Tenderizing Effect of *Cucumis trigonus* Roxb and *Carica papaya* on Emu Meat Chunks

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ABSTRACT

Among exotic meat species, ratites have received significant attention as producers of low-fat meats. Fresh emu meats were procured from local market and cut into small chunk of approximately 3 cm³ size and were randomly allotted for different treatments (0, 5, 7 and 10% W/V) of control, *cucumis trigonus* Roxb and *Carica papaya*. The pH value of *cucumis* solution was significantly (P<0.01) lower than other solution. The marinade absorption values were higher in papaya treated samples compared to *cucumis* treated samples. There was significant (P<0.05) reduction of pH in samples treated with *cucumis* compared to papaya. There was significant (P<0.01) reduction of WHC (water holding capacity) in *cucumis* treated samples compared to others. Moisture content tends to be higher in papaya treated samples. Significant (P<0.01) increase in collagen solubility and sarcoplasmic protein solubility, myofibrillar protein solubility and total protein solubility of *cucumis* and papaya treated samples compared to control. It is concluded that *cucumis* was found to be comparatively more effective on myofibrillar proteins, while papaya were found more effective on sarcoplasmic proteins.

Keywords: Emu meat, tenderness, herbal tenderizer, physicochemical

World meat consumption is outpacing the sale of other major agricultural commodities, especially in developing countries, according to the Agricultural Outlook 2012–2021 report by the United Nations Food and Agriculture Organization (FAO) and the Organization for Economic Co-operation and Development (OECD). Due to the spread of foot-and-mouth disease and BSE (bovine spongiform encephalopathy/mad cow disease) in bovines and the occurrence of dioxin in poultry in the 1990s, consumers are also concerned about the safety and quality of meat product (MacRae et al., 2005). Consumers also expect the meat products in the market to have the required nutritional value, be wholesome, fresh, and lean, and have adequate juiciness, flavor, and tenderness (Ngapo and Dransfield, 2006). Emu (*Dromaius novaehollandiae*) farming has become a popular and lucrative part of agricultural industry throughout the world. Today, the world population of emus is estimated to be around 2 million, out of which 1 million is estimated to be spread between Northern America, Peru, India and China and about 750 000 in Australia (Orumbayev, 2015). Appearance of ratite meat is similar to that of beef: red color and high level of hemicin iron, high level of unsaturated fatty acids (Chatli et al., 2015). The degree of tenderness can be related to those of connective tissue, myofibrils and sarcoplasmic proteins (Maiti et al., 2008). Several workers have attempted to improve tenderness of meat by using phosphate, inorganic salt, enzymes, electrolytes and pressure treatment (Sheard et al., 1999). Plant proteolytic enzymes such as papain, bromelin and ficin have been widely used for the tenderization of meat (Gerelt et al., 2000). Recently, proteolytic enzymes derived from papain and cucumis (*Cucumis Trigonus Roxb*) were also reported to be very effective for tenderization of chicken, sheep and buffalo meat.

Considering these points, study on tenderization of emu meat was conducted to standardize the optimum
concentration of different herbal tenderizer on emu meat chunks and evaluate the physico-chemical properties of emu meat after the different treatments.

MATERIALS METHODS

Fresh emu meats was procured from local market and were brought to the Department of Livestock Products Technology, West Bengal University of Animal and Fishery Sciences, Kolkata. The carcasses were deboned and lean meat were collected and packed in low-density polyethylene (LDPE) bags and stored in refrigerator at 4±1°C for 24 hrs. After 24 hrs chilling, muscle were taken out of refrigerator and cut into small chunk of approximately 3 cm³ size and were randomly allotted for different treatments.

Determination of optimum concentration of cucumis and papaya

Washed cucumis and papaya fruits were blend or homogenized with equal amount of chilled distilled water and filtered through muslin cloth. 3 cm³ uniform sized emu meat chunks were randomly divided into 4 groups and they were marinated with different concentration (0, 5, 7 and 10% W/V) of cucumis and papaya. For marination, required volume of marinates was diluted with the distilled water at 15% w/v. After thorough mixing by hand, the chunks were placed in polyethylene bags and kept at 4±1°C for 24 hrs. After 24 hrs of marination the chunks were washed, drained and were cooked in oven to an internal temperature of 75±1°C monitored using probe thermometer. Samples were evaluated for sensory attributes to select the best concentration of cucumis and papaya marinates. Minimum of six trials (n=6) were conducted and data obtained were analyzed statistically.

