



USING PLANT PROTEASE FROM WILD CANTALOUPE FRUIT (*CUCUMIS TRIGONUS ROX-B*) AS TENDERIZING AGENT OF AGED BULL MEAT

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Abstract

The present study was designed to examine the efficiency of different concentration of enzyme extract of cucumis fruit as tenderizing agent of aged bull meat compared to papain enzyme. After slaughter bull and dressing carcass, the three muscles namely, longissimus dorsi (LD), semi membranous (SM) and supra spinatus (SS) were separately immersed in 100 ml of chilled distilled water (control) and 1, 2 and 3% (w/v) enzyme extract solutions from cucumis and 0.2% (w/v) of papain enzyme solution. After that placed in plastic box and stored at room temperature for 30 min, then kept at 4°C for 24 hr., then stored in frozen at -18°C. The muscle samples were subjected to various physicochemical, biochemical and sensory evaluation of aged bull meat. The results obtained showed that there were significant ($P<0.05$) reduction of muscle pH and water holding capacity, increase ($P<0.05$) in collagen solubility, solubility of myofibrillar and total protein with slightly increase of sarcoplasmic solubility in LD, SM and SS muscle samples treated with extract of cucumis compared to control and papain enzyme. The electrophoretic pattern of muscles proteins illustrate extensive proteolysis and reduced protein band numbers in muscles samples treated with extract of cucumis and papain enzyme compared to control. It was observed that muscle samples treated with 2 and 3% of cucumis extract improvement ($P<0.05$) in color, flavor, tenderness and overall acceptability scores than those of the control and papain enzyme. It can be concluded that local cucumis fruit can be used as an effective tenderizing agent of meat toughness.

Key words: Bull meat, Tenderness, Plant enzyme, physicochemical, Sensory evaluation.

Introduction

Bulls in Iraq slaughtered after investment of production efficiency, bull meat in Iraq; obtained mostly from old bulls will be tough and poor quality characteristics. Meat toughness is an undesirable trait of palatable meat for consumer (kemp *et al.*, 2010). Toughness in meat primary occurs changes in myofibrillar proteins, which affected by rigor-mortis development in meat and enzymatic breakdown of the contractile proteins in post-slaughter muscles. Additionally to the thickness and amount of connective tissues with formation of the crosslinks between the molecules of collagen, which is the main component of connective tissue, it account for about 80% of connective tissue, the meat of old animals become tough in texture (Bailey *et al.*, 1989 and Chen *et al.*, 2006). Meat tenderness depends on the type of

muscle, post-slaughter factor and post-mortem pH and temperature (Anderson *et al.*, 2012). The chemical composition, structure and amount of connective tissue, generally depends on the age of animal, muscles anatomical location and the specific muscle types, they affect in meat tenderness (Bolumar *et al.*, 2013). Enzymes plant origin, such as papain, bromelain and ficin were often used for post-mortem meat tenderization. (Wada *et al.*, 2002). However, the highest plant protease concentration can cause meat decomposition and develop unfavorable taste due to over-tenderization (Chen *et al.*, 2006 and Rawdkuen *et al.*, 2013). Cucumis enzymes, which obtained from cucumis plant (*Cucumis trigonus* Rox-b) had been reported to have proteolytic activity and fruit of cucumis are used as a food-tenderizing agent (Hujjatullah and Baloch, 1970). Cucumis fruit is widely grown herb of India and grow with watermelon of Iraq.

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Cucumis fruit contains flavonoids compounds have antioxidant activity against lipid oxidation (Gill *et al.*, 2015).

The present study designed to investigate the effects of cucumis tenderizing enzyme extract by changes of physicochemical, biochemical and sensory properties of aged bull meat.

Materials and methods

Collection and drying local Cucumis

Fresh cucumis (*Cucumis trigonus* Rox-b) fruits were collected from local farm (Penjwen Region, Sulaimani, Iraqi). The collected fruits were washed with distilled water, cut into pieces and remove the seeds. The peels obtained were dried in an oven at 37°C. Dried peels were ground in laboratory milling machine to a fine passed through a 30-mesh sieve, the powder stored in the tight containers under refrigeration until used.

