemical activities of latex fluids are very diversified and are a complex blend of essential chemical compounds and enzymes **(El Moussaoui *et al*., 2001)** and work as an essential source of tenderization of meat **(Choudhary *et al*., 2024; Huet *et al*., 2006)**. The fluid is used commonly for digestion of protein, as it is a good source of enzyme viz., cysteine proteinase and contains as good as 80 per cent fraction of enzyme in the fluid of latex in papaya. Enzyme found in papaya and its peel is a good source of papain **(Issa-Zacharia *et al*., 2023; El Moussaoui *et al*., 2001)**. Among proteolytic enzymes, it has a broad span of specificity, and it is more heat stable comparatively **(Khaparde and Singhal 2001)**. It has several and multiple industrial applications in cosmetics, textiles, detergents, food, leather and pharmaceutical sectors **(Mamboya and Amri 2012; Qihe *et al*., 2006; Khaparde and Singhal 2001; Starley *et al*., 1999)**. Since papain is very effective compared to other proteases due to its high proteolytic activity. It is commonly used as a component in commercial meat tenderizers in the food industry **(Hatti-Kaul, 2001)**.
Papain has generally been purified using precipitation techniques **(Baines and Brocklehurst 1979)**, but the enzyme is still contaminated with other compounds **(Braia *et al*., 2013)**. Chromatographic methods such as ion exchange, covalent, or affinity chromatography were applied as substitute purification strategies **(Azarkan *et al*., 2003; Fukal *et al*., 1984; Funk *et al*., 1979)**. Most of them, as mentioned above, need several stages, lengthy processing periods, and expensive operations. For the purification of protein, industries are seeking fast and economical downstream processes, including those that can produce high yield and high purity**(Gupta *et al*., 2002)**. The above criteria could be met by a capable method viz., "aqueous two-phase system (ATPS)". The best reason for selection of above technology is that the probability of denaturation of protein is minimized, operational process is quick, cost of the raw material is less, easy to scale up, and condition or surrounding are mild for operation **(Singla and Sit 2023; Chen and Lee 1995; Andersson and Hahn-Hägerdal 1990)**.
The aim of this work is to extract the papain enzyme from papaya peel using ATPS and determine the best optimum level of papain extract for effective tenderization cut chicken and mutton using texture profile analysis and sensory evaluation.

MATERIALS AND METHODS

Raw materials & chemicals

The cut chicken and mutton meat were procured from the local market of Tezpur, Assam and brought to the Department of Food Engineering and Technology, Tezpur University, Assam. Chopped meat pieces were cleaned and washed manually & thoroughly with clean water to get rid of foreign impurities and blood clots.
Polyethylene glycol 6000 (PEG 6000) and ammonium sulphate ($(NH_4)_2SO_4$) were procured from Zenith India Pvt. Ltd., India.

Extraction and purification of papain

ATPS technique was used to extract papain from peel of papaya.

Preparation of crude enzyme

Ripe papaya peels were blended with buffer of extraction (0.01M sodium phosphate buffer & pH 7) in the ratio of 1:1 ratio. Further, passed through a muslin cloth for separation, which is called filtration. The obtained through filter was centrifuged at 7000 rpm for 15 min at 5°C. Further, the centrifuged supernatant called as "crude papain extract" and was subjected to ATPS technique for clarification/purification.

Aqueous two-phase system (ATPS)

ATPS was drawn up by mixing an adequate amount of PEG 6000 and $(NH_4)_2SO_4$. According to the **Singla and Sit (2023)**, 10% of the crude extract from papaya peel was mixed with 10% PEG 6000 and 18% $(NH_4)_2SO_4$, followed by addition of distilled water for optimal extraction of papain enzyme. The blend was whirlwind nicely and further, left to rest for 1 hour approximately at the ambient temperature. Further, blend was separated into the phases by centrifugation at 5000 rpm for the time of 20 min at the temperature of 5°C. Phases were divided into two parts PEG and salt solution at upper and lower layer, respectively. The obtained polymer phase was used as the meat tenderizer. To determine the activity of protein and protease enzyme, the obtained solution was dialyzed throughout the night with distilled water.

Sodium dodecyl-sulfate polyacrylamide gel electrophoresis

Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was utilized to determine the distribution of MW of the extracted materials following the **Laemmli method (1970)**. A buffer solution comprising 0.5 M Tris-HCl (pH 6.8), 4% SDS, and 20% glycerol was combined with the sample in a 1:1 ratio. For the reducing condition, 10% β-mercaptoethanol was added to the sample buffer. Subsequently, 20 µg of protein samples were loaded onto polyacrylamide gels, consisting of 12% running gels and 4% stacking gels. Electrophoresis was carried out at a constant current of 15 mA/gel. Following separation, the protein bands were stained using a 0.02% Coomassie Brilliant Blue R-250 solution, and subsequently de-stained using a mixture of acetic acid and methanol.

Gel permeation chromatography

The molecular weight (MW) of the extracted papain was assessed using gel permeation chromatography (GPC) employing an HSP gel RT 5.0 THF 3 µm column. Tetrahydrofuran was used as the mobile phase, flowing at a rate of 0.5 mL/min, while the temperature was maintained at 25°C. The GPC system utilized for the analysis was sourced from Waters Corporation, USA, following the methodology outlined by **Huang et al. (2010).** The extracted papain was dissolved in tetrahydrofuran at a concentration of 1 mg/ml, and a 20 µl volume of this solution was injected in triplicate. The relative MW was calculated by comparing the results to a calibration curve prepared using serial concentrations of polystyrene with a MW range of 162-22,000 Da.

Sample preparation

The meat from the chicken and mutton, which had been removed from the fat and the total connective tissues, was chopped into roughly equal chunk along the muscle fibers, each weighing around approximately 50g. Chopped meat parts were treated with certain treatment and separated into certain groups. Pieces were dipped in distilled water that had different concentrations of papain. The following sequences of treatments were performed for each:

Table 1 Different concentration of papain for the tenderization of cut chicken and mutton meat

Treatment	Concentration (%) in 100 mL of distilled water	Code
Control	-	R
Extracted enzyme	0.025	E1
	0.05	E2
	0.075	E3
	0.1	E4
	0.125	E5
	0.15	E6
	0.175	E7
	0.2	E8

Cooking

In this study, animals cut meat were boiled in a metal saucepan over an electric induction cooktop for a specified duration (Pigeon, Acer plus, India). The actual cooking period required minimal work, just observing the pot's progress and adding fuel as needed. To maintain simplicity, cuts of chicken and mutton were boiled for 15 minutes and 30 minutes, respectively. Boiling time is also significantly influenced by the meat's quality and cutting. After boiling, a texture profile evaluation and sensory assessment were done.

Texture Analysis

The texture of treated cut meat and cooked meat was analyzed by using Texture profile analysis (TPA) at the ambient or room temperature (25°C ± 2°C) with a model of texture analyzer (Model TA.XT Plus, Stable Microsystem, UK). Test samples (3×3×3 cm) were cut by using corer from the middle part of the meat, which were subjected to a twice cycle compression test. A cylindrical probe of 0.5 cm in diameter was used to compress test samples up to75 per cent per cent force of their original height. Analyzer was set at a pretest speed of 5.00 mm/sec, test speed of 1.0mm/sec, post test speed of 250 mm/sec, load capacity of 100kg and a trigger force of 0.049 N. Five TPA parameters were analyzed during analysis and are as follows: chewiness (N), cohesiveness, springiness, gumminess and harness (N).

