

EXTRACTION AND UTILIZATION OF PAPAYA EXTRACT AS MEAT TENDERIZER AND ANTIMICROBIAL ACTIVITY AGAINST *Salmonella typhimurium*

Muhammad Saeed¹, Sajjad ur Rahman², Muhammad Asim Shabbir¹, Nassrulla Khan¹ and Azam Shakeel^{1,*}

¹National Institute of Food Science and Technology, University of Agriculture, Faisalabad 38040, Pakistan;

²Institute of Microbiology, University of Agriculture, Faisalabad 38040, Pakistan.

*Corresponding author's e-mail: azam1087@gmail.com

Papaya extract from *Carica papaya* (dwarf solo) was found to curtail active growth of *Salmonella typhimurium*. Four different segments of *Carica papaya* fruit were processed and protein concentration of unripen peel (48.7%), unripen latex (58.5%), ripe peel (40.5%) and ripe latex (50.7%) were observed. The protease activity and pH of extract of unripe latex, unripe peel, ripe latex and ripe peel were recorded as “1648 - 9.20”, “1684.5 - 9.91” and “5 - 4.5”, “6 - 4.7” respectively. The proteolytic activity of unripe and ripe peel extract was observed as 9.20 and 9.91 at pH 4.5 and 4.7. The proteolytic activity of unripe and ripe latex extract was observed as 1648 and 1680.5 at pH 5 and 6 respectively. Anti-*Salmonella* activity of papaya extract was accessed through multiple dilution technique to find out minimum inhibitory concentration. Papaya extract from ripe peel showed minimum 25.31µg of MIC₅₀ and 50.62µg of MIC₉₀, followed by unripe latex with MIC₅₀ at 36.56µg and MIC₉₀ at 73.12µg. However, the MIC₅₀ for unripen peel was 60.93µg and MIC₉₀ at 121.87µg likewise the papaya extract derived from ripe latex showed MIC₅₀ at 63.37µg and MIC₉₀ at 126.75µg. Ripe peel and unripen latex had better potential to control *Salmonella typhimurium* growth. The beef kababs incorporated with unripe latex extract at various levels (0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/100g), showed significant reduction in moisture contents while protein, fat, ash, cooking yield and texture showed non-significant variation during storage.

Keywords: Papaya extract, beef kababs, protease activity, *Salmonella typhimurium*, minimum inhibitory concentration.

INTRODUCTION

Papaya mostly found in tropical and sub-tropical areas of the world however in Pakistan, province of Sindh and Punjab are rich in lush green orchards of papaya (Oad *et al.*, 2001). It is native to America (Pantoja *et al.*, 2002). Plant proteolytic enzymes are superior to bacterially derived enzymes as meat tenderizers because of safety concerns such as pathogenicity. Plant proteolytic enzymes can digest muscle proteins including collagen and elastin, which lessens the toughness of meat. However, the proper quantity of enzymes must be used because excessive amounts would result in meat decomposition (Rawdkuen *et al.*, 2012). It was suggested that papain with 0.025% concentration at 3% level (w/w) can be utilized to develop better tenderness and functional properties of spent hen meat cuts for efficient utilization (Khanna and Panda, 2007). Cysteine endopeptidase, also known as thiol protease is a group of enzymes have been found abundantly in the latex of *Carrica papaya* having enzymes like glycylendopeptidase, caricain, chymopapain and papain that comprise a huge amount of whole enzyme fraction, which also have 80% whole of the enzyme (Azarkan *et al.*, 2003). These enzymes showed broad specificity, cleaving peptide bonds of basic amino

