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Effect of Tenderization on Histological, Physico-Chemical and Properties of Raw and Cooked Emu Meat Treated with Natural Tenderizers

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ABSTRACT

The effect of natural tenderization on histological, physico-chemical and sensory properties of raw and cooked emu meat were studied and evaluated to improved tenderness and flavour of emu meat. Fresh emu meats were procured from local market and cut into small chunk of approximately 3 cm^3 size and were marinated with different concentrations (0, 5, 7 and 10% w/v) of cucumis and papaya juice for 24 h at 4±1°C. Based on sensory evaluation results, 10% w/v cucumis and papaya juice sample were randomly allotted for different treatments for detailed study. The results obtained revealed remarkable variations in all the studied parameters in the emu meat using plant proteolytic enzymes compared with corresponding control. Sensory evaluation scores revealed a significant improvement in flavor, tenderness and juiciness of treated samples compared to control samples. The results showed that the muscle fibre diameter and sarcomere length value were slightly lower than control. There was significant (P<0.01) reduction of cooking yield and pH in samples treated with cucumis compared to papaya. Significant reduction (P<0.01) in shear force value all treated samples. There was significant (P<0.01) improvement in flavor, juiciness, tenderness and overall acceptability scores of all treated samples compared to control.

Key words: *Emu meat, Tenderness, Herbal tenderizer, Histological, Physicochemical, Raw meat, Cooked meat.*

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INTRODUCTION

Feeding more than 9 billion people around the world by 2050 will require a significant contribution from the livestock and meat sector. Exotic meats (i.e., meat produced from game or non domesticated animals/birds) are gaining popularity in the global meat market, where health-conscious consumers are becoming increasingly careful in choosing lean alternatives over traditional red meats (beef, buffalo meat, sheep and goat meat, and pork) or white meat (chicken). 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 [16]. 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[4,16]. These growing health concerns have led to the demand for low kilo joule and low-cholesterol products, with increasing emphasis on reducing the ratio of n-6/n-3 polyunsaturated fatty acids in the diet[4,13].

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[26]. Total population of emus in India is around 75,000. From a single farm in 1996, there are today 900 emu farms in 14 states, with a majority of them in Andhra Pradesh, Maharashtra and other progressive states of India. The reason for the growth of emu farms today is the increasing demand for the bird's meat and oil, which is believed to have medicinal properties, especially for treating joint pains[6]. Likewise in production, there are estimate of about 33,000 tonnes of emu meat being produced in India now, which is a fraction of the 1.9 million tonnes of chicken meat produced in the country every year (Science Tech entrepreneur 2009). According to Livestock Census 2012, the population of Turkey/Ostrich/Emu were 8285 in West Bengal, India. Because of lack of knowledge by consumers and meat handlers about ratite meats, these products remain in the "market introduction" stage of the product life cycle and have not moved into the "market growth" stage, where demand tends to exceed supply[3]. Emu meat has healthier properties such as significantly higher bioavailable iron, B vitamins, and creatine compared with red meats and low-fat, low-cholesterol, rich polyunsaturated fatty acids compared with white meat. In addition to its meat, emu oil has also been reported to have various uses, including potential therapeutic roles. However, emu meat is a new product that potentially can be developed for a niche market to compete with other meats[23].

Emu meat having moderately intense meat flavor and a gamey flavor ranging in intensity from slight to extremely intense. Additionally, other factors such as feed, blood loss during slaughter, or maturity may affect the incidence and intensity of gamey flavor. If blood loss was more complete, the gamey flavor might also diminish in intensity[21].

MATERIALS METHOD

Fresh emu meats were procured from local market and were brought to the Department of Livestock Products Technology. The carcasses were deboned and lean meat were collected and packed in low-density polyethylene (LDPE) bags and stored in refrigerator at

4 ± 1^0C for 24 hrs. . After 24 hrs chilling, muscle were taken out of refrigerator and cut into small chunk of approximately 3 cm^3 size and were randomly allotted for different treatments. Material method were followed with certain modification of Naveena *et al.*[20].