Sensory analysis

A sensory analysis of the tenderized meat treated with papain was conducted by a panel of 20 semi-trained experts. A nine points hedonic scale (1: dislike extremely and 9: like extremely) was used to determine colour, flavor and general acceptance of the boiled meat samples **(Tóth *et al*., 2022)**. Since this study being conducted on the tenderization of meat, therefore, more emphasis was given to texture with 1=toughest/hardest and 2= most favorable/acceptable to teeth and palate.

Statistical analysis

To determine significant difference within the data analysis of variance (ANOVA) technique was used. Duncan's Multiple Range Test was used with the help of SPSS software and significance of statistics was determined at 5% level ($p<0.05$).

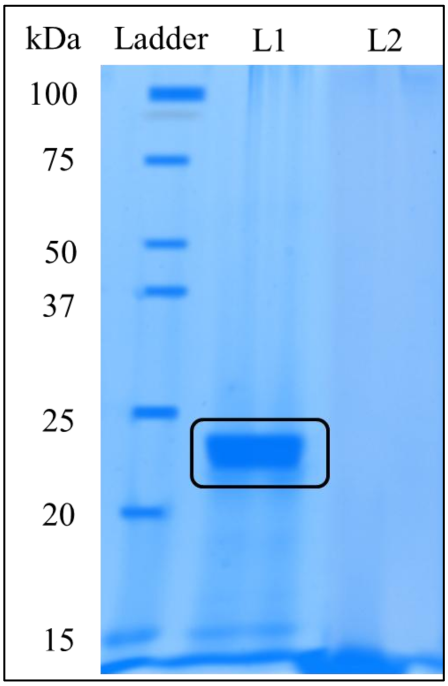
RESULTS AND DISCUSSION

Aqueous two-phase extraction (ATPS)

Papain from the peel of papaya was partitioned by ATPS technique. A blend with a configuration of 10% (w/w) PEG 6000 and 18% (w/w) salt $(NH_4)_2SO_4$ was examined and gave the results in terms of protease activity (1.43), purification factor (4.08), and system temperature (35°C) at constant pH 9.0. However, choosing the ATPS for separation and purification is easier when the molecular weight and hydrophobicity of the significant contaminants and protein is known. According to studies, papain from papaya peel is a good choice for meat tenderization on the protein catabolism of mutton, squid muscle and giant catfish **(Islam and Molinar-Toribio 2013; Lyon and Lyon 1990; Parkash *et al*., 2021)**.

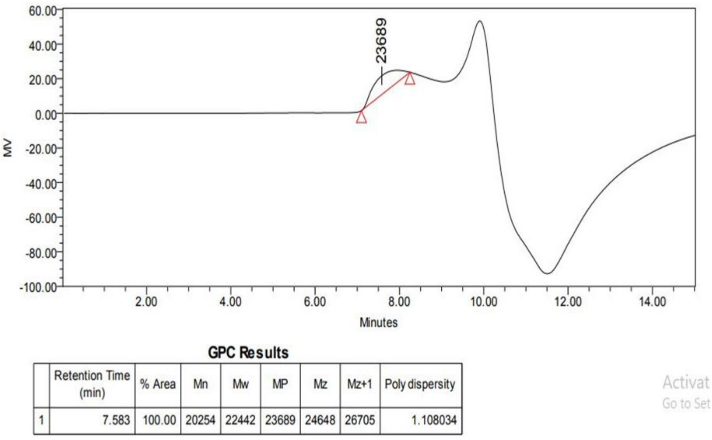
Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Electrophoretic patterns of the extracted papain, partitioned from the 10% PEG 6000 + 18% $(NH_4)_2SO_4$ ATPS fraction, were displayed in Figure 1. The migration of proteins from the crude protein sample into the top and bottom phases of the ATPS was clearly distinguishable. The molecular weights of the isolated enzymes ranged from 20 to 25 kDa, facilitating comparison with protein markers (Ladder). In the 10% PEG 6000 + 18% $(NH_4)_2SO_4$ ATPS, a prominent band corresponding to the papain enzyme was observed in the top phase (L1), while no band was present in the bottom phase (L2) as shown in Figure 1. In the composition of the bottom phase, no noticeable zones of a specific enzyme were observed, most likely due to the relatively low papain content, resulting in undetectable proteolytic activity. The different amounts of interfering proteins and other enzymes in each phase likely contributed to the variation in protein bands between the polymer and salt phases. However, it is crucial to accurately quantify which protein band corresponds to papain. Notably, previous studies by **Vernet *et al*. (1995)** reported that a SDS-PAGE examination revealed the purified papain isolated from papaya to have a molecular weight of approximately 23.4 kDa, which aligns with the obtained result. From the SDS-PAGE analysis, it is evident that the polymer phase of the 10% PEG 6000 ATPS fraction yielded the most favorable result.



where, L1 is10% PEG 6000, L2 is 18% $(NH_4)_2SO_4$,

Figure 1: SDS-PAGE of ATPS fraction



	Retention Time (min)	% Area	Mn	Mw	MP	Mz	Mz+1	Poly dispersity
1	7.583	100.00	20254	22442	23689	24648	26705	1.108034

Figure 2 Gel permeation chromatography of polymer phase (10% PEG)

Gel permeation chromatography

The results indicated that the polymer phase obtained from the 10% PEG 6000 + 18% $(NH_4)_2SO_4$ aqueous two-phase system (ATPS) fraction demonstrated the best overall performance. To further confirm the extraction and purification of papain, gel permeation chromatography analysis was conducted on the enzyme extracted from the polymer phase. The results, as depicted in Figure 2, revealed a molecular weight of 23.6 kDa for the extracted enzyme. This finding aligns with the work of **Vernet *et al*. (1995)**, who reported that purified papain isolated from papaya typically has a molecular weight of approximately 23.4 kDa. Therefore, the obtained result further supports the successful extraction and purification of papain from papaya peel using the ATPS method and can be used in various food applications.

Properties of tenderized meat

The key factors that influence how a customer perceives meat are its consistency, slicing characteristics, chewiness, juiciness, springiness, and hardness. The consumer's choice to repurchase the item can be significantly influenced by these factors. Throughout the production of meat, quality monitoring of the meat products is given the highest significance. Whole tissue and processed meats are subjected to texture analysis to get the optimum ingredient combinations, evaluate the effect of surface treatments, and identify variations in quality.

Texture profile of treated meat

The findings of the texture profile study, which are presented in Table 2 and Table 3, showed that using more enzyme concentration enhanced the tenderizing impact in both chicken and mutton meat cuts. This was consistent with prior research by **Ashie *et al*. (2002)**. As indicated in Figures 3 and 4, the lowest dose of 0.025% used in the experiment shows a significant difference ($p \leq 0.05$) in the tenderness of both chicken and mutton slices between the treated and control groups. Maybe the enhanced hydrophilicity of the papain-treated muscle tissue was the cause of this. Moreover, **Khanna and Panda (2007)** observed that papain treatment increased the hydrophilic properties of hen flesh. According to **Cavitt *et al*. (2004)**, the fat deposit and collagen present in the fillets described above provide another way to interpret the variation in hardness. Also, some customers who ingest larger dosages of papain may experience allergic responses **(Reed, 1975)**.