acids (Suster *et al.*, 2011). That is why used as meat tenderizing and clarifying agent in food industries and in processing to confirm proper fading of leather (Huet *et al.*, 2006). Papaya extract have antibacterial activity against *E. coli*, *S. aureus* and *C. albicans* (Tewari *et al.*, 2014) and *Listeria monocytogenes* (Eshamah *et al.*, 2014) and *S. dysenteria*, *P. aeruginosa*, *P. fluorescens*, *S. marcescens*, *P. vulgaris*, and *S. typhimurium* (Ogunjobi *et al.*, 2011). It has been reported that 90% of the estimated food-related deaths involve the pathogens *Salmonella* (28%), *Toxoplasma* (24%), *Listeria monocytogenes* (19%), Norwalk-like viruses (11%), *Campylobacter* (6%) and *Escherichia coli O157:H7* (3%) (CDC, 2011). According to previous research occurrence of *Listeria monocytogenes* in processed meat varies from 0 to 15% and in chicken was 20.6 to 24.1%. Different types of pathogenic bacteria and many other organisms might be hosted into meat during any process that may be in slaughter house, processing, cooking or any other, which causes spoilage and major loss for nutritional value that is affecting on human being. This is the main reason to decrease microbial potential that enhance the shelf-life (Gudbjomsdottir *et al.*, 2004). The basic purpose of present study was to increase the shelf life of beef kababs by

inhibiting the *Salmonella typhimurium* and improves the tenderizing property of beef kababs.

MATERIALS AND METHODS

Plant material collection: Unripe and ripe papaya (dwarf solo) was procured from local market of Faisalabad. Unripe latex, unripe peel, ripe latex and ripe peel were separated after cutting and seed removal. Unripe latex, unripe peel, ripe latex and ripe peel were kept into separate trays for the purpose of drying in oven at 40°C. The dried latex and peel were ground by blender and obtained in fine powder.

Preparation of papaya peel and latex protease (extract): Papaya (*Carrica papaya*) extract was prepared from unripe latex, unripe peel, ripe latex and ripe peel through a method of ethanol extraction in 70% ethanol (Sumathi and Gowthami, 2014). The ethanolic extraction was done at 28±1°C for 72 hours. The extracts were then decanted and filtered through a Whatman filter paper followed by membrane syringe filters and then ethanol was evaporated. Finely ground powder (5g) from each part was taken in each of the 250 ml beaker containing 100 ml ethanol. The mixture was kept at room temperature for 72 hours and stirred using sterile glass rod after every 4 hours. Each extract was filtered through Whatmann No. 1 filter paper and beakers were kept in a water bath at 50°C until the solvent gets evaporated. Finally, a greasy type material was obtained from the unripe latex, unripe peel, ripe latex and ripe peel extract. Each extract was transferred to sterile screw capped bottles, labeled and stored under refrigerated condition till use.

pH: The pH of papaya (unripe latex, unripe peel, ripe latex and ripe peel) extract was determined by using digital pH meter (InoLab 720, Germany) by following the method (981-12) as described in AOAC (2006).

Determination of protein content: Protein content in papaya extract was determined through Bradford method as described by Nitsawang *et al.* (2006).

Proteolytic activity: Proteolytic activity of papaya extract was determined by following the Arnon method as described by Nitsawang *et al.* (2006). The reaction mixture contained 200 µl of 50 mM cysteine–20mM EDTA (disodium salt), pH 8.0, 700 µl 50 mM Tris–HCl buffer, pH 8.0 and 100 µl enzyme solution. The mixture was incubated at 37°C for 5 min before starting the reaction by adding 1ml of 1% (w/v) casein solution. After 10 min, the reaction was stopped by adding 3ml of 5% (v/v) trichloroacetic acid (TCA) and then cooled for 1 h. The reaction mixture was centrifuged, and absorbance of the supernatant was measured at 275 nm. The reading was corrected for a blank in which the enzyme was added after addition of TCA.

Microbiological analysis of papaya extract against salmonella:

Source of Salmonella culture: A pathogenic *Salmonella typhimurium* culture was obtained from diagnostic laboratory of Institute of Microbiology, University of

Agriculture, Faisalabad. The *Salmonella typhimurium* indigenous strain (*SS-Stm-pd-2015*) was isolated from poultry droppings and maintained under slant culture tubes. The pure slant culture was recultivated onto *Salmonella-Shigella* (SS) agar and Xylose Lysine Deoxycholate (XLD) agar separately. The plates were then incubated at 37°C for 24 hours and characteristic colonies of *Salmonella* were selected. Motility test and Gram's staining test were performed to identify the pure growth of *Salmonella* spp.

Identification and Serotyping of Salmonella: Pure colonies were further confirmed by triple sugar iron test, indole test, methyl red test, Voges Proskauer test, citrate test and catalase test. *Salmonella* isolates were serotyped by the method based on slide agglutination for O and H antigens.