Washed cucumis and papaya fruits were blend or homogenized with equal amount of chilled distilled water and filtered through muslin cloth. 3cm^3 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@ 15% w/v. After thorough mixing by hand, the chunks were placed in polyethylene bags and kept at 4 ± 1^oC 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^oC monitored using probe thermometer. Samples were evaluated for sensory attributes. Minimum of six trials were conducted and data obtained were analyzed statistically. Detailed experiments were conducted to access the tenderness effect of selected concentration of 10% cucumis, papaya. 3 cm^3 uniform sized meat chunks randomly allotted to the following three treatments.

All the marinate were diluted with distilled water and marinated the meat chunks @ 15% w/v. After 24 hrs of marination at 4 ± 1^oC raw chunks (i.e. before cooking) were analyzed for and histological parameters viz., fibre diameter, Sarcomere length. Cooked meat chunks were also analyzed for cooking yield, pH, moisture, shear force value and sensory attributes.

Analytical procedure

pH determination:

The pH of the finely minced meat sample was determined by the method of Trout *et al.*[32] 10 gms 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).

Cooking yield:

The weight of samples were recorded before (raw weight) and after cooking. Cooked weight was divided by raw weight and the result was multiplied by 100 to give percent cooking yield.

Determination of moisture:

About 10 gms of minced meat were taken in an aluminium moisture cup and dried in a hot air oven for 18 h at 100^0C. After that the moisture cups were removed from the hot air oven, cooled in a desiccator and again weighed. The loss in weight was reported as moisture content. The process was repeated until constant weight of the sample was obtained.

Shear force value:

Cores of 1 cm^3 were taken from cooked samples after overnight cooling at 4 ± 1^oC and sheared using Warner Bratzler shear press (Model No. TA-XT Plus 11489., UK) with the fibres parallel to the longitudinal axis. The force required to shear the samples was observed and recorded (kg/cm^2).

Muscle fibre diameter:

Procedure of Tuma *et al.*[32] was followed for determination of muscle fibre diameter. One inch core of muscle tissue was fixed in 10% formal saline for 24 hrs and was blended in micro-blender at low speed for 30 sec. A drop of this was placed over a glass slide, covered with coverslip and observed under microscope with a 10X eye piece containing a calibrated micrometer. Diameter of each fibre was measured at 3 different locations. 50 such straight fibres were measured and average muscles fibre diameter was expressed in microns.

Sarcomere Length

Sarcomere length of the muscle fibre of emu was measured as per the method outlined by Cross *et al*.[2], with certain modifications. A 5 gram sample of emu drumstick, thigh and breast muscles were cut into small pieces and homogenised at low speed in 30ml chilled 0.25M sucrose solution (0.25M). A drop of the homogenate was then transferred onto a slide. The slide was examined under a phase contrast microscope (100x objective and 8x eyepiece) with oil immersion. If the fibres were not sufficiently broken apart, the sample was homogenized for an additional period of 20 - 30 seconds, taking care to ensure that the myofibrils were not homogenized to less than 10 sarcomeres. The length of the sarcomeres measured by starting from one edge of the slide and moving straight across using a calibrated micrometer.

Sensory evaluation:

Cooked products were served to experienced panelists consisting of the scientists and students of the department. Samples were evaluated for appearance and colour, flavour, juiciness, tenderness, overall acceptability using 8-point descriptive scale (where 8=extremely desirable, 1=extremely undesirable) as given in the score sheet (Annexure 1)[10].

Statistical analysis:

All the data which were obtained during the present investigation were analyzed statistically to draw valid conclusion in SPSS (Trial Version 20.0) software. Six replicates were performed for each experiment. The results were expressed in terms of mean and standard error (SE) of mean. A probability value of $p<0.05$ was describe as significant and $P<0.01$ was noted as highly significant. The relationship between different parameters was studied by simple regression and correlation.

RESULT AND DISCUSSION

Histological characteristic of raw chunks

The results of changes of histological character of emu meat chunks treated with cucumis and papaya are presented in Table 1, respectively.

Muscle fibre diameter:

There was significant difference ($P<0.05$) in muscle fibre diameter between control and treated samples. The values were slightly lower in all treated samples compared to control.