Table 2 Texture profile of treated cut chicken meat

Batch	Hardness (N)	Springiness	Cohesiveness	Gumminess	Chewiness (N)
R	29.83 ± 0.61^{a}	0.988 ± 0.054^{a}	0.543 ± 0.010^{a}	1073.09 ± 9.12^{a}	16.00 ± 0.92^{a}
E1	24.68 ± 0.51^{b}	0.871 ± 0.011^{b}	0.379 ± 0.002^{b}	665.78 ± 2.98^{b}	8.14 ± 0.24^{b}
E2	17.69 ± 0.49^{c}	0.790 ± 0.008^{c}	0.353 ± 0.011^{c}	651.14 ± 1.84^{b}	4.93 ± 0.20^{c}
E3	7.46 ± 0.35^{d}	0.768 ± 0.005^{cd}	0.340 ± 0.003^{c}	586.06 ± 2.95^{c}	1.94 ± 0.31^{d}
E4	5.3 ± 0.29^{e}	0.752 ± 0.019^{d}	0.265 ± 0.012^{d}	464.06 ± 2.35^{d}	1.05 ± 0.20^{e}
E5	4.63 ± 0.29^{f}	0.695 ± 0.012^{e}	0.242 ± 0.006^{e}	413.55 ± 1.99^{e}	0.77 ± 0.05^{f}
E6	3.51 ± 0.25^{g}	0.651 ± 0.005^{f}	0.217 ± 0.010^{f}	374.03 ± 4.09^{f}	0.49 ± 0.15^{g}
E7	3.28 ± 0.07^{g}	0.489 ± 0.008^{g}	0.205 ± 0.012^{f}	250.22 ± 3.08^{g}	0.32 ± 0.05^{h}
E8	1.53 ± 0.06^{h}	0.418 ± 0.004^{h}	0.185 ± 0.005^{g}	231.95 ± 4.50^{g}	0.11 ± 0.05^{ij}

values are presented as mean ± standard deviations. Means in a same row with different superscripts indicate significant difference ($p < 0.05$)

Table 3 Texture profile of treated cut mutton meat

Batch	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness
R	77.3 ± 0.56^{a}	0.573 ± 0.02^{a}	0.766 ± 0.011^{a}	3316.82 ± 85.44^{a}	34.37 ± 1.75^{a}
E1	27.07 ± 0.47^{b}	0.397 ± 0.015^{b}	0.634 ± 0.007^{b}	1265.89 ± 80.82^{b}	6.81 ± 0.33^{b}
E2	23.34 ± 0.36^{c}	0.336 ± 0.02^{c}	0.589 ± 0.009^{c}	1061.54 ± 52.91^{c}	4.61 ± 0.20^{c}
E3	21.84 ± 0.84^{d}	0.297 ± 0.01^{d}	0.566 ± 0.011^{cd}	619.01 ± 15.27^{d}	3.67 ± 0.01^{d}
E4	16.16 ± 0.58^{e}	0.275 ± 0.01^{d}	0.54 ± 0.030^{d}	340.40 ± 15.27^{e}	2.39 ± 0.06^{de}
E5	14.89 ± 0.41^{f}	0.234 ± 0.015^{e}	0.489 ± 0.021^{e}	247.73 ± 14.42^{f}	1.70 ± 0.04^{de}
E6	13.04 ± 0.76^{g}	0.175 ± 0.010^{f}	0.476 ± 0.020^{e}	187.83 ± 10.50^{fg}	1.08 ± 0.03^{de}
E7	10.57 ± 0.36^{h}	0.156 ± 0.009^{fg}	0.358 ± 0.015^{f}	166.76 ± 9.50^{g}	0.59 ± 0.01^{de}
E8	7.97 ± 0.67^{i}	0.137 ± 0.005^{g}	0.342 ± 0.007^{f}	78.06 ± 0.57^{h}	0.37 ± 0.07^{e}

values are presented as mean ± standard deviations. Means in a same row with different superscripts indicate significant difference ($p < 0.05$)

As per **Ha *et al*. (2012)**, the disintegration and hydrolysis of collagenous fibers in meat differed across plant extracts based on the kind and content enzymes viz., papain and bromelain. The breakdown of the muscles' fibrous structure showed noticeable alterations within the specified period. The issues noted by **Koohmaraie and Geesink (2006)** and **Chen *et al*. (2007)**, who demonstrated that softness can be influenced by the size of the myofibrils and the stiffness of the muscle fiber's structure which can be attributed to the breaking down or fragmentation of the crosslinks in the connective tissues into smaller sections. Because of a rise in collagen cross-linking, meat from young animals is less stuffer than old meat, which creates an issue for the satisfaction of consumers and the meat production sectors. Thus, according to **Ashie *et al*. (2002)**, reported that undesirable mushy texture on the surface of meat results post over-tenderizing of papain.