Antimicrobial activity of papaya extract against Salmonella typhimurium: The MICs of *Salmonella* isolate was determined according to the NCCLS guidelines for the multiple dilution method. Papaya extract from different parts were separately processed containing different levels of protein contents as peel of *Carica papaya* (48.7% protein), unripe latex (58.5%), ripe latex (50.7%), and ripe peel (40.5%). Specific antimicrobial activity was determined against *Salmonella typhimurium*. Brain heart infusion broth 50µL was added in each row of sterile 96 well Microtiter plate and 50µL of papain extract was added in each 1st well and performed serial dilution, 10µL of activated culture of *Salmonella typhimurium* having turbidity of 0.5 McFarland standard was added in each well and incubated this assembly for 24 hours at 37°C.

Utilization of papaya extracts in beef kababs: The raw beef, utilized in research program, was obtained from local slaughter house in Faisalabad at maximum two hours post-slaughter. The cow was more than 9 years old. The beef kababs were prepared by mincing the meat with meat mincer (Hobart®, USA) with 5 mm plate. Unripened papaya latex was added in minced meat by the following treatments and left for two hours for enzymatic actions. This minced meat was mixed with condiments (black pepper, garlic paste, salt, chopped onion, green chilies and whipped egg) as per recipe of commercial beef kababs and then manually molded to make a uniform kabab with 30 mm diameter. The pieces were breaded and fried in canola oil at 180°C until an internal temperature of 71°C was attained. The fried beef kababs were cooled at room temperature for 30 min, packed in polyethylene bags and stored under sterilized condition at room temperature till further analysis at 0, 5, 10 and 15 days storage as described by Kumar and Tanwar (2011).

Treatment plan

1. Unripe latex not mixed in minced beef
2. Unripe latex mixed in minced beef with a concentration of 0.5mg/100g
3. Unripe latex mixed in minced beef with a concentration of 1.0mg/100g

4. Unripe latex mixed in minced beef with a concentration of 1.5mg/100g
5. Unripe latex mixed in minced beef with a concentration of 2.0mg/100g
6. Unripe latex mixed in minced beef with a concentration of 2.5mg/100g
7. Unripe latex mixed in minced beef with a concentration of 3.0mg/100g

Physico-chemical analysis of beef kababs

Moisture, ash, fat and protein contents: Moisture, ash, fat and protein contents of beef kababs were determined by using Air Forced Draft Oven, Muffle Furnace, Soxtec System, Kjeldal Apparatus by following the method number (934-1, 942-05, 920-39, 984-13) respectively as described in AOAC (2006).

Cooking yield: It was determined by measuring the difference in the sample weight before and after cooking using the following formula as described by Ikhlas *et al.* (2011).

$$\text{Cooking yield} = \frac{\text{Weight after cooking}}{\text{Weight before cooking}} \times 100$$

Texture profile analysis: Texture analysis of beef kababs was done by the texture analyzer (Model TA-XT2). The display shows the results in term of force (g) and distance in term of millimeters (mm). The data was collected by its software for checking the results as described by (Piga *et al.*, 2005).

Sensory evaluation: Beef kababs were subjected to sensory analysis by ten panelists comprised of faculty members from National Institute of Food Science and Technology, University of Agriculture, Faisalabad. Kababs were evaluated for color, texture, flavor, juiciness and overall acceptability on 9-point hedonic scale by following the method as described by stone *et al.* (2012).

Statistical analysis: The data obtained was subjected to statistical analysis by applying the Two-Factor Factorial under Completely Randomized Design (CRD) to determine the level of significance using Statistix version 8.1. as described by Montgomery (2008).