The values of muscle fibre diameter in our experiment are in agreement with Kanthapanit and Chaosap[9]. Hiner *et al*.[8] indicated that meat having small fibres is more tender than meat having large fibres. Tuma *et al*.[32] also suggested that fibre diameter increases and tenderness decrease with increasing animal age. Reduction in muscle fibre diameter in the treated samples in our experiment can be correlated with reduction in shear force values and improvement in tenderness scores. In contrast to this, Brady[1] obtained a non-significant correlation between fibre diameter and shear force. Naveena *et al* [24] reported that there was a significant ($P < 0.05$) increase in the muscle fiber diameter of emu meat cubes during aging under both AP and VP conditions. The increase in the muscle fiber diameter with aging may be attributed to the changes associated with muscle fiber structure with aging. The mean muscle fiber diameter observed in the current study were similar to those reported for emu[21], chicken[19] and buffalo meat. Muscle fibre diameter values in our experiment are in agreement with Naveena *et al.* [20].

Sarcomere length

There was no significant difference in Sarcomere length between control and treated samples. However values were slightly lower in papaya treated samples.

However, the shear force measurement, which can be regarded as an instrument measurement of tenderness, was conducted. The sarcomere length of raw emu meat was negatively correlated with the shear force value of cooked (at 65°C). Dutson *et al.*[5] reported that difference in tenderness of these muscles was not constant at all sarcomere lengths, with the muscle decreasing in shear force at a faster rate due to increasing sarcomere length. This phenomenon is probably due to a toughening of both connective tissue and muscle fibers as the muscle shortens.

Physico-chemical characteristics cooked emu meat chunks

The results of changes of physico-chemical characteristic of emu meat chunks treated with cucumis and papaya are presented in Table 2.

Cooking yield

There was a significant ($P<0.01$) reduction in cooking yield in cucumis treated samples compared to others. However in papaya treated samples there was a marginal increase in the yield and the values differ significantly. Reduction in the yield of cucumis treated samples may be due to lower pH, which in turn reduced the water holding capacity. Increase in the yield of papaya treated sample is in agreement with Labell[12] who reported reduction in shrinkage of microwaved meat and poultry by 5-20% after treatment with papaya powder. Naveena[17] also reported increase in the yield the smoked spent hen meat treated with 30% GE. Increase in the yield of papaya treated samples can be positively correlated with higher pH and WHC. Ketnawa and Rawdkuen,[9] found that in addition, all three bromelain extract treated samples were significantly different from the control samples in the cooking yield ($p < 0.05$). Cooking yield values in our experiment are in agreement with Naveena *et al.*[20] observed that significant reduction in cooking yield in cucumis treated samples compared to others treatments.

Moisture:

The moisture content values ranged from 66.20, 66.36 and 66.58 percent respectively for control, cucumis and papaya treated samples and did not differ significantly between control and treated samples. Moisture values in our experiment are in agreement with Naveena *et al.*[20] who also observed non significant difference between all treated samples. However, Ketnawa and Rawdkuen[9], found markedly decrease in the moisture content in the bromelin extract treated samples when compared with the untreated samples. Maiti and Ahlawat[14] reported significantly higher moisture content of raw gizzard than that of goat heart.

pH:

The pH values were significantly ($P<0.01$) lower in cucumis treated samples compared to papaya. However, there was a marginal increased increase in pH of papaya treated samples compared to control. Significant reduction in pH of cucumis treated samples may be attributed to lower pH of the cucumis extract (4.8-5.0). Slightly higher pH of papaya treated samples could be due to higher pH of their extracts. Negbenebor *et al.*[25] also reported that treatment of meat patties treated with GE did not alter the pH significantly. pH values in our experiment are in agreement with Naveena *et al.*[20].