Analytical procedure

pH determination

The pH of the finely minced meat sample was determined by the method of Trout et al. (1992). 10 g of sample was homogenized with 50 ml distilled water using mortar and pestle. Then the pH of the suspension was recorded using digital pH meter (Systronics µ pH System 361).

Marinade absorption

The marinade absorbed by the chunks after marination was calculated by following formula.

\[
\text{Marinade absorption} \% = \frac{\text{Weight after marination} - \text{weight before marination}}{\text{Weight of chunks before marination}} \times 100
\]

Water Holding Capacity (WHC)

Water holding capacity of meat sample was estimated by following the method as described by Nakamura and Kotah (1985) and Dal Bosco et al. (2001) with certain modifications. Water holding capacity was estimated by centrifuging 1 g of muscle sample placed on tissue paper inside a centrifuge tube for 4 minutes at 1,500g (approx. 1000 rpm) in Remi centrifuge machine. The weight of centrifuged meat sample was taken after removing the tissue paper and sample was dried at 70°C for 12 hours. After drying, weight of dried meat sample was noted to determine the quantity of water remained in meat sample after centrifugation.

Calculation

Water holding capacity (%) =

\[
\frac{(\text{Wt. of meat sample after centrifugation}) - (\text{Weight of meat sample after drying})}{\text{Initial weight of meat sample}} \times 100
\]
Determination of moisture

About 10 g of minced meat were taken in an aluminum moisture cup and dried in a hot air oven for 18 h at 100°C. After that the moisture cups were removed from the hot air oven, cooled in a desiccators and again weighed. The loss in weight was reported as moisture content. The process was repeated until constant weight of the sample was obtained.

Determination of crude protein

A 0.2 to 0.3 g of moisture free oven dried samples was taken in digestion tubes. After that, 3 to 4 g of catalyst mixture (CuSO$_4$ + K$_2$SO$_4$ in the ratio of 1:5) and 10 ml concentrated sulphuric acid were added to the sample and the tubes were placed in the preheated (300°C) digestion block of KEL PLUS KES 4L (M/s Pelican Equipment, Chennai) along with the manifolds. Then the Scrubber System was immediately switched on. After some time, if there was no frothing then the temperature was increased to 420°C. The tubes were kept in the block for 1 to 2 hours until the color of the samples were turned into bluish green. Once the bluish green colour appears the tubes were removed and placed in the cooling stand. An acid blank was also run along with the samples for correction of any nitrogen contribution by the acid itself. After cooling, the digested samples were quantitatively transferred into a volumetric flask (250 ml) with repeated washing with distilled water. The nitrogen content of the samples was estimated by distilling a suitable aliquot in the distillation unit of the instrument (KEL PLUS CLASSIC DX; M/s Pelican Equipment, Chennai) with 25 ml of Boric acid indicator and 40 ml of 40% NaOH. The distillated solution was titrated against 0.1N H$_2$SO$_4$ (Standardized) and once the color changes from bluish green to permanent pale pink the burette reading was noted as titrant value. The nitrogen content was multiplied by 6.25 to obtain the CP content of the sample.

Calculation

Crude Protein (%) =

\[
\frac{X \times (\text{Titrant Value}) \times (\text{normality of acid}) \times 25 \times 100 \times 6.25}{\text{Sample Wt.} \times 1000}
\]

Collagen content

Collagen content was determined by the International Standards Method (ISO, 1978). A standard curve was prepared containing known amount of hydroxyproline, absorbance (Model systronic PC based 2202 double beam Spectrophotometer) of samples was measured at 558 nm, and hydroxyproline concentration was calculated from the standard curve. Hydroxyproline content was reported as a percentage of mass and multiplied by 7.25 to convert to collagen content. Hydroxyproline (HP) content of meat sample was determined based on the procedure of Neuman and Logan (1950), with some modifications.

