Chapter-6

Tenderization of cut meat using papain enzyme extracted from papaya peel

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6.1 Introduction

For many people, meat is their preferable source of animal protein as it's the most valuable livestock product. Meat is either eaten as processed meat or as a component of home-style meal preparations. Rigomortis, a natural process that occurs after animal slaughter, causes the muscle protein fibers to shorten and the tissue to become tough **[39]**. 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 **[30]**. It varies by animal species, breed, age, sex, and specific skeletal muscle tissue. In particular, myofibrils and intermediate filaments in muscle fibers as well as the collagen-rich endomysium and perimysium of the intramuscular connective tissue are responsible for tenderness **[1]**. Increase in the cross linkage in the connective tissues of aged animals is the key parameter responsible for toughness of the meat **[3]**. Post addition of meat tenderizers, meat may be cooked more quickly and at a lower temperature, which prevents the development of cancer-causing heterocyclic amines and polycyclic aromatic hydrocarbons **[3,25]**.

Meat can be tenderized in a number of 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 **[26]**. Several tenderizing compounds, including enzymes, chlorides, phosphates, and others, have reportedly been used effectively to tenderize old and tough meat **[38]**. 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. Traditionally, papaya peel was used as the meat tenderizer. 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 **[25]**. The chemical activities of fluid of latex are much diversified and are a complex blend of essential chemical compounds and enzymes **[14]** and works as an essential source of tenderization of meat **[23]**. 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 **[14]**. Papain is an enzyme found in papaya and its peel is a good source of papain **[40]**.

Cysteine protease is a papain in an active endolytic form (EC 3.4.22.2). Among proteolytic enzymes, it owns a broad span of specificity and it is more heat stable comparatively **[27]**. It has several and multiple industrial application in cosmetics, textiles, detergents, food, leather and pharmaceutical sectors **[27,32,36,41]**. Since papain is more effective than other proteases, it is a common component in commercial meat tenderizers in the food business **[20]**. According to Khanna & Panda **[26]**, chicken meat pieces may be made more soft and have better functional qualities by adding papain at a concentration of 0.025%. Also, Mendiratta et al. **[33]**, observed that pH, protein, and fat have faced no significant difference with papain treated chickens and moreover, noticeably, overall acceptability, tenderness and juiciness scored high. According to Navid et al. **[34]**, meat's softness and juiciness was enhanced when 2 per cent papaya leaf meal fed to wasted layer chickens some days earlier to slaughter.

Papain has generally been purified using precipitation techniques **[6]**, but the enzyme is still contaminated with other compounds **[8]**. Chromatographic methods such as ion exchange, covalent, or affinity chromatography were applied as substitute purification strategies **[5,16,17]**. The majority 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 **[19]**. 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 are less, easy to scale up, and condition or surrounding are mild for operation **[2,12]**.

The above ATPS system is capable of and has proved narrow down processing of protein by integration of multiple unit operation viz., clarification, concentration, and purification of the targeted item in single unit operation. This system is made in such a way that it consist of one salt and one polymer or both the polymers **[21,43]**. High output separation and purification of enzymes have been successfully achieved through the use of ATPS **[24,29,44]**. Additionally, if the high degree of purity of enzyme is not intended then crude enzyme can be used for the same purpose. Moreover, crude enzyme would be economical as well.

The ultimate aim to undertake 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.

6.2 Materials and methods

6.2.1 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.

6.2.2 Extraction and purification of papain

The ATPS (Aqueous Two-Phase System) technique was applied for the extraction of papain from papaya peels, as described in Chapter 4 (Sections 4.2.2 and 4.2.3). The results obtained in Chapter 4 revealed that a combination of 10% of the crude papain extract derived from papaya peels with 10% PEG 6000 and 18% $(NH_4)_2SO_4$ yielded the most efficient extraction of the papain enzyme. This extracted enzyme was subsequently employed for meat tenderization, showcasing the practical application of the ATPS-based extraction method.

6.2.3 Sample preparation

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

Table 6.1: Different concentration of papain for the tenderization of cut chicken and mutton meat

Treatment	Concentration (%) in 100 mL of distilled water	Code
Control (untreated)	-	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

6.2.4 Texture analysis

Texture of meat chopped pieces 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 upto 75 per cent force of their original height. Analyser 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)

6.2.5 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, we'll estimate that the majority of cuts of chicken and mutton can be boiled in 15 minutes and 30 minutes, respectively, however the actual time required may vary depending on the treatment of meat cuts. Boiling time is also significantly influenced by the meat's quality and cut. After boiling, a texture profile evaluation and sensory assessment were done.