Shear force values:

There was a significant ($P<0.01$) reduction in shear force values of all treated samples compared to control. Significant reduction in shear force values of cucumis treated sample are in agreement with Kumar and Berwal[11] who had also reported significant reduction of values in spent hen meat treated with ammonium sulfate extracted cucumis powder. Significant reduction of shear force values with GE treatment were also reported in sheep[15,30] buffalo[28] and spent hen meat[18]. Takagi *et al*.[29] also reported significant reduction in shear force values in beef meat treated with papain. Maiti and Ahlawat[14], reported that ginger and kachri alone and in combination significantly reduced the shear force value of marinated as well as cooked chicken gizzard and goat heart.

Reduction in shear force values of all enzyme treated samples in our experiment might be due to increased proteolysis and collagen degradation which is evident from higher collagen solubility, nitrogen extractability and protein solubility values. Shear force values in our experiment are in agreement with Naveena *et al.*[20] reported significant reduction of shear force value in all enzyme treated samples compared to control.

Sensory attributes:

The results of changes of physico-chemical characteristic of emu meat chunks treated with cucumis and papaya are presented in Table 3, respectively. The mean score of appearance did not differ significantly between control and all treated samples.

There was significant ($P<0.01$) improvement in flavor, juiciness, tenderness and overall acceptability scores of all treated samples compared to control. Significant improvement in tenderness and overall acceptability scores of spent layer hens treated with cucumis powder was reported by Kumar and Berwal[11]. Yadav has also reported improvement in juiciness, texture and overall acceptability of buffalo meat treated with crude extract from cucumis.

Significant improvement of tenderness scores of papain treated samples were also reported by Gerelt *et al*.[7]. Increase in tenderness scores of all treated samples in our experiment can be positively correlated with significantly reduced shear force values. Naveena *et al*.[20] also reported that meat chunks treated with different enzyme extracts received significantly higher score for flavour, juiciness, tenderness and overall acceptability as compared with that of control in buffalo meat.

The result of physico-chemical characteristics and sensory attributes of this experiment clearly indicate the tenderizing effect of cucumis and papaya. Significant reduction in shear force values 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. Cucumis and papaya treated samples scored almost equally.

Table 1: Histological characteristics of raw emu meat chunks treated with cucumis and papaya for 24 hrs

Parameters	Control	Cucumis	Papaya
Muscle fibre diameter (µm)	54.25±0.02[b]	52.54±0.08[a]	52.68±0.04[a]
Sarcomere length (µm)	2.13±0.02	2.17±0.01	2.10±0.02

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

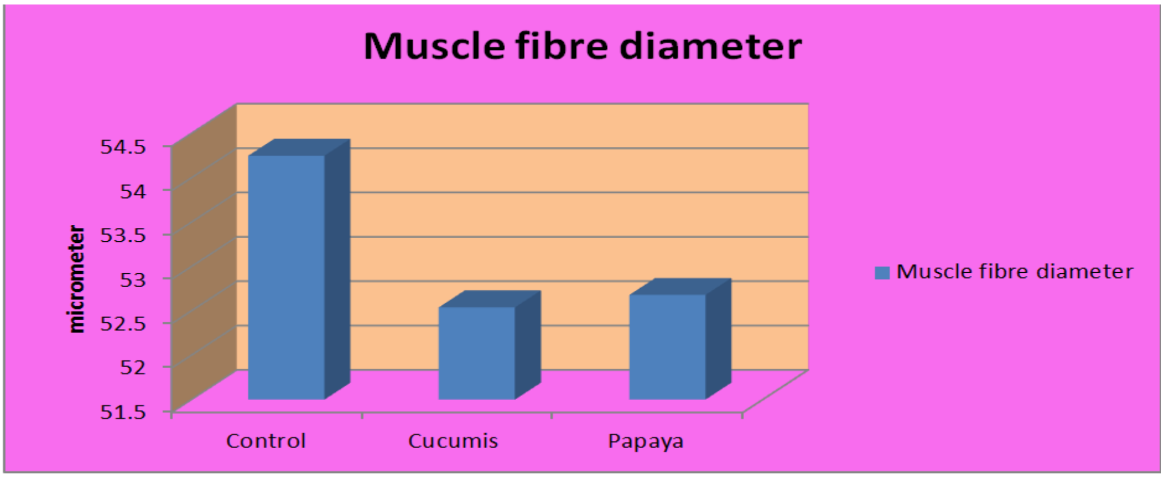

Fig. 1: Physico-chemical (muscle fibre diameter) characteristics of raw emu meat chunks treated with cucumis and papaya for 24 hrs