Collagen solubility

Solubility of Hydroxyproline and thus of collagen was determined by the method described by Mahendrakar et al. (1989), with some modification. A 5 gm muscle tissue was taken in 250 ml beaker and immersed in water bath after covering the beaker with petridish. Water bath was then heated to boiling temperature and kept for 30 min. The cooked meat from beaker was then taken out and cut into small pieces and homogenized with 50 ml distilled water at 40±1°C in blender for 2 min. The extract was then centrifuged at 4000 rpm for 30 min. Aliquots of cooked out juice and centrifugate were hydrolyzed for 18 hrs at 105°C in hot air oven. Soluble HP was calculated according to Williams and Harrision (1978).

\[
\% \text{ HP solubilized} = \frac{(\text{gm HP in drip} + \text{gm HP in cooked meat})}{\text{gm HP in raw muscle}} \times 100
\]

\[
\% \text{ collagen solubility} = 7.14 \times \% \text{ HP solubilized}
\]

(Dransfield et al., 1983)

Protein solubility

The protein solubility was determined according to the procedures of Joo et al. (1999) with slight modifications. To determine the solubility of the sarcoplasmic and total (sarcoplasmic and myofibrillar) proteins, two extractions were conducted. Sarcoplasmic proteins were extracted from 2g muscle using 20 mo of ice-cold 0.025M
potassium phosphate buffer (pH 7.2). The samples were minced, homogenized and kept overnight at 4°C with frequent shaking. Samples were centrifuged at 5000 rpm for 20 min and protein concentration in the supernatant was determined by the Biuret method. Total protein was extracted from 2 g muscle using 40 ml of ice-cold 1.1 M potassium iodide in 0.1 M phosphate buffer (pH 7.2) homogenization, centrifugation and protein determination were carried out as described above. Myofibrillar protein concentrations were obtained by difference between total and sarcoplasmic protein solubility.

**RESULTS AND DISCUSSION**

The results of effect on sensory attributes of different concentration of cucumis and papaya marinade in emu chunks are presented in Table 1 and 2. Appearance, colour and juiciness of emu chunks for control, 5%, 7% and 10% treated samples were not significant. There was significant (P<0.01) increase in flavour for 10% cucumis treated samples from control, 5% and 7% cucumis treated samples. There was a significant (P<0.01) increase in tenderness scores for 10% treated sample than control and 7%, however the difference between control and 5% was non-significant. Mean for overall acceptability scores were significantly (P<0.01) higher in 10% treated samples than control.

Significant improvement in tenderness and overall acceptability scores in buffalo meat roast treated with 2% (W/W) cucumis extract was reported by Naveena et al. (2004). Kumar and Berwal (1998) reported similar results in spent layer hens treated with 4% (W/W) of cucumis

**Table 1**: Effect of different concentration of cucumis on sensory attributes of emu chunks. (Mean ± SE)*

<table>
<thead>
<tr>
<th>Sensory attributes</th>
<th>Control</th>
<th>Concentration of cucumis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5.0%</td>
</tr>
<tr>
<td>Appearance and colour</td>
<td>6.55±0.05</td>
<td>6.45±0.01</td>
</tr>
<tr>
<td>Flavour</td>
<td>6.33±0.01a</td>
<td>6.63±0.01b</td>
</tr>
<tr>
<td>Juiciness</td>
<td>6.02±0.13</td>
<td>6.05±0.01</td>
</tr>
<tr>
<td>Tenderness</td>
<td>6.21±0.10a</td>
<td>6.16±0.01a</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>6.17±0.11a</td>
<td>6.46±0.02b</td>
</tr>
</tbody>
</table>

*: Mean values bearing same superscripts row-wise (alphabets) do not differ significantly; #: 8-Point descriptive scale (1=Extremely undesirable; 8=Extremely desirable); Number of observations, n = 24.