Preparation of muscles samples

Fresh aged bull meat (more than 5 years old) were procured (pre-rigor state) from government abattoir at maximum 3hr post-slaughter and were brought to the Department of Animal Sciences, College of Agricultural Sciences, University of Sulaimani, Sulaimani, Iraq. Longissimus dorsi (LD), Semimembranosus (SM) and Supraspinatus (SS) muscles were excised from loin, round and chuck cuts, the external fat and visible connective tissues trimmed from muscles, then packed in polyethylene bags and kept in refrigerator at 4°C for 24 hrs. After chilling, muscles were taken out of refrigerator and cut into small pieces of approximately 3cm³ size, the pieces of muscles (LD, SM and SS) were separately divided into 5 groups and immersed with different concentrations of cucumis and 0.2% (w/v) of papain enzyme extract solutions.

Enzyme treatment of muscle samples

100g of each muscle pieces (LD, SM and SS) muscles were separately immersed in 100 ml of chilled distilled water (control); enzyme extract solutions at concentrations of 1, 2 and 3% (w/v) of cucumis and 0.2% (w/v) of papain enzyme extract solution. Muscle samples were taken out from the enzyme extract solutions, washed and drained, then placed in polyethylene bags and tightly sealed, kept in the refrigerator at 4°C for 48 hrs., then stored in frozen (-18°C). The muscles samples evaluated for physicochemical properties and sensory traits of aged bull meat.

Analysis muscles samples

pH determination

Muscle samples (10g) homogenized with 50 ml of chilled distilled water and the pH values measured with

pH meter (W.T.W 2F40-114, Germany) at room temperature (27°C). The pH meter initially calibrated with pH 7 and pH 4 buffers before used in pH determination (Wardy *et al.*, 2009).

Water holding capacity (WHC)

Water holding capacity of muscles samples estimated according to method in Wardlaw *et al.*, (1973). 20g of grinded muscle samples put into a centrifuge tube containing 30 ml of NaCl buffer solution (0.6M) and stirred with a glass rode for 1 min. The tube was then kept at 4 ± 1°C for 15 min, stirred again and then centrifuge at 3000×g for 25 min at 5°C (Labnet- Germany). The supernatant was poured into measuring cylinder and the volume was recorded, and the WHC was expressed as a percentage of initial volume.

Protein solubility

Sarcoplasmic and total protein solubility were determined according to procedure, which describe by Joo *et al.*, (1999). Sarcoplasmic proteins were extracted from 2g minced muscle using 20ml of ice-cold 0.025 M potassium phosphate buffer (pH 7.2). The samples were homogenized and kept overnight at 4°C with frequent shaking. Samples were centrifuged at 1500×g for 20 min and protein concentration in the supernatant was determined by the Biuret method. Total protein (sarcoplasmic + myofibrillar) was extracted from 2g muscle using 40 ml ice-cold 1.1 M potassium iodide in 0.1 M phosphate buffer (pH 7.2), then homogenization, centrifugation and total protein determination were carried out as described above. Myofibrillar protein concentrations obtained by difference between total and sarcoplasmic protein solubility.

Collagen solubility

Extraction of soluble and insoluble collagen for the treated and untreated muscles samples performed by modified Hill procedure (1966) and method described by Wattanachant *et al.*, (2004). Muscle samples (4gm) homogenized with 12 ml of ¼ strength Ringer's solution (15mM KCl, 32.8mM NaCl and 0.5mM CaCl₂). The homogenate heated at 77°C for 70 min in water bath with frequent shaking. Remove mixture from water bath and cooling for 30 min at room temperature, then centrifuged at 6000×g for 10 min at 4°C. The supernatant (soluble collagen) separated from precipitate (insoluble collagen). The precipitate homogenized with 8 ml of ¼ strength Ringer's solution then centrifuged again for 10 min with supernatant combined. The precipitate and supernatant hydrolyzed separately with 40 ml of 6M HCl at 120°C for 16 hrs. (Overnight) using autoclave. Allow slow exhaust of pressure of prevent overflowing, they