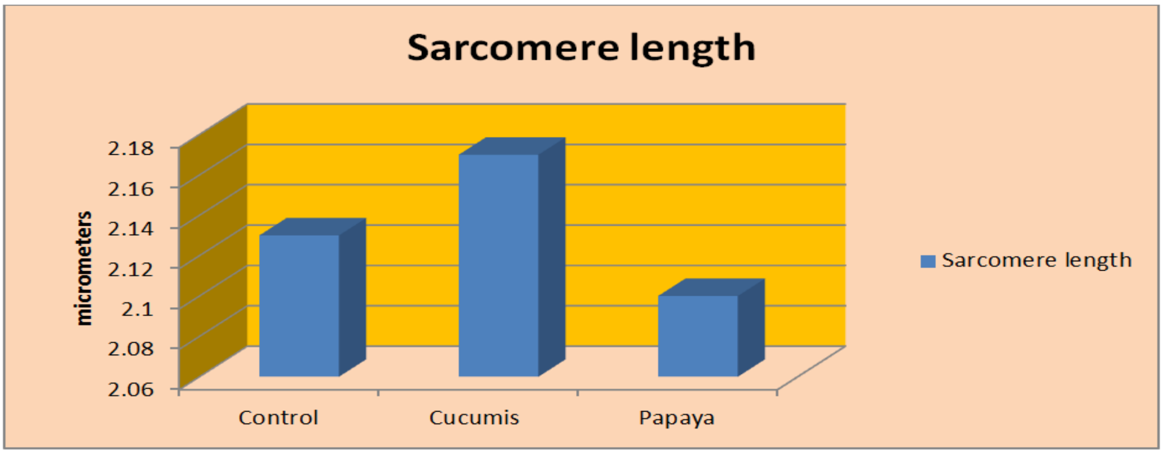

Fig. 2: Physico-chemical (Sarcomere length) characteristics of raw emu meat chunks treated with cucumis and papaya for 24 hrs

Table 2: Physico-chemical characteristics of cooked emu meat chunks treated with cucumis and papaya for 24 hrs. (Mean ± SE)*

Parameters	Control	Cucumis	Papaya
Cooking yield	58.19±0.02[b]	56.28±0.01[a]	58.76±0.01[c]
Moisture	66.20±0.02	66.38±0.01	66.58±0.01
pH	5.89±0.01	5.65±0.01	5.94±0.01
Shear force	4.54±0.01[b]	2.39±0.01[a]	2.45±0.01[a]

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

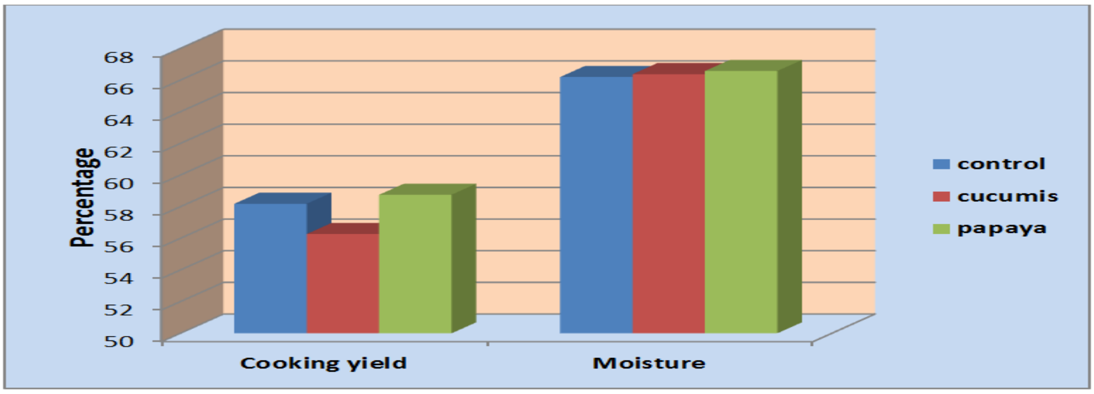

Fig. 3: Physico-chemical characteristics (cooking yield and moisture) of cooked emu meat chunks treated with cucumis and papaya for 24 hrs