**Table 2**: Effect of different concentration of papaya on sensory attributes of emu chunks. (Mean ± SE)*

<table>
<thead>
<tr>
<th>Sensory attributes</th>
<th>Control</th>
<th>Concentration of papaya</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5.0%</td>
</tr>
<tr>
<td>Appearance and colour</td>
<td>6.21±0.10</td>
<td>6.31±0.01</td>
</tr>
<tr>
<td>Flavour</td>
<td>6.11±0.02a</td>
<td>6.15±0.02a</td>
</tr>
<tr>
<td>Juiciness</td>
<td>6.07±0.02a</td>
<td>6.10±0.01a</td>
</tr>
<tr>
<td>Tenderness</td>
<td>6.06±0.01a</td>
<td>6.10±0.01a</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>6.05±0.02a</td>
<td>6.12±0.01b</td>
</tr>
</tbody>
</table>

*: Mean values bearing same superscripts row-wise (alphabets) do not differ significantly; #: 8-Point descriptive scale (1=Extremely undesirable; 8=Extremely desirable), n=24
powder. Yadav (1982) has also reported improvement in juiciness, texture and overall acceptability of buffalo meat treated with crude extract from cucumis.

The score for appearance and colour did not differ significantly between control and papaya treated samples. There was significantly (P<0.01) improvement in the flavor scores in 7 and 10% papaya samples than control. There was a significant (P<0.01) increase in tenderness scores for 10% papaya treated samples than control. Mean scores were significantly (P<0.01) higher in all papaya treated samples than control.

Naveena (2000) reported that marination of tough meat chunks in 0.2% (w/w) papain was optimum for desirable tenderness and sensory attributes of roasted products. Bawa et al. (1981) reported increase in tenderness of spent hens with increasing levels of papain. Based on results, 10% cucumis and papaya treatment was selected for detailed study.

pH of marinade solution

The results of changes in pH of marinade and marinade absorption of cucumis and papaya are presented in Table 3, respectively.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Cucumis</th>
<th>Papaya</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH of marinade solution</td>
<td>6.56 ± 0.01a</td>
<td>6.24 ± 0.02a</td>
<td>6.48 ± 0.01b</td>
</tr>
<tr>
<td>Marinade absorption</td>
<td>23.27 ± 0.17c</td>
<td>21.16 ± 0.03b</td>
<td>22.25 ± 0.08b</td>
</tr>
</tbody>
</table>

*: Mean values bearing same superscripts row-wise (alphabets) do not differ significantly. n=24

The pH value of cucumis solution was significantly (P<0.01) lower than other solution. Significantly lower pH of cucumis solution could be due to the low pH of added cucumis extract (4.8-5.0). The results of our experiment were in close agreement with Garg and Mendiratta (2006) who observed that pH value of cucumis solution decreases significantly in pork chunks.

Marinade absorption

The marinade absorption values differ significantly (P<0.01) between treated samples. However, values were higher in papaya treated samples than cucumis treated samples. This may be due to lower pH of cucumis extract. The lower pH might have caused overall reduction in reactive groups of proteins available for water holding (Hedric et al., 1994).

pH of marinated emu meat chunks

The results of changes of pH of emu meat chunks treated with cucumis and papaya are presented in Table 4, respectively.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Cucumis</th>
<th>Papaya</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.64 ± 0.05b</td>
<td>5.58 ± 0.04a</td>
<td>5.72 ± 0.04b</td>
</tr>
<tr>
<td>Water holding capacity (%)</td>
<td>68.90 ± 0.16b</td>
<td>65.80 ± 0.07a</td>
<td>69.02 ± 0.08b</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>75.42 ± 0.19</td>
<td>75.76 ± 0.05</td>
<td>76.02 ± 0.04</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>21.75 ± 0.14a</td>
<td>21.56 ± 0.22</td>
<td>21.37 ± 0.39</td>
</tr>
<tr>
<td>Collagen content (mg/g tissue)</td>
<td>6.92 ± 0.03a</td>
<td>7.16 ± 0.04</td>
<td>7.13 ± 0.03</td>
</tr>
<tr>
<td>Collagen solubility (% total collagen)</td>
<td>22.55 ± 0.04a</td>
<td>26.39 ± 0.14c</td>
<td>25.78 ± 0.15b</td>
</tr>
<tr>
<td>Sarcolemmic protein solubility (mg/g)</td>
<td>35.53 ± 0.06a</td>
<td>38.13 ± 0.05a</td>
<td>37.58 ± 0.17b</td>
</tr>
<tr>
<td>Myofibrillar protein solubility (mg/g)</td>
<td>55.18 ± 0.06a</td>
<td>79.59 ± 0.032c</td>
<td>65.71 ± 0.06b</td>
</tr>
<tr>
<td>Total protein solubility (mg/g)</td>
<td>79.64 ± 0.85a</td>
<td>102.43 ± 0.20b</td>
<td>97.62 ± 0.31b</td>
</tr>
</tbody>
</table>