RESULTS AND DISCUSSION

Unripe latex extract, unripe peel extract, ripe latex extract and ripe peel extract was extracted through 70% ethanol extraction method from immature and fully mature papaya respectively. Chaiwut *et al.* (2007) extracted the proteases from papaya peel extract and papaya latex with extracting methanol (75%), ethanol (70%) and 2-propanol (67%). The proteolytic yield (57.6%) was obtained through ethanol (70%) greater than other precipitants. pH of the Unripe latex extract, unripe peel extract, ripe latex extract and ripe peel extract were 5.00, 4.5, 6.0 and 4.7 respectively. pH of peel extract was less as compared to latex extract is due to the presence of acidic component in peel (Table 1). The results were comparable to the previous report where the proteases

were extracted from fresh and dried papaya peel through different extracts and measured the pH in fresh and dried papaya peel were 5.61 ± 0.04 and 4.31 ± 0.13 respectively (Chaiwut *et al.*, 2010). In addition, papaya fruit extract displayed a pH of 4.30 after tapping papain (Akin-Osanaiye *et al.*, 2008). Protein was determined separately in unripe latex extract, unripe peel extract, ripe latex extract and ripe peel extract having protein contents 58.5, 48.7, 50.7 and 40.5 g/100ml respectively (Table 3). According to previous research proteases present in papaya were papain, chymopapain, glycyI endopeptidase and caricain having total protein content 69-89% (Barrett and Rawling, 1998). Total protease activity was determined by using casein as substrate and found 1648U, 9.20U, 1680.5U, 9.91U in unripe latex extract, unripe peel extract, ripe latex extract and ripe peel extract respectively (Table 1). Specific activity is actually the ratio between the proteolytic activity (unit) and protein (mg). Specific activity of unripe latex extract was greater than other respective extracts. The results are similar to the findings of Chaiwut *et al.* (2007) who determined the total proteolytic activity in papaya peel extract and dried papaya latex protease were 44u/5g, 1623u/g respectively. Specific activity of dried papaya latex (4.95) was greater than the papaya peel extract (1.07).

Table 1. Values of total proteolytic activity (U) and specific activity (U/g) of different extracts of papaya.

Papaya extract	pH	Total Proteolytic activity (U)	Specific activity (U/g)
Unripe latex	5.0	1648	4.67
Unripe peel	4.5	9.20	1.08
Ripe latex	6.0	1680.5	4.32
Ripe peel	4.7	9.91	1.00

Isolation of *Salmonella typhimurium*: The pure culture isolate of *Salmonella typhimurium* (SS-Stm-pd-2015) was found Gram negative coccobacilli and motile in nature. Biochemical results of *Salmonella typhimurium* (SS-Stm-pd-2015) are shown in (Table 2) confirms the purity of the sample isolate. Triple sugar iron test was performed to access the fermentation characteristics of three sugars, glucose: lactose: and sucrose. Samples were glucose fermenter and produced H₂S shown by the black butt. Identification of *Salmonella typhimurium* (SS-Stm-pd-2015) sample was done in accordance with Mondal *et al.* (2008).

Table 2. Biochemical tests of *Salmonella typhimurium* (SS-Stm-pd-2015).

Biochemical test	Results
Triple sugar iron	+
Indole	-
Methyl red	+
Voges proskauer	-

Citrate	+
Catalase	+

Unripe latex	58.5%	36.56 µg	73.125 µg
Ripe latex	50.7%	63.37 µg	126.75 µg
Ripe peel	40.5%	25.31 µg	50.62 µg

Antimicrobial activity of papaya extract against Salmonella:

Four different segments of *Carica papaya* fruit were used to determine the anti-*Salmonella* activity. These four parts of *Carica papaya* were separated and processed through ethanol extraction technique and the papaya extract containing protein concentration in unripen peel was 48.7%, unripen latex 58.5%, ripe peel 40.5% and ripe latex was 50.7%.

Papaya extract extracted from *Carica papaya* (Papaya plant) was found to curtail active growth of *Salmonella typhimurium* carrying bacteriostatic and bactericidal potential. Papaya extract extracted from ripe latex showed MIC₅₀ at 63.37µg and MIC₉₀ at 126.75µg. Results from MIC of ripe peel were 25.31µg (MIC₅₀) and 50.62µg (MIC₉₀). The MIC₅₀ was recorded at 36.56µg and MIC₉₀ was at 73.12µg in unripe latex whereas, the MIC₅₀ from unripen peel was 60.93µg and MIC₉₀ at 121.87µg against *Salmonella typhimurium* was recorded. The overall results revealed the papaya extract from ripe peel and unripen latex had better potential to control *Salmonella typhimurium* under *in-vitro* condition. The results were found to convincingly promote the best source of papain activity to be described under *in-vivo* trails. Table 2 indicates the complete results of the MIC of *Carica papaya* on the *Salmonella typhimurium* (SS-Stm-pd-2015). Antibacterial activity of papain against *streptococcus mutans* ATCC 25175 was determined with the use of minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) using dilution method. Minimum inhibitory concentration (MIC) of papain against *Streptococcus mutans* was 7.5% and minimum bactericidal concentration (MBC) was against *Streptococcus mutans* 15%. This study shows the antibacterial activity of papain against *Streptococcus mutans* (Gartika *et al.*, 2014).

