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Effect of Proteolytic Enzymes (Bromelain and Papain) on Sensory and Chemical Quality of *Sukuti* (an Indigenous Dried Meat Product of Nepal)

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Abstract

The present work was undertaken to compare the effect of bromelain and papain on the sensory quality of *sukuti* (Nepalese indigenous dried buffalo meat). Buffalo lean meat (round cut) was purchased from the local market of Dharan and used for the preparation of *sukuti* after injection of 10% m/m enzyme (bromelain and papain) solution at the concentration (0-100 mg/L) and resting for 4h followed by drying at 65°C up to the moisture of 5%. The optimized concentration of each enzyme was selected by sensory evaluation based on color, flavor, texture, and overall acceptability. The proximate composition, collagen content, and collagen solubility were studied for two optimized samples with enzyme treatment and control. The best tenderizing effect of papain and bromelain enzyme was found at 40 mg/L and 10 mg/L respectively from the sensory analysis and the sensory attributes for these concentrations were significantly ($p<0.05$) higher than other samples. The soluble collagen content increased from 0.44 mg/g tissue in untreated meat to 0.52 for bromelain treated and to 0.98 mg/g for papain treated samples. The collagen solubility for the untreated sample was 4.74% which increases to 7.80% for bromelain and 13.82% for papain-treated samples. The protein content of optimized papain and bromelain treated samples was significantly decreased ($p<0.05$) from 82.44 to 80.25% and 81.43% respectively for papain and bromelain treated samples. There were no significant changes in fat, ash, and moisture on enzyme treatment.

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1. Introduction

Sukuti is an indigenous dried buffalo lean meat product of Nepal. In the traditional method, lean meat is cut into strips and hung over the fireplace in the kitchen and subjected to heat and smoke of the burning woods till the strips become adequately dry and hard. It can also be prepared by air drying or sun drying but drying and smoking over a fireplace impart more characteristics, pleasant taste, and texture than the sun-dried or air-dried counterpart. Most of the *sukuti* available in the local market are air-dried, sun-dried or smoke-dried, which should be cooked before consumption. Steamed-and-dried *sukuti* is also available in the market that need not be cooked before consumption. However, it is not very common in the market. There are several styles of eating *sukuti* but one of the more general styles is by roasting it in burning coal. The *sukuti* chips are then mixed with tomato chutney, ground spices, chilly, sauce, onion, garlic, salt, etc. the traditionally prepared *sukuti* has been known for its hard leathery texture due to more cross-linked connective tissues and is difficult to eat. Thus, it is unsatisfactory to the consumer (Zainal et al., 2013). Therefore, multiple methods were attempted to encounter this problem and tenderizing meat with enzymes becomes one of the abundant methods to improve the tenderness. It is due to the hydrolytic activity of enzymes on connective tissue leading to the better tenderization of the tough meat.

The useful and common characteristic of meat is tenderness. Meat tenderness varies with species, age, sex, breed, and part of muscle tissue. Tenderness originates from myofibrils, intermediate filaments, and the amount of connective tissue. The biochemical and structural components are most important for predicting the tenderness of meat (Istrati, 2008).

The tenderness of meat tissue depends mainly on the length of the sarcomere, intramuscular connective tissue, and intramuscular fat (Arshad et al., 2016). The meat industry is always seeking a suitable method to enhance meat tenderness. Different physical, chemical, and biochemical techniques are being investigated for beef meat due to the inconsistency among bovine muscles (Mărgean et al., 2017). Different research has been done using calcium chloride, salts, phosphates, and enzymes and physical methods like pressure treatment, electrical stimulation, and blade tenderization. The main objective of these treatments is to reduce the detectable connective tissue. Among many tenderization techniques, use of enzymes obtained from plants (e.g., such as ficin from figs latex, bromelain from pineapple stem and papain from pineapple latex) is efficient (Manohar, 2016).

The enzymatic activity of tenderizing enzymes is affected by immersion time, enzyme concentrations, pH, temperature, and mode of application (dipping or injection). Changes in pH may promote myoglobin oxidation and meat stability (Santos et al., 2020). Broad-spectrum enzymatic activity has been shown by papain in the pH range 5–8 and at 65°C (Scaman, 2003). The use of bromelain in meat showed desirable results at 10 mg/100 g meat with an increase in temperature from 4 to 70°C (Kolczak et al., 2008).