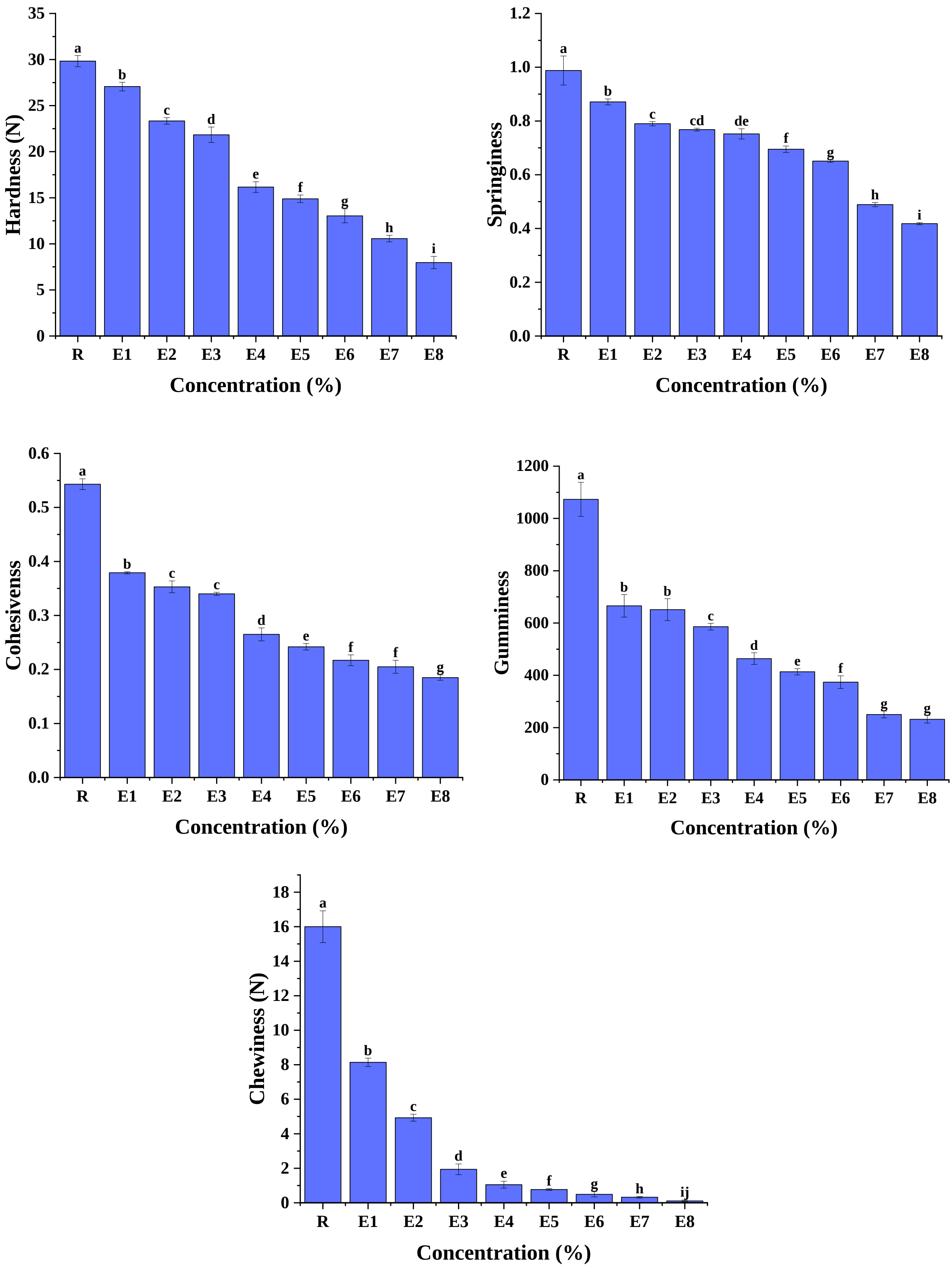


Figure 3: Texture profile of papain treated cut chicken meat

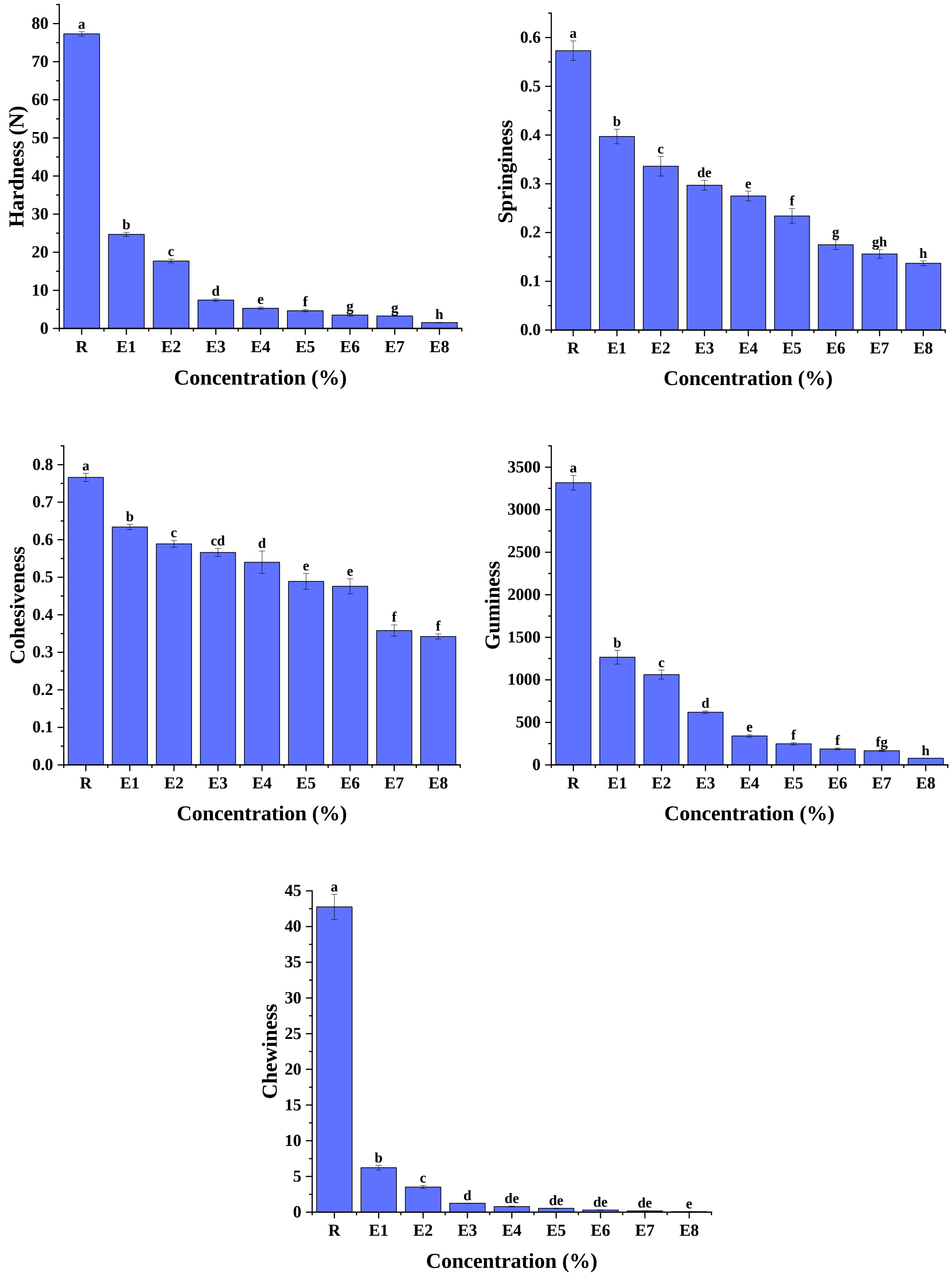


Figure 4: Texture profile of papain treated cut mutton meat

Texture profile of boiled meat

The texture of the meat, which is a product of how the animal was raised and how the meat was prepared, has a significant impact on its quality. Due to the extremely complex structure of animal muscle tissue and the different processing steps raw meat undergoes, including the technique in which it is slaughtered, storage time and temperature, salting and any treatment, might vary the texture and quality of the meat. The temperature and duration of cooking might also have an impact on the physical properties of meat quality (as displayed in Figure 5 and Figure 6). Heat can alter the meat's ability to store water as well as several chemical changes related to muscle fibers and connective tissues. As a result, the meat will have a much drier texture and be less juicy and tender. As shown in Table 4 and Table 5, it was noted that there was significantly reduction in the texture properties of meat with increasing in the concentration of papain enzyme treatment.

Table 4 Texture profile of cooked cut chicken meat

Batch	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)	Gumminess
R	33.45 ± 1.07[a]	0.484 ± 0.009[a]	0.695 ± 0.008[a]	11.27 ± 0.54[a]	11.36 ± 0.47[a]
E1	30.80 ± 0.75[b]	0.359 ± 0.001[b]	0.546 ± 0.005[b]	6.04 ± 0.12[b]	5.69 ± 0.32[b]
E2	28.82 ± 0.50[c]	0.278 ± 0.008[c]	0.528 ± 0.008[c]	4.24 ± 0.20[c]	4.58 ± 0.26[c]
E3	27.71 ± 0.84[cd]	0.277 ± 0.004[c]	0.487 ± 0.005[d]	3.74 ± 0.18[d]	3.84 ± 0.12[d]
E4	23.44 ± 1.14[e]	0.270 ± 0.007[c]	0.447 ± 0.010[e]	2.83 ± 0.16[e]	2.97 ± 0.09[e]
E5	22.11 ± 0.84[e]	0.243 ± 0.009[d]	0.423 ± 0.007[f]	2.27 ± 0.04[f]	2.58 ± 0.09[f]
E6	18.22 ± 0.53[f]	0.231 ± 0.008[d]	0.407 ± 0.007[g]	1.71 ± 0.10[g]	1.97 ± 0.05[g]
E7	15.65 ± 0.44[g]	0.201 ± 0.008[e]	0.358 ± 0.007[h]	1.13 ± 0.08[h]	1.56 ± 0.03[h]
E8	12.83 ± 0.29[h]	0.152 ± 0.003[f]	0.289 ± 0.003[i]	0.56 ± 0.01[i]	0.56 ± 0.006[i]

values are presented as mean ± standard deviations. Means in a same row with different superscripts indicate significant difference ($p < 0.05$)