Table 3. Minimum inhibitory concentration (MIC) of *Carica papaya* on the *Salmonella typhimurium* (SS-Stm-pd-2015).

Papaya extract source	Protein %age	MIC ₅₀	MIC ₉₀
Unripe peel	48.7%	60.93 µg	121.87 µg

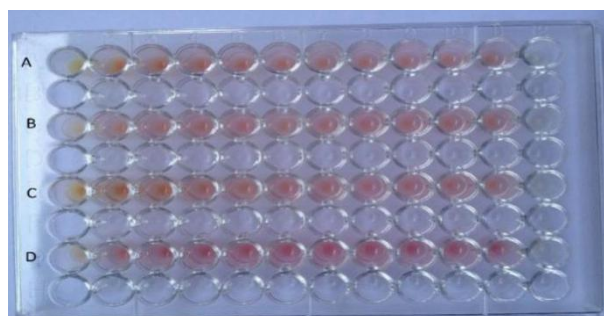


Figure 1.96 wells microtiter plate for minimum inhibitory concentration (MIC) of *Carica papaya* on the *Salmonella typhimurium* (SS-Stm-pd-2015).

Proximate analysis of beef kababs

Effect of proteolytic extract on ash (%) and fat of beef kababs: Ash content in beef kababs represents inorganic residue remaining after organic matter has been burnt away. Ash content ranging in beef kababs were 4.29 to 4.14% (Fig. 2). Non-significant decreasing trend was seen between control beef kababs and treated beef kababs. Ash content was decreased non-significantly due to loss of juiciness in beef meat by the action of proteolytic extract that damaged the muscle tissue of beef. Ash contents in beef kababs showed non-significant results during storage period (Table 5). Istrati *et al.* (2012) determined the marinating effect of proteolytic enzyme on quality of beef meat and found the result, loss of juiciness of beef meat depending on heat treatment and nature of enzymes. Bound water was low in papain treated beef as compared to control due to destruction of muscle tissue. The results are similar to the findings of Abdeldaiem and Hoda (2013) who studied a method for improving the tenderness of aged camel meat by ginger extract which is also a tenderizer and the effect of ginger extract decreased the ash contents (4.30% to 4.09%) of camel meat. Fat contents ranging in beef kababs were 9- 8%. There was non-significant trend was seen in fat (%) of beef kababs with the addition of protease extract (fig. 2).

Table 4. Effect of proteolytic extract on proximate composition, cooking yield and texture of beef kababs.

Storage days	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Cooking yield (%)	Texture (N)
0	54.56a	23.52a	9.00a	4.28a	86.21a	72.43a
5	52.52b	23.33b	8.97b	4.26b	85.14b	73.28a
10	50.53c	23.21c	8.93c	4.23c	84.19c	75.11b
15	48.51d	23.06d	8.71d	4.21d	83.19d	74.64c
Level of significance	**	NS	NS	NS	NS	NS

Means carrying same letters within a column or row do not differ significantly (P < 0.05)

However, non-significant trend was seen in fat (%) of control and treated beef kababs during storage period from 0 to 15 days. Non-significant trend was also seen in ether extract and total ash contents of duck patties stored at ambient and refrigeration temperature (Biswas *et al.* 2011).