In the present study, we attempted to prepare a tender *sukuti*, which otherwise has a particularly tough and leathery texture, by using various concentrations of bromelain and papain. After treatment with enzymes, the *sukuti* obtained was analyzed for sensory parameters, proximate components, and the amount of hydroxyproline to represent collagen. The goal of this research was to help improve protein intake in the elderly and young Nepali via savory traditional meat product *sukuti* that can be eaten without excessive difficulty

2. Materials and Method

Fresh buffalo meat of round-cut (5 kg) was collected within 1-2 h post-slaughter from the local market of Dharan and was packed in low-density polyethylene (LDPE) bags to prevent moisture loss and contamination during transportation. The sample was transported to the laboratory and stored in a refrigerator at 4°C for 24 h for rigor-mortis set up before the further experimental process. Enzymes papain (P3375-25G) and bromelain (B4882-10G) from Sigma Aldrich were made available by Central Campus of Technology, Hattisar, Dharan.

2.1 Preparation of meat for enzyme treatment

Fresh buffalo meat was washed (by potable water) and trimmed (on a clean table) to remove fatty tissue from lean meat. The block meat was sliced with a sharp knife to dimension 1 cm × 1 cm × 15 cm strips. The strips were mixed and divided into the required number of portions.

2.2 Preparation of enzyme solution and treatment

Enzyme solution with concentrations 10, 20, 40, 60, 80, 90, and 100 mg/L were prepared for both the enzymes in 1 L distilled water each at 65°C. Three meat strips weighing 120 g each were taken and were injected with 10% weight of meat samples using a sterilized disposable syringe as per Moon (2018) and rested for 4 h then dried at 65ºC to moisture 5% using moisture balance technique. The dried samples were packaged in an airtight plastic package.

2.3 Optimization of enzyme concentration

The prepared samples of *sukuti* (treated with bromelain and papain) and control (without enzyme treatment) were coded and subjected to sensory evaluation using a 9-points hedonic rating (Ranganna, 1986). These samples were evaluated for texture, color, flavor, and overall acceptability, and the scores obtained were statistically analyzed to get an optimum concentration of enzymes.

2.4 Analytical methods

pH was determined using the method cited in Buyukyavuz (2014). Moisture was determined by the hot-air oven method, fat content was determined by the Soxhlet extraction method and crude protein was determined by the Micro-Kjeldahl method as described in Ranganna (1986). The ash content was determined by the dry ashing method as described in *AOAC* (2005).

The water holding capacity of meat was determined by the filter press method according to Subba (2010).

0.3 g of meat sample was placed in an ordinary filter paper and pressed between the pair Plexi plates and held for 5 min. The ratio of meat film area to the area on filter paper wet with meat fluid was calculated. A ratio of > 0.5 is regarded as good and < 0.4 as poor.

The hydroxyproline content of the meat sample was determined based on the procedure of Neuman & Logan (1950). 2 g meat sample was hydrolyzed with 40 ml of 6 N HCl for 18 h. The hydrolysate was filtered and the volume adjusted to 50 ml with distilled water followed by measurement of Absorbance at 540 nm and the hydroxyproline content was determined by referring to a standard curve. The collagen content was determined by multiplying hydroxyproline content by a factor of 7.52 and was expressed in mg/g tissue.

$$\text{Sample Hydroxyproline} = \frac{\text{sample absorbance} + 0.00921}{0.0572} \times \frac{2}{50}$$

$$\text{Collagen content(mg/g)} = \text{hydroxyproline} \times 7.52$$

2.5 Sensory evaluation

The prepared *sukuti* samples were cut into pieces of equal length of 2 cm and evaluated in terms of appearance, flavor, texture, and overall acceptability on the nine-point hedonic scale as per Ranganna (1986). The coded samples were randomly presented to 10 semi-trained panelists.

2.6 Data Analysis

The analyses were carried out in triplicate. Data were checked for homogeneity then analyzed by ANOVA using Genstat programming (Genstat Discovery Edition 3, 2010). Means of the data were compared using the LSD (Least Significant Difference) method at a 5% level of significance.

3. Results and Discussion

Optimizations were done for the concentration of enzymes (papain and bromelain) used for the tenderization of *sukuti* based on sensory evaluation and collagen content. The best-obtained products by sensory evaluation were subjected to Physicochemical analysis.

3.1 The Physico-chemical composition of raw meat

The physical and chemical composition of buffalo meat used for this work are presented in Table 1

Table 1: Physico-chemical composition of raw meat

Parameters	Values*
pH	5.6 (0.06)
Moisture (%)	78.2 (0.31)
Protein (%)	20.2 (0.2)
Fat (%)	1 (0.1)
Ash content (%)	1.13 (0.05)
Water holding capacity	0.52 (0.02)

* Values in Table 1 are the means of triplicates. Figures in the parentheses are the standard deviations.