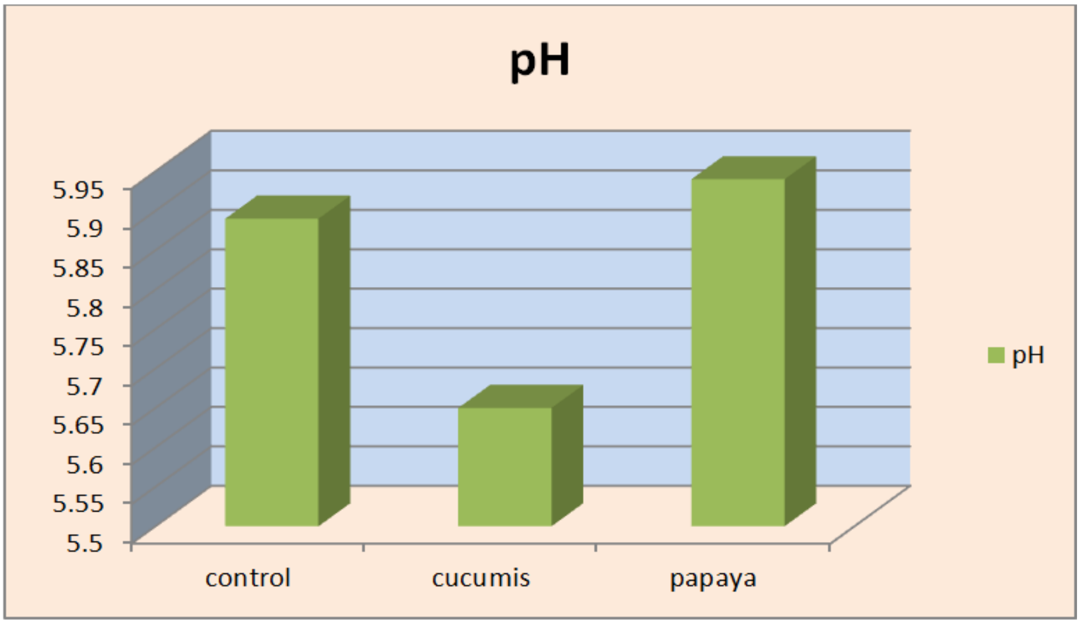

Fig. 4: Physico-chemical characteristics (pH) of cooked emu meat chunks treated with cucumis and papaya for 24 hrs