removed from autoclave and allow hydrolyzed to cool to room temperature. Add a small amount of charcoal to the supernatant and precipitate to clarify solution, and pH adjusted to 7.0, with an equal volume of 6M NaOH. Filter samples through Whatman No.1 filter paper into a graduated cylinder and diluted supernatant to 100 and residuals to 500 ml with distilled water. Mix dilutions well and store in refrigerator. The hydroxyl proline concentrations of the diluted samples were determined by measuring the absorbance at 558 nm against a standard curve of hydroxyl proline according to the method described by Bergman and Loxley, (1963). The hydroxyproline (HOP) concentration calculated as follows:

$$\text{Mg (HOP)/gm tissue} = \frac{(\text{HOP})\mu\text{g/ml} \times \text{dilution factor}^*}{[\text{Sample wt. (gram)}]} \times 1/1000.$$

* Dilution factor for supernatant (100 ml) and residual (500 ml).

Soluble and insoluble collagen content were calculated by multiplying hydroxyproline content by 7.52 and 7.25 respectively, and were expressed as mg/gm tissue (Cross *et al.*, 1973). Then reports total collagen (soluble + insoluble collagen) and collagen solubility (%) (Soluble collagen / total collagen \times 100).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE performed by the procedure of Laemmli *et al.*, (1970). 2g of minced muscles subjected to different treatment conditions were all mixed with 18 ml of 5% (w/v) SDS solution (85°C). The mixture was then homogenized. The homogenate mixture incubated at 85°C in a water bath for 1 hr to dissolve the protein. It was then centrifuged at 8000 \times g for 5 min at room temperature using a centrifuge (Cooler centrifuge, Labnet, Germany) to remove the un-dissolved debris. The supernatants were mixed at a ratio 1:1 (v/v) with the sample buffer (0.5M Tris-HCl, pH 6.8 containing 4% SDS, 20% glycerol and 10% Beta-mercatoethanol (BME) and then boiled for 3 min. The samples (20 μ g protein) were loaded into a polyacrylamide gel containing (10% running and 4% stacking gels). Then, they were subjected to electrophoresis set at a constant current of 15 mA per gel using a Mini Protean Tetra Cell unit (Bio-Rad Laboratories, Richmond, CA, USA). After electrophoresis, the gels were stained overnight with staining solution (0.02% (w/v) Coomassie Brilliant Blue R-250) in 50% (v/v) methanol and 7.5% (v/v) acetic acid. The protein patterns were made visible after de-staining the gel until a clear background was achieved.

Sensory evaluation

Muscles samples pieces removed from frozen storage and thawed under refrigeration at 4°C for 24 hrs. Prior to sensory evaluation. Then cooked for 20 min in an oven at 180°C to an internal temperature of 75 \pm 1°C monitored using a probe thermometer and served warm to 7 experienced panel members consisting of meat scientists and post-graduate students of the department of animal science with previous experience. The sensory traits were evaluated using 8-point descriptive scale were one and eight were the extremes of each trait (8-extremely desirable color, extremely desirable flavor, extremely desirable juicy, extremely desirable tender and extremely desirable acceptable, respectively) were used to evaluate color, flavor and aroma, juiciness, tenderness and overall palatability. Panelists were required to cleanse their palate between samples with drinking water (Keeton, 1983).

Statistical analysis

The obtained data was statistically analyzed analysis with the SAS program (SAS, institute, 2010). General linear model (GLM) within SAS (2010) program. Factorial Complete Randomized Design (CRD) was used to study the effect of treatments and muscle types on studied traits. Duncan's multiple range test (Duncan, 1955) was used to compare significant differences among means with each factor on all studied traits.