The analysis showed that the meat used was of good quality in terms of water holding capacity (Subba, 2010). Factors like breed, age, frame size, sex, and weight at the slaughter of an animal, diet, production system, exercise, weather, stress, pre-slaughter condition, and slaughtering condition are affecting the composition of meat (Sañudo et al., 2013)

3.2 Optimization of papain enzyme concentration

Eight samples of dried meat were prepared with variation in the concentration of papain enzyme (00, 10, 20, 40, 60, 80, 90, and 100 mg/L) and the samples were coded as P00, P10, P20, P40, P60, P80, P90, and P100 respectively. The samples were subjected to sensory evaluation. The mean of the sensory score given to each quality attribute of each sample was calculated (Figure 1)

The statistical analysis sample treated with 40 mg/L (P40) got the highest sensory score in all parameters and was significantly different than others ($p<0.05$). This variation in the sensory parameter might be due to the enzyme concentration and enzyme penetration power. Youn and Yang (1975), showed that penetration values increased proportionally with increasing enzyme concentration but Dawson and Wells (1969) showed that at higher concentration of enzyme the meat was extremely tender and not acceptable to the panel. Papain tenderization of the adult beef determined improvement of meat tenderness, flavor and juiciness can be significantly improved using papain enzyme at a low dose to avoid advanced tenderization leading to soft structure, low resistance to mastication (Istrati, 2008).

3.3 Optimization of bromelain enzyme concentration

Eight samples of dried meat were prepared by making a variation in the concentration of bromelain enzyme (00, 10, 20, 40, 60, 80, 90, and 100 mg/L) and the samples were coded as B00, B10, B20, B40, B60, B80, B90, and B100 respectively and subjected to sensory evaluation (Figure 2). The sample B10 (10 mg/L bromelains) got the highest value of mean sensory score and found significantly different ($p<0.05$) from other samples at a 5% level of significance. The tenderizing effect of bromelain was due to the hydrolysis of muscle protein via fragmentation of the myosin heavy chain (Ketnawa & Rawdkuen, 2011 and Bille & Taapopi, 2008). The heating process also affects the tenderization process. Heating of meat above 60°C shows increased meat tenderness due to denaturation of myofibrillar and connective protein, change in the structure of endomysium and perimysium as well as solubilization of collagen fiber (Kolczak et al., 2008).