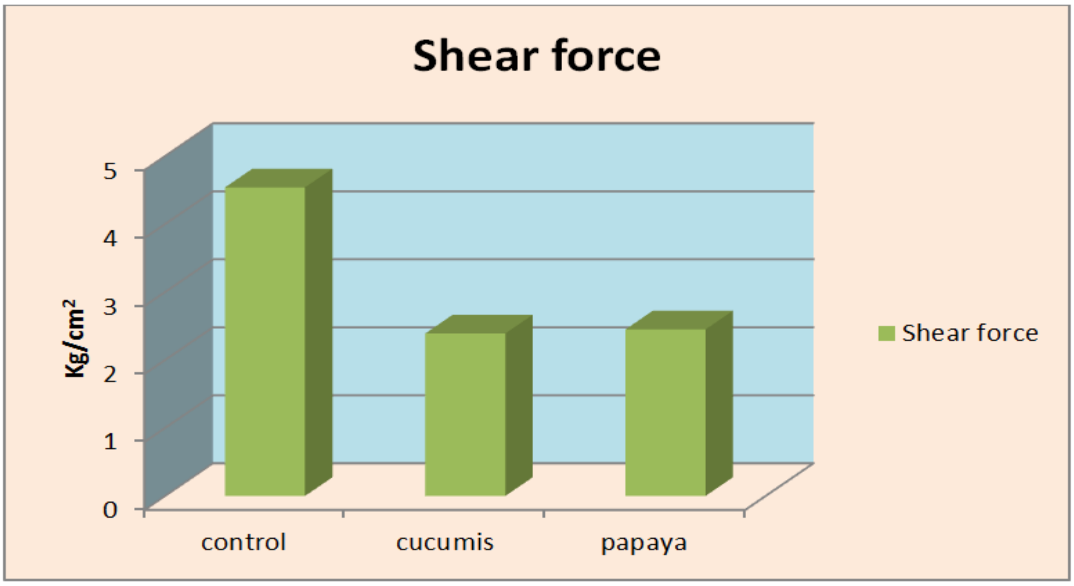

Fig. 5: Physico-chemical characteristics (Shear force) of cooked emu meat chunks treated with cucumis and papaya for 24 hrs

Table 3: Effect of sensory attributes of cooked emu meat chunks treated with cucumis and papaya for 24 hrs. (Mean ± SE)*

Parameters	Control	Cucumis	Papaya
Appearance and colour	6.60±0.02	6.85±0.01	6.71±0.01
Flavour	6.04±0.01[a]	6.74±0.02[c]	6.55±0.02[b]
Juiciness	6.56±0.02 [a]	6.75±0.02 [c]	6.67±0.02 [b]
Tenderness	5.50±0.03[a]	6.70±0.01[c]	6.59±0.01[b]
Overall acceptability	5.43±0.02[a]	6.99±0.03[c]	6.52±0.01[b]

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

Number of observations n=24

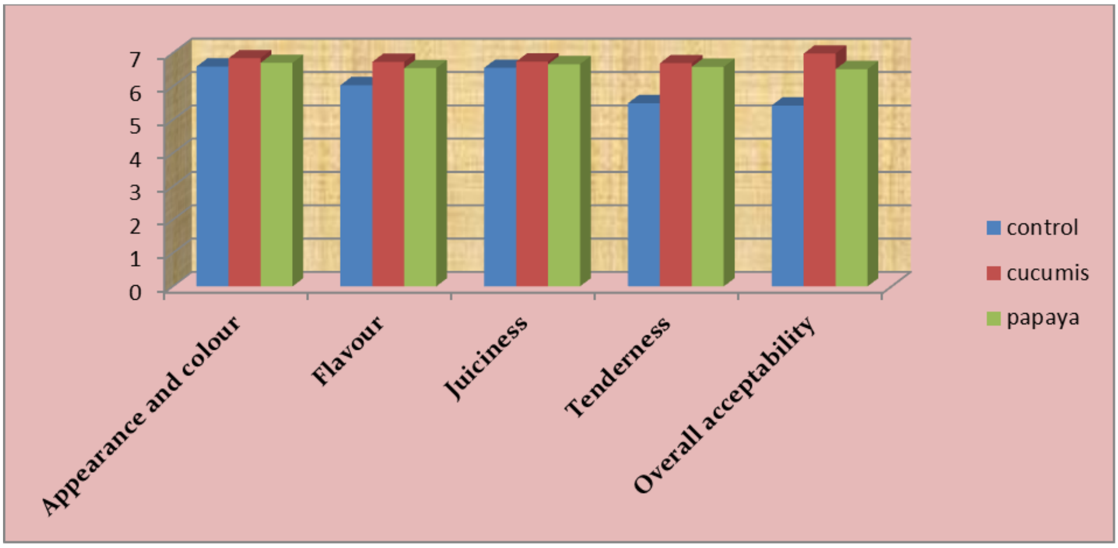

Fig. 6: Effect of sensory attributes of cooked emu meat chunks treated with cucumis and papaya for 24 hrs

CONCLUSION

Treatment of tough emu meat chunks with 10% cucumis (w/v) marinates and 10% papaya (w/v) marinates were found to be optimum for desirable tenderization. Cucumis and papaya caused significant reduction in shear force values and improved the sensory attributes of emu meat. Sensory attributes like flavour, juiciness, tenderness and overall acceptability for cucumis treated emu meat chunks were higher than control. Increased in muscle fibre diameter samples treated with cucumis and papaya.

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