*: Mean values bearing same superscripts row-wise (alphabets) do not differ significantly; Number of observations n=24

There was significant (P<0.05) reduction of pH in samples treated with cucumis compared to papaya. Reduction in the pH of cucumis treated samples might be due to low pH of cucumis extract (4.8-5.0). Slightly higher pH of papaya treated samples was probably due to higher pH (6.51) of the extract form papaya. Maiti and Ahlawat (2011) observed that treatment with kachari (cucumis)
reduced the pH of chicken gizzard and goat heart. Garg and Mendiratta, (2006) found that pH of cucumis solution lower significantly in pork meat. The pH values in our experiment are in agreement with Naveena et al. (2004) who reported marked reduction in pH of cucumis treated samples compared to other treated samples in buffalo meat.

**Water holding capacity**

The results of changes of WHC of emu meat chunks treated with cucumis and papaya are presented in Table 4, respectively. There was significant (P<0.01) reduction of WHC in cucumis treated samples compared to others. The reduction in WHC of cucumis treated samples might be due to lower pH and this drop in pH may be responsible for overall reduction of reactive groups of proteins available for water holding (Forrest et al., 1994). Slightly improved WHC in papaya treated samples may be due to higher pH. This is an agreement with Bouton et al. (1973) who reported linear increase in WHC with increase in pH. Grojadonov et al. (1973) found improvement in hydration by treatment with proteolytic enzymes. Foda et al. (1976) also observed increased in WHC in camel meat on enzymatic processing with bromelain, ficin and trypsin. It may also be due to slight denaturation of sarcoplasmic proteins which play an important role in determining WHC (Joo et al., 1999). Slight denaturation of sarcoplasmic proteins was evident by the comparatively lower sarcoplasmic protein solubility in our experiment. Lower WHC values might be due to marination of small size chunks for 24 hrs which causes more drip loss and reduces water binding (Offer and Knight, 1988). WHC values in our experiment are in agreement with Naveena et al. (2004) who reported significant reduction of WHC in cucumis treated sample compared to others in buffalo meat.

**Moisture**

Although moisture content tends to be higher in papaya treated samples, no significant difference was observed between control and treated samples. Slightly higher moisture content of papaya treated samples was probably due to increase in hydration capacity with treatments. Moisture values in our experiment are in agreement with Naveena et al. (2004) and Maiti and Ahlawat (2011) observed moisture content of buffalo meat and goat heart/chicken gizzard respectively did not differ significantly between control and treated samples.

**Crude protein**

The values did not differ significantly between control and treated samples. Crude protein values in our experiment are in agreement with Naveena et al. (2004) & Maiti and Ahlawat (2011) observed that crude protein content of buffalo meat and goat heart/chicken gizzard respectively did not differ significantly between control and treated samples.

**Collagen content**

The collagen content values were 6.92, 7.16 and 7.13 mg/g tissue for control, cucumis and papaya treated samples respectively. There was no significant difference between control and treated samples. Slightly higher collagen content in enzyme treated samples might be contributed by the enzyme used in the study (Woessner, 1961). Total quantity of collagen in muscle tissue does not increase with increase in the chronological age of meat animal. Collagen content values in our experiment are in agreement with Naveena et al. (2004) found that there was no significant difference in collagen content in treated samples of buffalo meat.