Results and discussion

pH values

Muscles samples of LD, SM and SS immersed with cucumis extract had lower ($P < 0.05$) pH values compared to the control and papain enzyme (Table 1). This result may be due to low pH of local cucumis extract (5.16), which was probably caused by the lower pH of the treated muscle samples. Moreover, enzyme extract of cucumis hydrolysis of the muscle may release amino acid that can decrease the pH for treated samples. The pH values in our experiment correspond with Naveena *et al.*, (2004), Verma *et al.*, (2018) who reported that treatment with cucumis extract reduced the pH of buffalo, and emu chuck meat. The results recorded a higher of pH values of papain enzyme treated muscles samples, were probably due to higher pH (6.22) of the papain extract. The results (Table 1) indicated presence of a significant ($P < 0.05$) differences in pH values among LD, SM and SS muscles for each treatment, it may be due to the differences in the rate of glycolysis process as well as the differences in muscle type (Warriss, 2000). The rate of glycolysis process affected by several factors including

animal type, age, muscle type and pre-post slaughter of animal condition. Also, it may be probably due to the differences in glycogen content for each muscle (Lawrie, 2002).

Water holding capacity (WHC)

Results in Table 1 showed that there was a significant ($P<0.05$) reduction of WHC in all the treated muscles of LD, SM and SS with different concentration of cucumis extract, especially when the concentration of cucumis extract increased compared to the control and papain enzyme. The reduction of WHC of cucumis treated muscles samples may be due to lower pH of cucumis extract (Table 1) and this decline in pH might be responsible for overall reduction of reactive group of proteins available for water binding (Forrest *et al.*, 1994). This result is in an agreement with Naveena *et al.*, (2004), Ketnawa and Rawdkuen, (2011) who reported reduction of WHC in cucumis extract or bromelain extract treated meat samples in buffalo and beef meat. It was observed in our experiment that reduction WHC percentages in cucumis treated muscles of LD, SM and SS might be due to the muscles from old animal that have lower WHC (Syed zaindden, 1994), and may be due to slight denaturation of sarcoplasmic proteins, which play an important role in determining WHC (Joo *et al.*, 1999). Besides the degree of WHC was due to the myofilament space into the extra-cellular spaces (Ketnawa and Rawdkuen, 2011). It was observed from results in Table 1 higher WHC in papain treated muscles of LD, SM and SS might be due to higher pH of papain extract (6.22). This result was agreement with Naveena *et al.*, (2004) who reported higher WHC in papain treated meat chunk of buffalo meat. It was observed from the table SS muscle samples have a high ($P<0.05$) WHC as compared with LD and SM muscles; this may be due to higher pH (6.21) of SS muscle (Table 1). Such result is to different muscle function, fiber type and pre-post glycolysis process (Han *et al.*, 2009).

Protein solubility

It was observed high significantly ($P<0.05$) of myofibrillar and total protein solubility values in all cucumis extract and papain enzyme for LD, SM and SS muscles compared to the control Table 2. The results revealed that cucumis extract treated muscles at concentration of 3% of cucumis extract had a higher myofibril and total protein solubility values, whereas the lower values was found in the control treatment. Davey and Gitbert, 1968, reported that the regularly aligned filaments of myofibrils might have helped to prevent cucumis extract penetration, thus making the action seemingly resistant to extraction. The results showed that the protein solubility changes were due to myofibrillar protein degradation, an increase in solubility of cucumis extract treated samples for LD, SM and SS muscles may be due to an increase in permeability of myofibrils, which will then disintegrate easily. Besides, sarcoplasmic protein solubility values of cucumis extract treated muscle samples for LD, SM and SS muscles gave slightly increase compared to the control. The increase in protein solubility with cucumis treatments was also reported by Naveena *et al.*, (2004) and Yerma *et al.*, (2018) who reported increase in protein solubility of cucumis extract treated muscle samples of buffalo and emu chunk meat. Less solubility of sarcoplasmic proteins solubility in treated muscles of LD, SM and SS corresponded with Kang and Bice (1970) who reported that water-soluble proteins are more resistant to enzyme degradation than other fraction. Joo *et al.*, (1999) reported that water soluble protein solubility increase with increasing pH, but salt soluble protein solubility showed that the weakest correlation. It seems from results that papain treatment for LD, SM and SS muscles had moderate increase in protein solubility values compared to the control. Increase in protein solubility with papain treatment also reported by Naveena *et al.*, (2004) in buffalo meat. It was observed from results that protein solubility values for cucumis treated muscle