Table 5 Texture profile of cooked cut mutton meat

Batch	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness
R	101.43 ± 1.41[a]	0.703 ± 0.003[a]	0.664 ± 0.005[a]	5654.11 ± 26.65[a]	47.41 ± 1.26[a]
E1	85.94 ± 0.83[b]	0.636 ± 0.005[b]	0.581 ± 0.004[b]	4778.86 ± 16.92[b]	31.84 ± 0.37[b]
E2	83.99 ± 0.59[c]	0.492 ± 0.011[c]	0.574 ± 0.009[b]	4503.54 ± 14.01[c]	23.77 ± 0.18[c]
E3	83.64 ± 0.53[c]	0.473 ± 0.009[d]	0.546 ± 0.011[c]	4078.7 ± 14.04[d]	21.68 ± 0.47[d]
E4	73.76 ± 0.69[d]	0.432 ± 0.012[e]	0.528 ± 0.013[d]	3739.56 ± 12.01[e]	16.88 ± 0.32[e]
E5	58.36 ± 0.55[e]	0.405 ± 0.003[f]	0.523 ± 0.004[d]	3189.14 ± 15.27[f]	12.36 ± 0.24[f]
E6	21.78 ± 0.46[f]	0.382 ± 0.016[g]	0.51 ± 0.009[e]	3140.42 ± 24.70[g]	4.25 ± 0.15[g]
E7	17.58 ± 0.29[g]	0.357 ± 0.005[h]	0.431 ± 0.007[f]	920.84 ± 10.01[h]	2.71 ± 0.09[h]
E8	17.04 ± 0.53[g]	0.333 ± 0.008[i]	0.366 ± 0.012[g]	884.33 ± 13.57[i]	2.08 ± 0.03[h]

values are presented as mean ± standard deviations. Means in a same row with different superscripts indicate significant difference ($p < 0.05$)

Based on research by **Lyon and Lyon (1990)**, the textural characteristics of poultry meat varied significantly depending on how it was cooked. Generally, when meat is heated, the collagen in the muscle itself softens, making connective tissues softer **(Baldwin, 2012)**. As per, **Kang and Rice (1970)**, the sarcomere fraction and the tougher connective tissue resolvable activity were more significantly influenced by the papain enzyme. According to **Parkash *et al*. (2021)**, applying pressure and papain together improved tenderness and increased connective tissue solubility. **Khanna and Panda (2007)** stated that the imbue of papain enzyme combined with forking technology was preferable approach for tenderizing hen meat pieces. Sodium tripolyphosphate and papain have a synergistic impact on improving the softness of chicken gizzards, as demonstrated by **Grover *et al*. (2005)**. According to **Hay (1952)**, the hardness and elasticity of the collagen fibre, its ease of conversion to soft soluble gelatin by boiling, and its insufficiency as a dietary protein are the characteristics of collagenous tissue that most worry us from the perspective of the characteristics of meat. He emphasized that collagen is thoroughly digested by the proteolytic enzyme and that, when it is often accompanied by a significant surplus of protein with high biological value, it will be used effectively on its own.