6.2.6 Sensory analysis

A nine members/specialists team was made for sensorial analysis of papain treated tenderized meat. A nine points hedonic scale (1: dislike extremely and 9: like extremely) was used to determine color, flavor and general acceptance of the boiled meat samples. Since, this study being conducted on the tenderization of meat, therefore, more emphasis was given to texture with 1=toughest/hardest and 2= most favourable/acceptable to teeth and palate.

6.2.7 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 ($p < 0.05$)

6.3 Result and Discussion

6.3.1 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 **[25,32,35]**.

6.3.2 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.

6.3.2.1 Texture profile of treated meat

The findings of the texture profile study, which are presented in Tables 6.2 and 6.3, 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 **[4]**. Since the experiment's lowest dose of 0.025% could produce a statistically notable difference ($p \leq 0.05$) in the tenderness of both chicken & mutton slices between the control and treated groups. Maybe the enhanced hydrophilicity of the papain-treated muscle tissue was the cause of this. Moreover, Khanna & Panda **[26]** observed that papain treatment increased the hydrophilic properties of hen flesh. According to Cavitt et al. **[10]**, 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 **[37]**.

Table 6.2: 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 column with different superscripts indicate significant difference ($p < 0.05$).

Table 6.3: 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 column with different superscripts indicate significant difference ($p < 0.05$).

As per Ha et al. **[20]**, 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 of time. The problems highlighted by Koohmaraie & Geesink **[28]** and Chen et al. **[12]**, who furnished that softness canbe altered by an extent of the myofibrilia and sturdiness of structure of the muscle fiber alters the actomyosin hardness, can be explained as an outcome of the connective tissues crosslink being divided or differentiated into small saclesections. 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 Ashie et al. **[4]**, reported that undesirable mushy texture on the surface of meat results post over-tenderizing of papain.