Table 1: pH values and water holding capacity (WHC) in longissimus dorsi (LD), semimembranosus (SM) and supraspinatus (SS) muscles of aged bull meat treated with different concentration of enzymes extract from Cucumis fruit and papain enzyme (Mean \pm S.E.).

Treatment	pH values			WHC %		
	LD	SM	SS	LD	SM	SS
Control	a B5.82 \pm 0.004	a C5.71 \pm 0.005	a A5.91 \pm 0.003	b B24.13 \pm 0.013	b C21.49 \pm 0.03	b A24.56 \pm 0.025
1% CE	c B5.76 \pm 0.006	c C5.64 \pm 0.004	c A5.83 \pm 0.004	c B23.20 \pm 0.032	c C19.80 \pm 0.022	c A23.34 \pm 0.018
2% CE	d B5.73 \pm 0.004	d C5.62 \pm 0.004	d A5.81 \pm 0.003	d B22.64 \pm 0.01	d C18.75 \pm 0.011	d A22.81 \pm 0.008
3% CE	e B5.70 \pm 0.004	e C5.60 \pm 0.004	e A5.77 \pm 0.004	e B22.20 \pm 0.015	e C18.00 \pm 0.027	e A22.36 \pm 0.015
0.2% Papain	b B5.80 \pm 0.005	b C5.68 \pm 0.005	b A5.87 \pm 0.004	a B24.43 \pm 0.01	a C21.93 \pm 0.013	a A24.95 \pm 0.018

Means having different small letters (abc.) among treatments for each muscle are significantly different ($P<0.05$). Means having different capital letters (ABC) among muscles for each treatment are significantly different ($P<0.05$).

Table 2: Protein solubility (mg/g) in LD, SM and SS muscles of aged bull meat treated with different concentrations of enzyme extract from cucumis fruit (CE) and papain enzyme (Mean \pm S.E).

Treatment	Sarcoplasmic protein			Myofibrillar protein			Total protein		
	LD	SM	SS	LD	SM	SS	LD	SM	SS
Control	e A26.63 \pm 0.085	e C24.43 \pm 0.063	e B25.48 \pm 0.085	e A60.70 \pm 0.173	e C51.05 \pm 0.119	d B57.93 \pm 0.125	e A87.33 \pm 0.125	e C75.48 \pm 0.103	e B83.45 \pm 0.065
1% CE	d A28.38 \pm 0.111	d C26.30 \pm 0.129	d B27.35 \pm 0.065	d A61.98 \pm 0.131	d B53.33 \pm 0.095	c A61.93 \pm 0.075	d A90.35 \pm 0.065	d C79.38 \pm 0.165	d B89.28 \pm 0.085
2% CE	c A31.26 \pm 0.065	c C29.03 \pm 0.085	c B30.23 \pm 0.125	b A74.13 \pm 0.085	b C65.30 \pm 0.071	b B73.28 \pm 0.149	b A105.38 \pm 0.111	b C94.33 \pm 0.111	b B103.50 \pm 0.108
3% CE	a A34.55 \pm 0.065	a C31.33 \pm 0.18	a B32.35 \pm 0.104	a A76.00 \pm 0.041	a C67.23 \pm 0.18	a B75.13 \pm 0.246	a A110.55 \pm 0.065	a C98.55 \pm 0.065	a B107.48 \pm 0.165
0.2% Papain	b A32.45 \pm 0.065	b C30.40 \pm 0.091	b B31.48 \pm 0.111	c A64.03 \pm 0.111	c C53.75 \pm 0.087	c B62.15 \pm 0.144	c A96.53 \pm 0.111	c C84.15 \pm 0.087	c B93.63 \pm 0.085