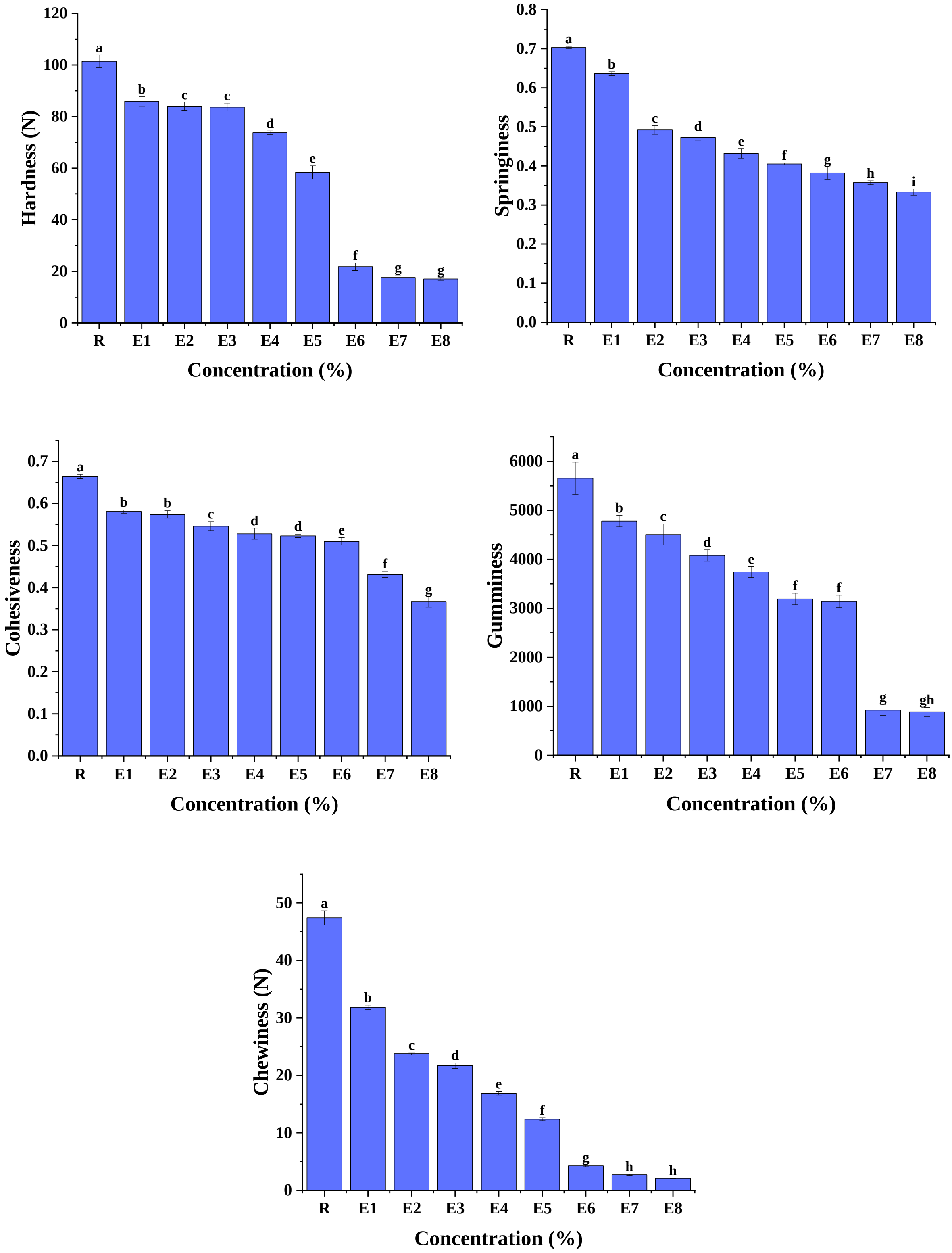


Figure 5: Texture profile of cooked cut chicken meat

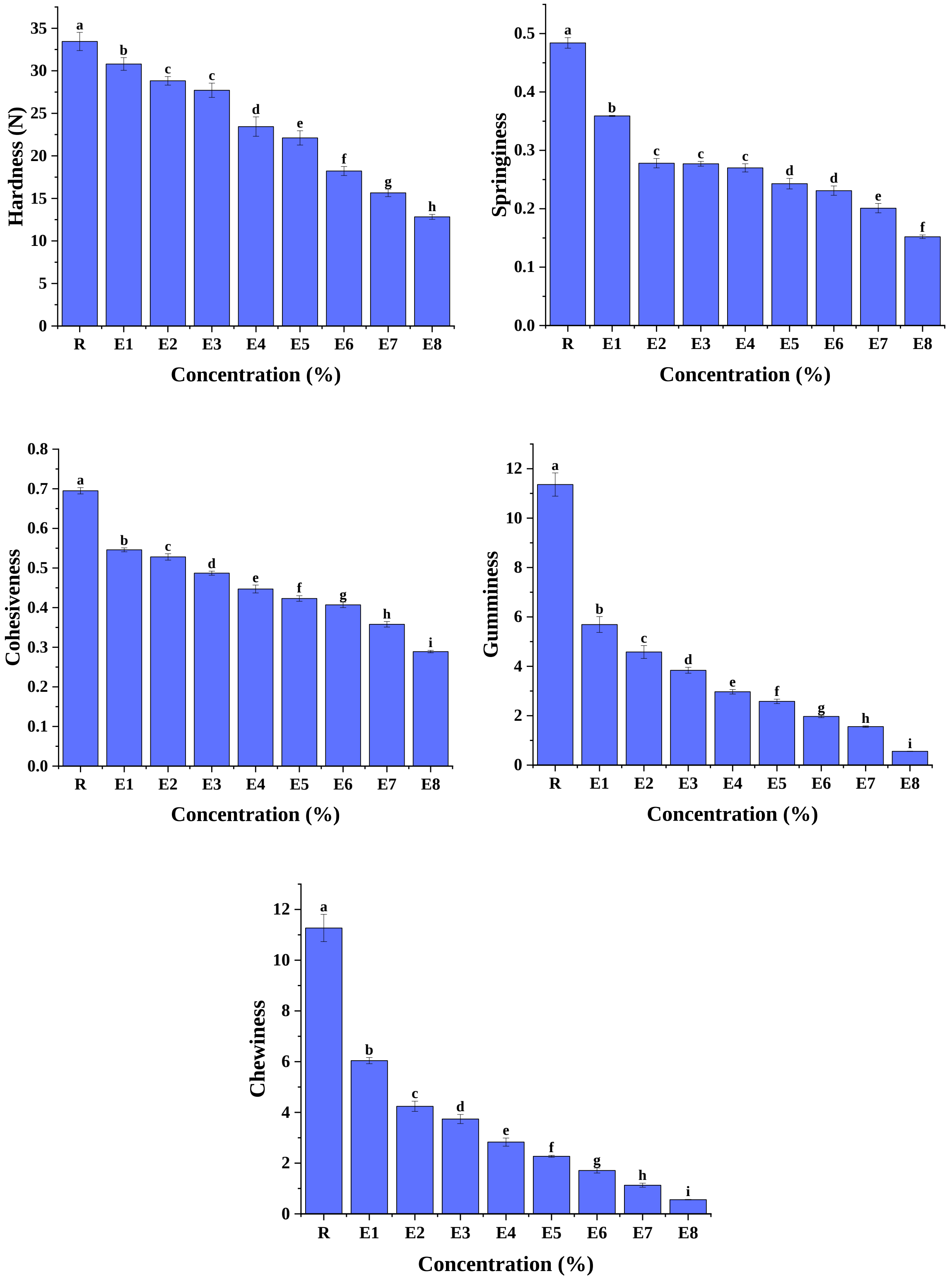


Figure 6: Texture profile of cooked cut mutton meat

Sensorial evaluation

Although meat's physical properties are generally evaluated analytically, sensory assessment is the only method that can accurately predict how the meat will taste when consumed, therefore it is essential. Because of this, it's crucial to conduct a textural profile and a thorough quantifiable analysis using an experience specialists panel **(Brandt *et al*., 1963; Szczesniak *et al*., 1963)**. In sensory analysis, the palatability of enzymatically tenderized chicken and mutton that had been thermally cooked by boiling was evaluated. The significant amount of muscle fiber fragmentation was seen as the amount of papain added increased; also, some of the enzymatic meat pieces dipped in papain softened after boiling compared to control meat and had very little chewing resistance. Moreover, several samples showed patches with texture resembling paste after boiling, perhaps because of exceeding the required tenderization period. It was seen from Figures 7 and Figures 8 that the color and aroma of the papain treated meat were not considerably impacted. The papain-tenderized samples were juicier than the control samples because of the minimal losses. One of the key factors impacting consumers' overall satisfaction with meat products has been recognized as texture **(Fletcher, 2002)**. Both types of meat had a significant variation in both texture and appearance. According to the sensory evaluation, the 0.05% papain concentration was chosen for chicken meat cut and 0.125% papain concentration was chosen for mutton meat, since there was noticeable gap in tenderness in between the control samples ($p>0.05$).