**Collagen solubility**

There was a significant (P<0.01) increase in collagen solubility of all treated samples compared to control. Significant improvement in the collagen solubility of cucumis treated samples was consistent with the findings of Yadav (1982). He reported significantly higher collagen solubility in buffalo meat treated with cucumis. The increased collagen solubility of cucumis treated in our experiment was consistent with the finding of Naveena et al (2004) who reported a significant increase in collagen solubility of buffalo meat with cucumis and papain treatment. Higher collagen solubility values were observed in all treated samples compared to control.

**Protein solubility**

The mean values of sarcoplasmic protein solubility
Tenderization of emu meat chunks with Cucumis and Papaya

The mean values of total protein solubility (mg/g) were 79.64±0.85, 102.43±0.20 and 97.62±0.31 for control, cucumis and papaya treated samples respectively. The values were significantly (P<0.01) higher in all the treated samples compared to control. The results of changes of protein solubility of emu meat chunks treated with cucumis and papaya are presented in Table 4, respectively. Increase in protein solubility of all the enzyme treated samples in our experiment are consistent with those reported by some earlier workers (Buckley et al., 1974; Kim et al., 1981). Foda et al. (1976) also reported significantly higher solubility of actomyosin in enzyme treated samples than in meat subjected to natural ageing.

Increase in solubility in proteins in our experiments can also be correlated with histological changes. Increase in the solubility of treated samples might be due to increase in permeability of myofibrils, which will disintegrate easily. Whereas in control samples, regularly aligned filaments of myofibrils prevent buffer penetration, thus making actin seemingly resistant to extraction (Davey and Gilbert, 1968; Hasselbach and Schneider, 1951). This might also be a reason for difference in the extractability of control and treated samples. Results of lower solubility of sarcoplasmic protein in our experiments are in good agreement with Kang and Rice (1970) who reported that water soluble proteins are more resistant to enzyme degradation than other fractions. Joo et al. (1999) reported that water soluble protein solubility increases with increasing pH but Salt Soluble Protein solubility showed the weakest correlation. Lan et al. (1993) reported that besides pH and muscles fibre type, extraction conditions also have large influence on the protein solubility.

In our experiment even though cucumis treated samples have lower pH than others, significantly higher solubility might be due to higher proteolysis. According to Xiong and Berekke (1990), solubility decreases as protein reaches their isoelectric point (5.00) but pH of cucumis treated samples are well above this isoelectric point. Fretheim et al. (1986) reported that cutaneous trunci (white) muscles myosin was more soluble than myosin from M. masseter (red) below pH 5.7, whereas the solubilities were similar at pH 6.0. Differences in SSP solubility with pH could be caused by differential filament forming ability of myosin isoforms. pH may have differential effects on the solubility of proteins other than myosin that may influence the overall solubility of SSP (Warner et al., 1999).

Naveena and Mendiratta (2001) reported significant improvement of SSP and WSP extractability in spent hen meat treated with 3% (w/v) ginger extract. Increases in the protein solubility of papaya treated samples in our experiments are consistent with the findings of Kang and Rice (1970). Protein solubility values in our experiment are in agreement with Naveena et al. (2004).

The result of physico-chemical characteristics and sensory attributes of this experiment clearly indicate the tenderizing effect of cucumis and papaya. In general, there was significant increase in collagen solubility, protein solubility, sarcoplasmic and myofibrillar protein solubility in all enzyme treated samples compared to control. Even though cucumis and papaya enzymes were found effective on both myofibrillar proteins and collagen, cucumis had showed comparatively more effect on actomyosin toughness whereas papaya had showed higher effect on collagen. This might be due to different class of enzymes they belongs to. As cucumis belongs to serine protease and papaya belongs to thiol proteases, their action may differ. Samples treated with cucumis were rated superior and most preferred by the panelists, which can be attributed to desirable cucumis flavour.

CONCLUSION

There was significant reduction of pH and WHC in samples treated with cucumis compared to papaya. There was significant increased of collagen solubility, total protein solubility and sarcoplasmic solubility of cucumis and papaya treated samples. There was significant increased in myofibrillar solubility samples treated with cucumis compared to papaya. It is concluded that cucumis was found to be comparatively more effective on myofibrillar proteins, while papaya were found more effective on sarcoplasmic proteins.
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REFERENCES


Tenderization of emu meat chunks with Cucumis and Papaya