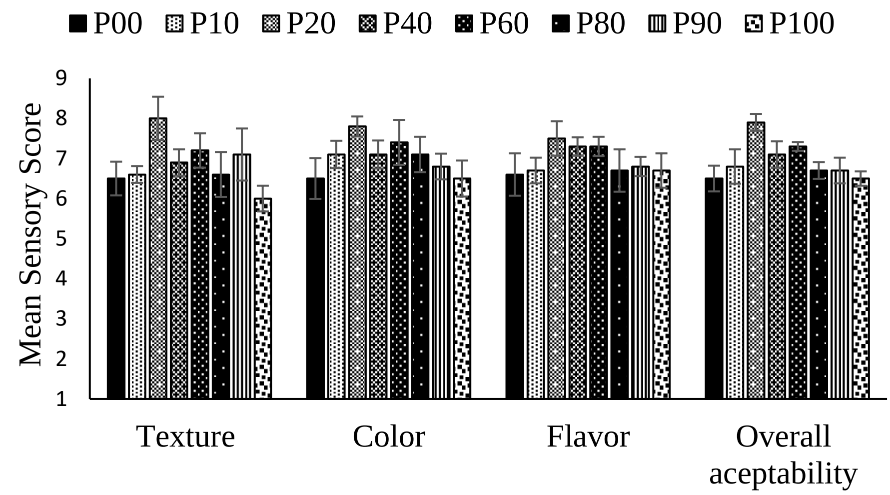

Figure 1: Mean sensory score of treatments at a different level of papain enzyme

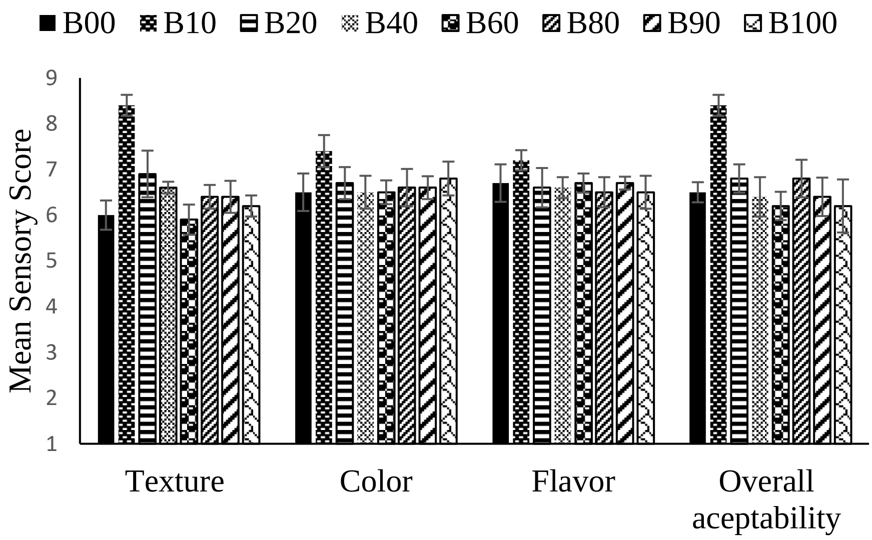

Figure 2: Mean sensory score of treatments at a different level of bromelain enzyme

3.4 Chemical Composition of products

The proximate analysis of the two optimized products (P40, B10), and a control sample were subjected for proximate analysis, and the data obtained were shown in Table 2. The analysis showed that the protein content of control was significantly higher than the protein content of enzyme-treated products. According to Haslaniza et al. (2010) at a lower concentration of enzymes, a small number of soluble peptides were produced and some amount of them were lost resulting in a low nitrogen content value.

3.5 The collagen content of optimized products

The collagen contents of optimized products are presented in Table 3. The statistical analysis showed a significant ($p<0.05$) variation of collagen content in between enzyme-treated and untreated samples.

The analysis showed that the control samples were the least tender samples while the sample treated with papain enzyme was significantly tenderer than other samples (Table 3). Papain enzyme had higher soluble collagen content and percent solubility compared to the bromelain enzyme. The control sample had the most insoluble collagen and was statistically higher than all other samples. Structural alteration occurs by the action of the enzyme on collagen cross-link and the collagen solubility increases (Rawdkuen & Benjaku, 2012). It might be due to an increase in permeability of the connective tissue due to which collagen disintegrates easily. The conversion of collagen to gelatin is an important quality indicator of tender meat. Collagen is the determining factor in the textural variations among various muscles and species. The solubility of connective tissue is more associated with sensory characteristics rather than the total amount of connective tissue (Naveena et al., 2011). Papain is more effective for increasing collagen solubility than the other proteases like bromelain, ficin, and *B. subtilis* proteases (Sullivan & Calkins, 2010; Qihe et al., 2006).

Table 2: Proximate analysis of the optimized products and control sample

Samples	**Parameter***			
	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
P40	$9.34^{c} \pm 0.08$	$80.25^{b} \pm 0.17$	$3.55^{b} \pm 0.05$	$4.55^{c} \pm 0.01$
B10	$9.65^{d} \pm 0.003$	$81.43^{a} \pm 0.20$	$3.21^{a} \pm 0.18$	$4.51^{a} \pm 0.006$
Control	$8.63^{a} \pm 0.08$	$82.44^{c} \pm 0.13$	$3.49^{b} \pm 0.07$	$4.53^{b} \pm 0.006$

**Values in Table 2 are the means of triplicates. Figures in the parentheses are the standard deviation. Means within a given column with a common superscript do not differ significantly.*

Table 3: Collagen content of enzyme-treated samples

Samples	**Collagen content (mg/g tissue)***			
	Soluble	Insoluble	Total	Solubility (%)
P40	$0.98^{b} \pm 0.01$	$6.11^{b} \pm 0.006$	$7.09^{b} \pm 0.02$	$13.82^{c} \pm 0.88$
B10	$0.52^{a} \pm 0.006$	$6.14^{a} \pm 0.006$	$6.66^{a} \pm 0.01$	$7.80^{b} \pm 0.07$
Control	$0.44^{a} \pm 0.006$	$8.91^{c} \pm 0.006$	$9.35^{c} \pm 0.01$	$4.74^{a} \pm 0.06$

**Values in Table 3 are the means of triplicates. Figures in the parentheses are the standard deviation. Means within a given column with a common superscript do not differ significantly.*

4. Conclusion

For tenderization of dried meat (*sukuti*), less amount of bromelain (10 mg/L) is effective as compared to papain (40 mg/L) for sensory quality enhancement. The total collagen and soluble collagen content decrease more in bromelain treated than papain treated *sukuti*. The solubility of collagen was found to be more in papain treatment than bromelain treatment resulting in more loss of protein in papain-treated samples.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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