Effect of proteolytic extract on moisture (%) and protein (%) of beef kababs: Moisture and protein contents ranged in beef kababs were 54.29-48.83 and 24-22.5 respectively. Non-significant trend was seen on moisture (%) and protein (%) of beef kababs in control beef kababs and treated beef kababs (Fig. 2). However, a significantly decreasing trend was seen in moisture content while non-significant in protein during storage from 0 to 15 days. The results are in close agreement with the findings of Naveena *et al.* (2004). He studied the raw buffalo meat chunk, treated with cucumis, ginger and papain and found 20.06, 19.14 and 19.42 protein values respectively. In controlled chunk, 20.08% protein was observed, as the chunks were cooked, protein level increased by decreasing the moisture. The moisture content in raw buffalo chunk was 76.51% and after cooking it was 54.29%. The results are in close agreement with the findings of Garg and Mendiratta (2006) that prepared the enrobed pork chunks in microwave oven with different tenderizer like ginger extract, cucumis extract and papain and stored for 15 days under refrigerated conditions and observed that papain and ginger treated chunks having significantly decreased in moisture contents during storage.

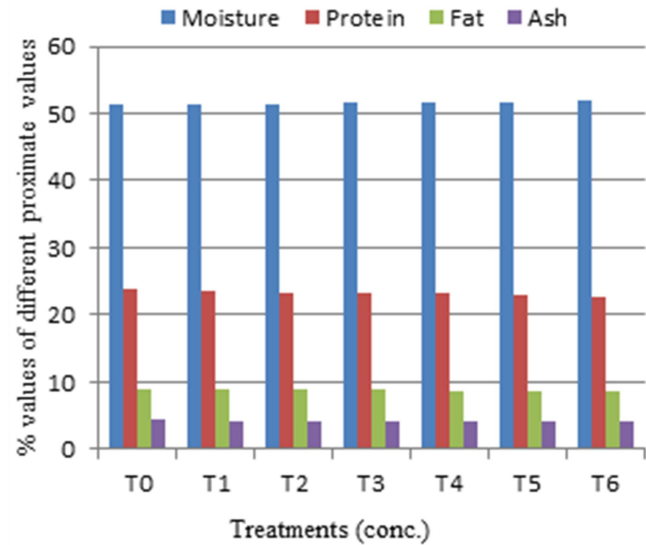


Figure 2. Effect of protease on proximate composition of beef kababs.

Cooking yield: Cooking yield values were higher in beef kababs treated with protease extract as compared to control but the difference was not statistically significant (Fig. 3). In the previous study, different plant extracts like kachri, Ginger rhizome were used and its tenderizing efficacy was compared with most popular enzyme papain. Cooking yield increased with papain 0.2% (w/w) and ginger 5% (w/v) and decreased with cucumis 2% (w/w) but this change was not statistical significant (Naveena *et al.* 2004). Non-significant trend was observed during the storage period from 0 to 15 days. In the previous study, the results are similar to the findings of Istrati *et al.* (2012) who observed the effect of papain, bromelain, papaya, pineapple on the beef muscles and also found the thermal loss and cooking yield during storage. Cooking yield ranged from 87.35 to 90.05 which was not significantly differed.

Texture: The results indicated that texture of the beef kababs ranged from 93.73 to 58N among the beef kababs prepared by using different levels of protease extract (Fig. 3). 360 Nmm energy is utilized to measure the hardness (92.01N) of beef kababs prepared without protease extract and less than 360 Nmm energy is used in treated beef kababs because the texture of beef kababs become soft due

Table 5. Effect of proteolytic extract on sensory evaluation of beef kababs.

Storage days	Colour	Flavour	Juiciness	Texture	Overall acceptability
0	6.80a	7.00a	6.91a	7.57a	7.00a
5	6.73b	6.84b	6.72b	7.41b	6.71b
10	6.50c	6.51c	5.99c	7.15c	6.40c
15	6.32d	6.19d	5.00d	6.89d	5.99d
Level of Significance	**	**	**	**	**

Means carrying same letters within a column or row do not differ significantly (P < 0.05).

to degradation of myofibrillar and collagen protein with the addition of protease extract (Fig. 7). Firmness significantly decreased with protease extract. Non significance trend was seen in beef kababs during storage from 0 to 15 days (Table 3). In the previous study, raw beef, chicken and squid was treated with bromelain extract (0 to 20%) and found the hardness 570 N, 111 N and 314 N, respectively (Ketnawa and Rawdkuen, 2011).