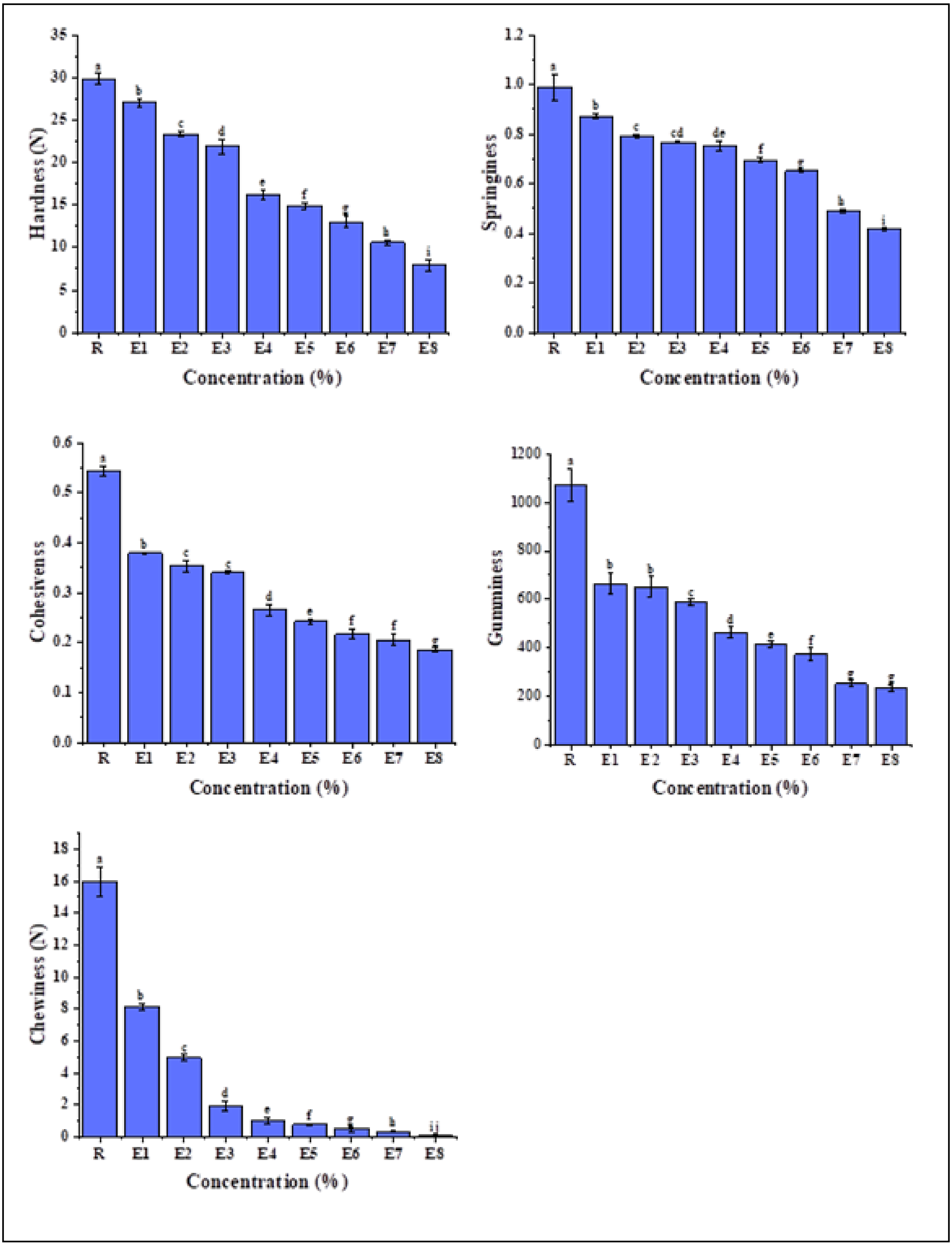

Figure 6.1: Texture profile of papain treated cut chicken meat

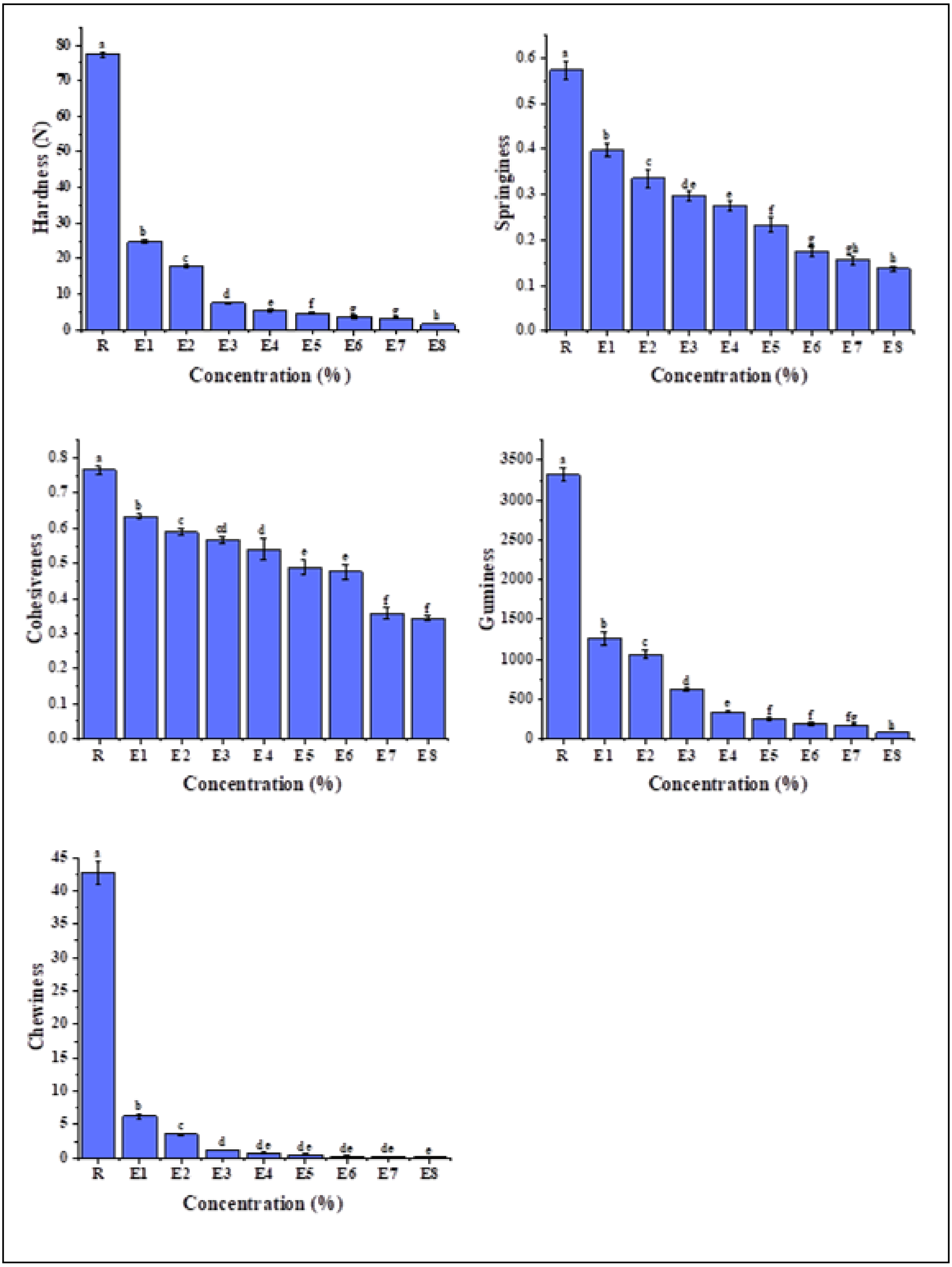

Figure 6.2: Texture profile of papain treated cut mutton meat

6.3.2.2 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 physical properties of meat quality. Heat can alter the meat's ability to store water as well as a number of chemical changes related to the 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 6.5 and Table 6.6, it was noted that there was significantly reduction in the texture properties of meat with increasing in the concentration of papain enzyme treatment.

Table 6.4: 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 represented as mean ± standard deviations. Means in a same column with different superscripts indicate significant difference ($p < 0.05$).

Table 6.5: 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 represented as mean ± standard deviations. Means in a same column with different superscripts indicate significant difference ($p < 0.05$).

Based on research by Lyon & Lyon **[31]**, 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 **[7]**. Enzyme papain furnished greater influence for the sacromere fraction and harder connective tissue resolvable activity (Kang and Rice, 1970). According to Prakash et al. **[35]**, applying pressure and papain together improved tenderness and increased connective tissue solubility. Khanna & Panda **[26]**, stated that imbue of papain enzyme combined with forking technology was preferable approach for tenderising hen meat pieces. Sodium tripolyphosphate and papain have a synergistic impact on improving the softness of chicken gizzards, as demonstrated by Grover et al. **[18]**. According to Hay **[22]**, the hardness and elasticity of the collagen fiber, 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 rather effectively on its own.