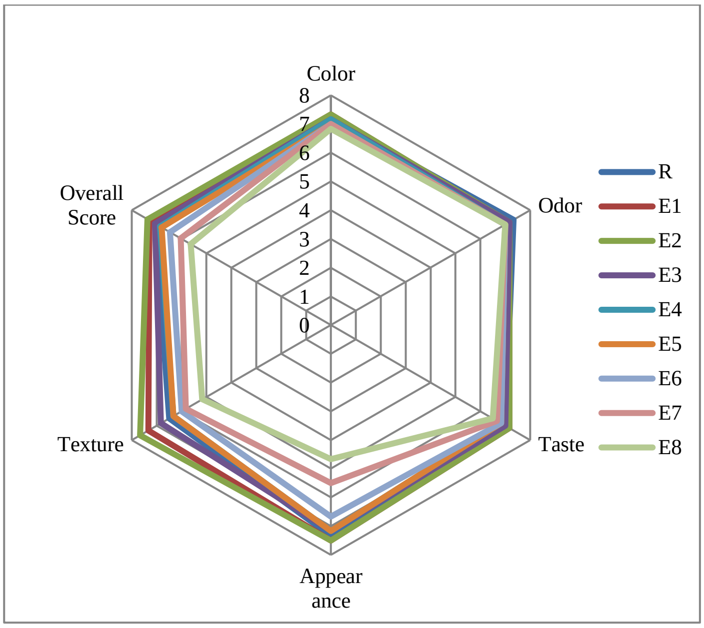


Figure 7 Sensory analysis of cooked chicken meat

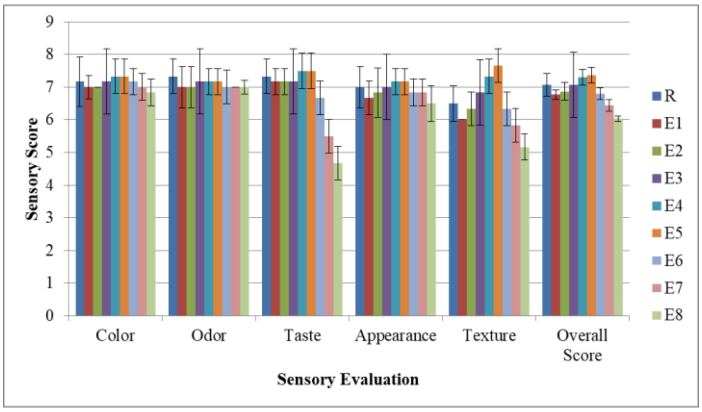


Figure 8 Sensory analysis of cooked mutton meat

CONCLUSIONS

The extraction of papain enzyme from ripe papaya peel using ATPS proved effective in improving the tenderness of meat. Papain demonstrated its potency as a protease by specifically targeting and breaking the peptide bonds in protein molecules, leading to their degradation into amino acids. The study observed that higher levels of papain and longer treatment times resulted in a notable weakening of the meat structure. The tenderization of the meat cuts through papain treatment led to improvements in tenderness, flavour, and juiciness. Sensory evaluation indicated that a papain concentration of 0.05% was optimal for chicken meat cuts, while a concentration of 0.125% was preferable for mutton meat, as both showed significant differences in tenderness compared to the control samples. It is advisable to use the lowest possible doses of papain to avoid excessive tenderization, which may result in overly soft meat with low mastication resistance and a pasty texture. In future investigation may be conducted on the effect papain derived from papaya peel on rigor mortis of the muscles for better understanding of the mechanism. The application of this technology has the potential to benefit meat producers and processors in meeting consumer expectations for meat products. Further this study investigated that using papain, which is derived from papaya peel, as an economical and sustainable tenderization agent in the meat sector. This could decrease food waste and create new opportunities for the creation of meat products with additional value.

Ethics approval and consent to participate: Not Applicable
Consent for publication: Not Applicable
Conflict of interest: The authors declare no conflict of interest.
Funding information: No external funding was received to carry out the present work.
Availability of Data or Materials: The authors declare that data will be made available on request.
Author contributions
Mohit Singla: Methodology, Investigation, Visualization, Formal analysis, Writing-original draft; **Bhaskar Jyoti Kalita**: Methodology, Investigation; **Nandan Sit**: Conceptualization, Validation, Writing-review and editing, Visualization, Supervision, Project administration.

Acknowledgements: The authors acknowledge the financial help received from UGC-SAP (DRS-I), Government of India and DST-FIST, Government of India and AICTE, Government of India for carrying out the work.

REFERENCES

Abdalla, H. O., Ali, N. N. A., Siddig, F. S., & Ali, S. A. M. (2013). Improving tenderness of spent layer hens meat using papaya leaves (Carica papaya). *Pak Vet J*, 33(1), 73–76

Amri, E., Mamboya, E. A. F. (2012). Papain, a plant enzyme of biological importance: A review. *Am. J. Biochem. Biotechnol*, *8*(2), 99-104. https://doi:10.3844/ajbbsp.2012.99.104

Andersson, E., & Hahn-Hägerdal, B. (1990). Bioconversions in aqueous two-phase systems. *Enzyme and Microbial Technology*, *12*(4), 242-254. https://doi.org/10.1016/0141-0229(90)90095-8

Ashie, I. N. A., Sorensen, T. L., & Nielsen, P. M. (2002). Effects of papain and a microbial enzyme on meat proteins and beef tenderness. *Journal of Food Science*, *67*(6), 2138-2142. https://doi.org/10.1111/j.1365-2621.2002.tb09516.x

Azarkan, M., El Moussaoui, A., Van Wuytswinkel, D., Dehon, G., & Looze, Y. (2003). Fractionation and purification of the enzymes stored in the latex of Carica papaya. *Journal of Chromatography B*, *790*(1-2), 229-238. https://doi.org/10.1016/S1570-0232(03)00084-9

Baines, B. S., & Brocklehurst, K. (1979). A necessary modification to the preparation of papain from any high-quality latex of Carica papaya and evidence for the structural integrity of the enzyme produced by traditional methods. *Biochemical Journal*, *177*(2), 541-548. https://doi.org/10.1042/bj1770541

Baldwin, D. E. (2012). Sous vide cooking: A review. *International Journal of Gastronomy and Food Science*, *1*(1), 15-30. https://doi.org/10.1016/j.ijgfs.2011.11.002

Braia, M., Ferrero, M., Rocha, M. V., Loureiro, D., Tubio, G., & Romanini, D. (2013). Bioseparation of papain from Carica papaya latex by precipitation of papain–poly (vinyl sulfonate) complexes. *Protein Expression and Purification*, *91*(1), 91-95. https://doi.org/10.1016/j.pep.2013.07.008

Brandt, M. A., Skinner, E. Z., & Coleman, J. A. (1963). Texture profile method. *Journal of Food Science*, *28*(4), 404-409. https://doi.org/10.1111/j.1365-2621.1963.tb00218.x

Cavitt, L. C., Youm, G. W., Meullenet, J. F., Owens, C. M., & Xiong, R. (2004). Prediction of poultry meat tenderness using razor blade shear, Allo-Kramer shear, and sarcomere length. *Journal of Food Science*, *69*(1), SNQ11-SNQ15. https://doi.org/10.1111/j.1365-2621.2004.tb17879.x

Chen, J. P., & Lee, M. S. (1995). Enhanced production of Serratia marcescens chitinase in PEG/dextran aqueous two-phase systems. *Enzyme and Microbial Technology*, *17*(11), 1021-1027. https://doi.org/10.1016/0141-0229(95)00030-5

Chen, X. L., Xie, B. B., Lu, J. T., He, H. L., & Zhang, Y. (2007). A novel type of subtilase from the psychrotolerant bacterium Pseudoalteromonas sp. SM9913: catalytic and structural properties of deseasin MCP-01. *Microbiology*, *153*(7), 2116-2125.

Choudhary, R., Kaushik, R., Chawla, P., & Manna, S. (2025). Exploring the extraction, functional properties, and industrial applications of papain from Carica papaya. *Journal of the Science of Food and Agriculture*, *105*(3), 1533-1545. https://doi.org/10.1002/jsfa.13776

El Moussaoui, A., Nijs, M., Paul, C., Wintjens, R., Vincentelli, J., Azarkan, M., & Looze, Y. (2001). Revisiting the enzymes stored in the laticifers of Carica papaya in the context of their possible participation in the plant defence mechanism. *Cellular and Molecular Life Sciences CMLS*, *58*, 556-570. https://doi.org/10.1007/PL00000881

Elzalaky, E. (2024). Methods for Enhancing Meat Tenderness a Main Key Factor of Meat Palatability: A Review. *Alexandria Journal of Food Science and Technology*, *22*(1), 25-36.

Fayaz, H., Ahmad, S. R., Qureshi, A. I., Hussain, S. A., & Nazir, T. (2024). Application of Enzymes in Processed Meat Products. In *Hand Book of Processed Functional Meat Products* (pp. 323-347). Cham: Springer Nature Switzerland. https://doi.org/10.1007/978-3-031-69868-2_13

Fletcher, D. L. (2002). Poultry meat quality. *World's Poultry Science Journal*, *58*(2), 131-145. https://doi.org/10.1079/WPS20020013

Fukal, L., Káš, J., & Paluska, E. (1984). Chromatographic separation of papain evaluated by immunochemical methods. *Journal of Chromatography A*, *285*, 365-372. https://doi.org/10.1016/S0021-9673(01)87776-1

Funk, M. O., Nakagawa, Y., Skochdopole, J., & Kaiser, E. T. (1979). Affinity Chromatographic Purification of Papain a Reinvestigation. *International Journal of Peptide and Protein Research*, *13*(3), 296-303. https://doi.org/10.1111/j.1399-3011.1979.tb01883.x

Grover, R. K., Sharma, D. P., & Ahlawat, S. S. (2005). Standardization of chicken gizzard pickle using sodium tripolyphosphate and papain as tenderizer. *Indian Journal of Poultry Science*, *40*(2), 202-205.