Sensory evaluation: Sensory evaluation is the most important quality parameter for the acceptance or rejection of product. Beef kababs incorporated with protease or unripe latex extract of papaya affect the sensory attributes like flavour, juiciness, texture and overall acceptability other than colour Sakandar *et al.* (2014). The lowest scores were given to flavour and juiciness with the addition of protease in beef kababs. There was significantly ($P<0.05$) reduction trend was observed in flavour and juiciness with the incorporation of protease. The highest scores were given to the texture and overall acceptability with the addition of protease. Significantly ($P<0.05$) improvement was observed in texture and overall acceptability with the incorporation of protease (Fig. 4) All the sensory parameter of beef kababs were significantly decreased during storage (Table 4). Flavour score decreased may be due to appearance of foreign beef taste sensation by increasing storage period and enzyme concentration. Reduction of juiciness may be due to breakage of protein bond by increasing enzyme concentration that enhances the juice loss from meat and texture become soft. Overall acceptability scores were significantly higher in treated beef kababs than control and decreased significantly during storage. The current results are in line with the previous results of Naveena *et al.* (2004), Naveena (2002) and Istrati *et al.* (2011) found that flavor, juiciness, tenderness and overall acceptability scores of buffalo meat and Garg and Mendiratta (2006) pork chunk decreased significantly during storage. Thomas *et al.* (2008) prepared the shelf stable pork sausages using hurdle technology and stored for 3 to 9 days at ambient temperature and pork sausages retained good acceptability for 6 days.

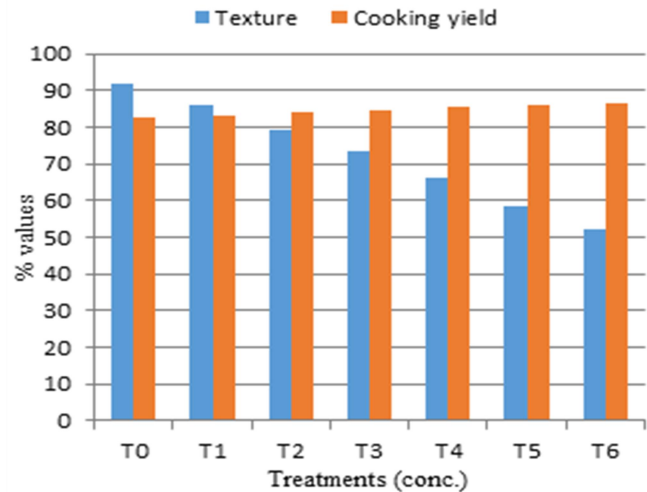


Figure 3. Effect of protease on texture and cooking yield of beef kababs.

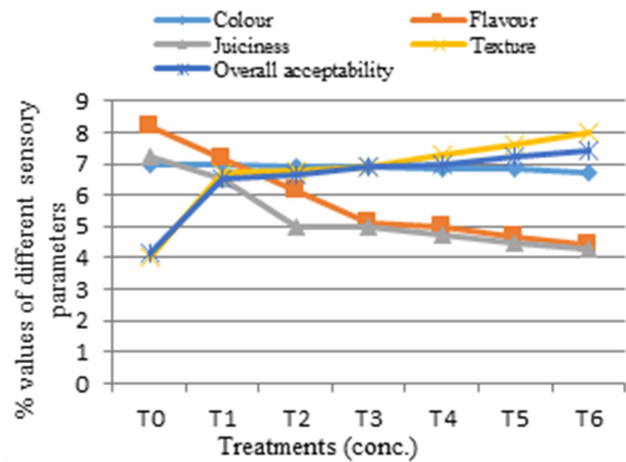


Figure 5. Effect of protease on sensory parameters of beef kababs.

Conclusion: The results of present study revealed that papaya extract is extracted from unripe latex, unripe peel, ripe latex and ripe peel. Unripe latex extract had more specific activity than other respective components. Therefore, unripe latex extract was used to improve the meat tenderness. Texture and overall acceptability in beef kababs were improved significantly while Juiciness and colour decreased significantly and colour remains constant. Basic purpose of the present study was designed to improve the tenderness and increased the shelf life of beef kababs by inhibiting the pathogenic microorganisms like *Salmonella typhimurium* and others.

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