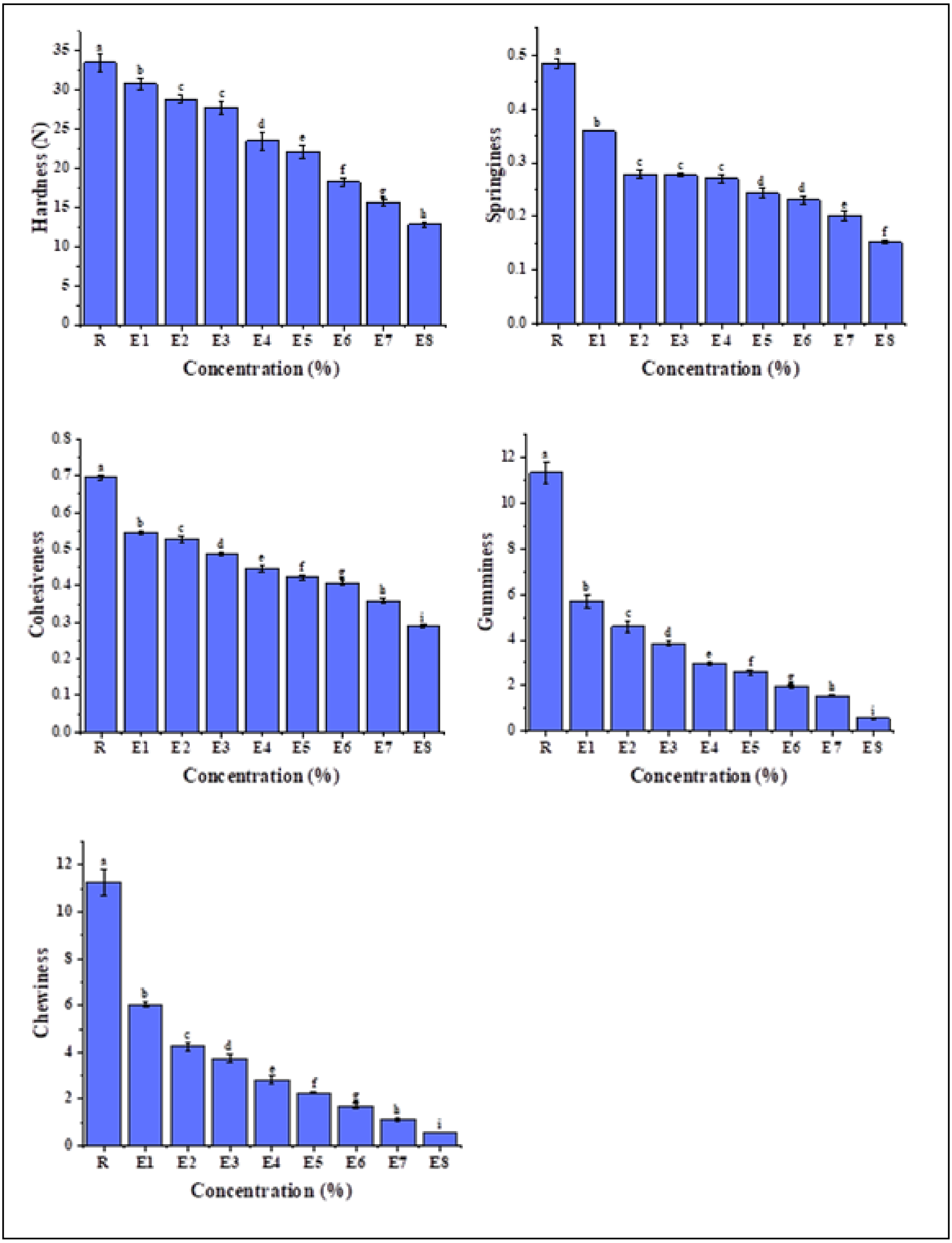

Figure 6.3: Texture profile of cooked cut chicken meat

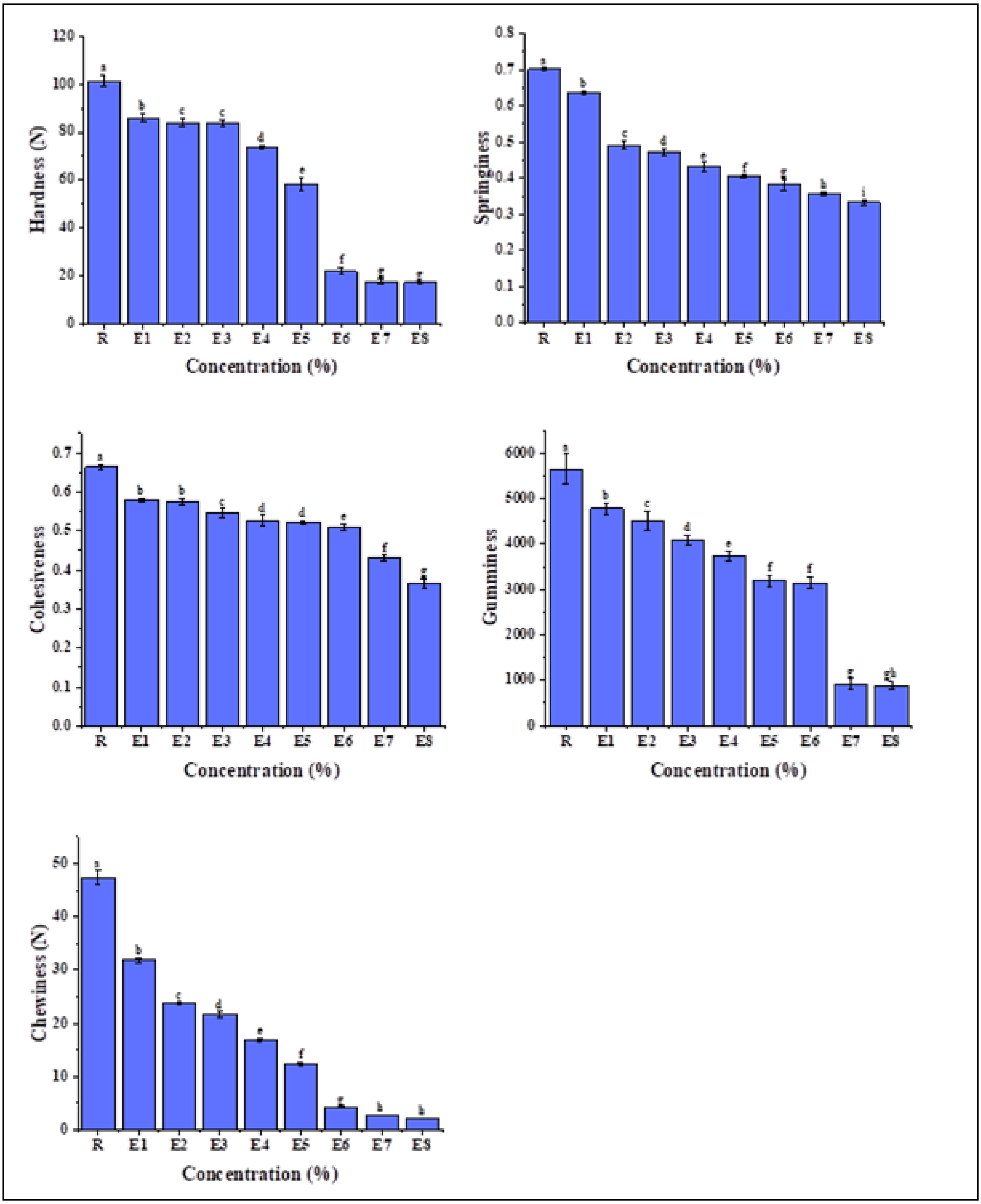

Figure 6.4: Texture profile of cooked cut mutton meat

6.3.3 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 a crucial to conduct a textural profile and a thorough quantifiable analysis using an experience specialists panel **[9,42]**. In sensory analysis, the palatability of enzymatically tenderized chicken and mutton that had been thermally cooked by boiling was evaluated. A 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 compare to control meat and had very little chewing resistance. Moreover, several samples showed patches with texture resembling paste after boiling, perhaps as a result of exceeding the required tenderization period. It was seen from Figures 6.5 and Figures 6.6 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 **[15]**. 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 ($p > 0.05$).