Gupta, R., Beg, Q., Khan, S., & Chauhan, B. (2002). An overview on fermentation, downstream processing and properties of microbial alkaline proteases. *Applied Microbiology and Biotechnology*, *60*, 381-395. https://doi.org/10.1007/s00253-002-1142-1

Ha, M., Bekhit, A. E. D. A., Carne, A., & Hopkins, D. L. (2012). Characterisation of commercial papain, bromelain, actinidin and zingibain protease preparations and their activities toward meat proteins. *Food Chemistry*, *134*(1), 95-105. https://doi.org/10.1016/j.foodchem.2012.02.071

Hatti-Kaul, R. (2001). Aqueous two-phase systems: A general overview. *Molecular Biotechnology*, *19(3)*, 269-277. https://doi.org/10.1385/MB:19:3:269

Hay, P. P. (1952). Effects of a meat tenderizer on less tender cuts of beef cooked by four methods.

Huang, X. D., Liang, J. B., Tan, H. Y., Yahya, R., Khamseekhiew, B., & Ho, Y. W. (2010). Molecular weight and protein binding affinity of Leucaena condensed tannins and their effects on in vitro fermentation parameters. *Animal Feed Science and Technology*, *159*(3-4), 81-87. https://doi.org/10.1016/j.anifeedsci.2010.05.008

Huet, J., Looze, Y., Bartik, K., Raussens, V., Wintjens, R., &Boussard, P. (2006). Structural characterization of the papaya cysteine proteinases at low pH. *Biochemical and Biophysical Research Communications*, *341*(2), 620-626. https://doi.org/10.1016/j.bbrc.2005.12.210

Islam, M. N., & Molinar-Toribio, E. M. (2013). Development of a meat tenderizer based on papaya peel. *J Revistade ID Techno (RIDTEC)*, *9*(2), 24-29.

Issa-Zacharia, A., Muhimbula, H. S., Bikuba, A. N., & Matondo, J. D. (2023). Development of Natural Liquid and Powdered Meat Tenderizer Based on Papaya Peel, Ginger and Garlic. https://doi.org/10.11648/j.jfns.20241202.13

Jayawardena, R., Morton, J. D., Brennan, C. S., Bhat, Z. F., & Bekhit, A. E. D. A. (2022). Meat co-products. In *Alternative Proteins* (pp. 329-359). CRC Press.

Kang, C. K., & RICE, E. E. (1970). Degradation of various meat fractions by tenderizing enzymes. *Journal of Food Science*, *35*(5), 563-565. https://doi.org/10.1111/j.1365-2621.1970.tb04809.x

Khanna, N., & Panda, P. C. (2007). Effect of papain on tenderization and functional properties of spent hen meat cuts. *Indian Journal of Animal Research*, *41*(1), 55-58.

Khaparde, S. S., & Singhal, R. S. (2001). Chemically modified papain for applications in detergent formulations. *Bioresource Technology*, *78*(1), 1-4. https://doi.org/10.1016/S0960-8524(00)00178-4

Koohmaraie, M., &Geesink, G. H. (2006). Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Science*, *74*(1), 34-43. https://doi.org/10.1016/j.meatsci.2006.04.025

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*(5259), 680-685. https://doi.org/10.1038/227680a0

Lyon, B. G., & Lyon, C. E. (1990). Texture profile of broiler pectoralis major as influenced by post-mortem deboning time and heat method. *Poultry Science*, *69*(2), 329-340. https://doi.org/10.3382/ps.0690329

Nagodawithana, T., Reed, G., (Eds.). (1975), *Enzymes in Food Processing,(3rd Ed). Academic Press Limited, 7-36*

Parkash, J., Sharma, D., & Yadav, S. (2021). Application of natural tenderizers (papain and ginger) in buffalo calf meat. *Pharma Innov*, *10*(3S), 203-205.

Qihe, C., Guoqing, H., Yingchun, J., & Hui, N. (2006). Effects of elastase from a Bacillus strain on the tenderization of beef meat. *Food chemistry*, *98*(4), 624-629. https://doi.org/10.1016/j.foodchem.2005.06.043

Sachdev, A. K., & Verma, S. S. (1990). Tenderization of spent hen meat. *Indian Poult Rev*, *21*, 21-25.

Shi, H., Shahidi, F., Wang, J., Huang, Y., Zou, Y., Xu, W., & Wang, D. (2021). Techniques for postmortem tenderisation in meat processing: effectiveness, application and possible mechanisms. *Food Production, Processing and Nutrition*, *3*, 1-26. https://doi.org/10.1186/s43014-021-00062-0

Simpson, B. K., Nollet, L. M., Toldrá, F., Benjakul, S., Paliyath, G., & Hui, Y. H. (Eds.). (2012). *Food biochemistry and food processing*.(2ndEdn). John Wiley & Sons.

Singla, M., & Sit, N. (2023). Isolation of papain from ripe papaya peel using aqueous two-phase extraction. *Journal of Food Measurement and Characterization*, *17*(2), 1685-1692. https://doi.org/10.1007/s11694-022-01741-3

Singla, M., & Sit, N. (2023). Theoretical Aspects and Applications of Aqueous Two-Phase Systems. *ChemBioEng Reviews*, *10*(1), 65-80. https://doi.org/10.1002/cben.202200026

Starley, I. F., Mohammed, P., Schneider, G., & Bickler, S. W. (1999). The treatment of paediatric burns using topical papaya. *Burns*, *25*(7), 636-639. https://doi.org/10.1016/S0305-4179(99)00056-X

Szczesniak, A. S., Brandt, M. A., & Friedman, H. H. (1963). Development of standard rating scales for mechanical parameters of texture and correlation between the objective and the sensory methods of texture evaluation. *Journal of Food Science*, *28*(4), 397-403. https://doi.org/10.1111/j.1365-2621.1963.tb00217.x

Tóth, M., Kaszab, T., & Meretei, A. (2022). Texture profile analysis and sensory evaluation of commercially available gluten-free bread samples. *European Food Research and Technology*, *248*(6), 1447-1455. https://doi.org/10.1007/s00217-021-03944-2

Vernet, T., Berti, P. J., de Montigny, C., Musil, R., Tessier, D. C., Ménard, R., Magny, M. C., Storer A.C., & Thomas, D. Y. (1995). Processing of the papain precursor: the ionization state of a conserved amino acid motif within the pro region participates in the regulation of intramolecular processing. *Journal of Biological Chemistry*, *270*(18), 10838-10846. https://doi.org/10.1074/jbc.270.18.10838

Weiss, J., Gibis, M., Schuh, V., & Salminen, H. (2010). Advances in ingredient and processing systems for meat and meat products. *Meat Science*, *86*(1), 196-213. https://doi.org/10.1016/j.meatsci.2010.05.008