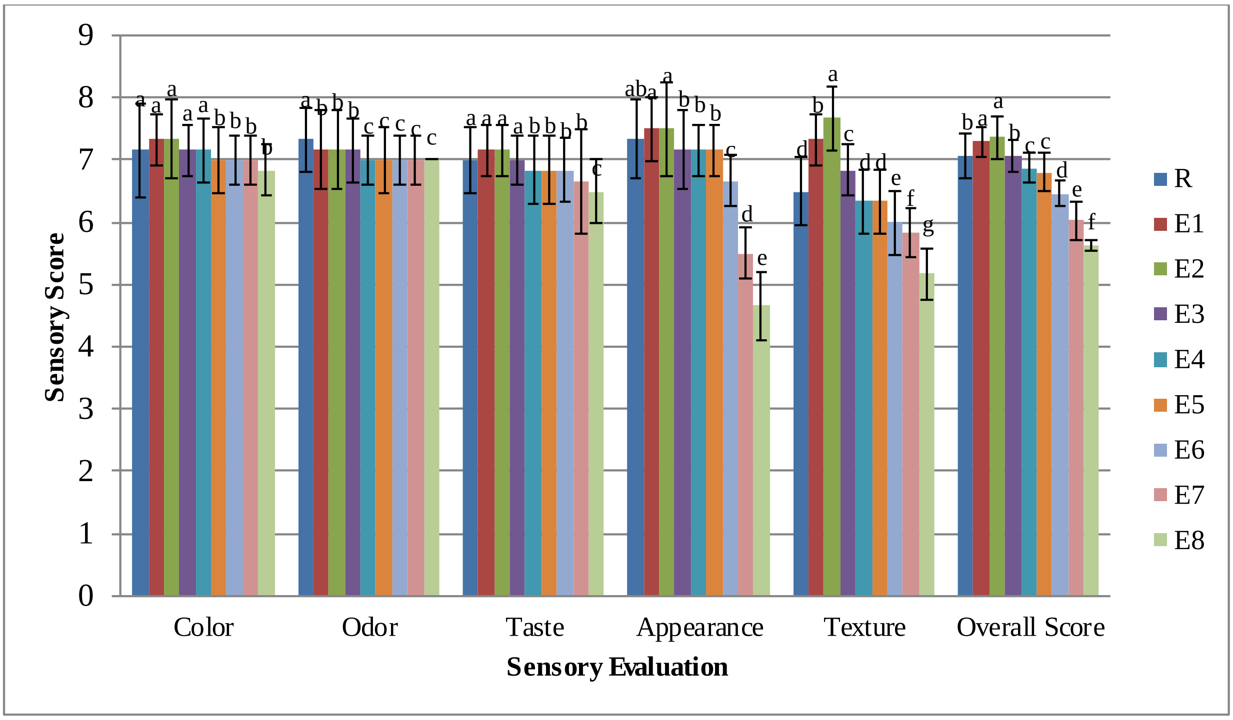

Figure 6.5: Sensory analysis of cooked chicken meat

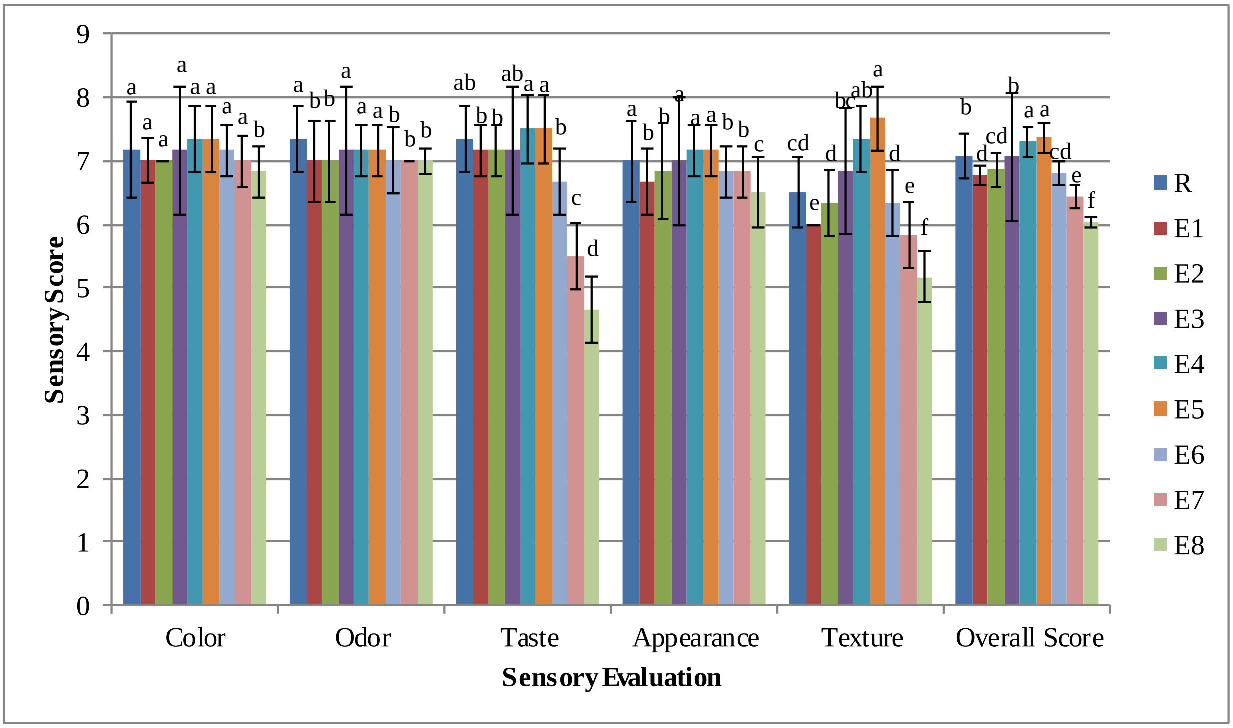

Figure 6.6: Sensory analysis of cooked mutton meat

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