Means having different small letters (abc.) among treatments for each muscle are significantly different ($P < 0.05$). Means having different capital letters (ABC) among muscles for each treatment are significantly different ($P < 0.05$).

samples were higher compared to papain treated muscle samples for LD, SM and SS muscles. In our experiment even though cucumis treated muscles samples, have lower pH than others, higher protein solubility might be due to higher proteolysis. It was observed that LD muscles had higher ($P < 0.05$) myofibrillar protein, total protein and sarcoplasmic protein solubility values compared to the SM and SS muscles. Differences in protein solubility may be due to the difference in structure of the muscles; also, differences in protein solubility might be due to the differences in pH values for each muscle (Warner *et al.*, 1999).

Collagen content and solubility

Results in Table 3 showed a significant ($P < 0.05$) reduction in collagen content in all treatment with cucumis extract treated samples for LD, SM and SS muscles, especially when concentration of cucumis extract increased as compared with control and papain enzyme treatment. It was observed that from the results, papain

treatment had moderate values of collagen content than those of cucumis treated muscles samples and control treatment. This is might be attributed to the cucumis enzymes protease causing a degradation of the insoluble collagen weakening of the cross-linkages in collagen molecules, which led to increase of soluble collagen content in the muscles and improve meat tenderness. The reduction collagen content of cucumis treated muscles samples in our experiment was consistent with finding of Wada *et al.*, (2004) who reported a significant increase of soluble collagen content in beef meat and sheep meat treated with kiwifruit actinidin protease. In addition to protease activity, the high acid content in cucumis extract might breakdown the connective tissue structure by possible enhancing the action of collagenase, which are active at low pH (Warriss, 2000). It was observed that total collagen content in SM and SS muscles were higher than LD muscles (Table 3), it may be due to the considerable differences in the collagen content in these muscles (Sazili *et al.*, 2004), in addition to the differences in the physiological function and location among muscles (Lawrie, 2002). LD muscle contains lower collagen than SM and SS muscles; therefore LD muscle was tenderness than SM and SS muscles Yanar *et al.*, (1999). It was observed from results in Table 4 that all cucumis extract treated muscles samples had significantly ($P < 0.05$) collagen solubility percentages in LD, SM and SS muscles compared to control treatment. It seems that 3% of cucumis extract gave a higher ($P < 0.05$) collagen solubility percentage than those of other treatments. It was observed that papain treatment surpassed control and 1% cucumis extract in collagen solubility. These results indicated that muscles samples treated with cucumis extract had higher collagen solubility compared to the control and papain treatment, which may be attributed to the proteolytic activity of cucumis protease in cucumis extract. Besides, increased collagen solubility of cucumis treated muscles samples might be due to an increase in permeability of the connective tissue, which will disintegrate easily, in addition, cucumis enzyme in cucumis extract may promote structural alterations through action on intermolecular cross-links (Rawdkuen and Benjakul, 2012). Moreover, Naveena *et al.*, (2011) stated that the solubility of connective tissue further than total amount of connective tissue is more highly associated with sensory traits. Higher collagen solubility in our experiment was harmony with Naveena *et al.*, (2004) and Verma *et al.*, (2018) who reported significantly higher collagen solubility in buffalo and emu meat chunk treated with cucumis extract and papain respectively compared to the control. The result given in Table 4 showed that LD muscle had

Table 2: Collagen content (mg/g) and collagen solubility (%) in LD, SM and SS muscles of aged bull meat treated with enzyme extract from Cucumis fruit (CE) and papain enzyme (Mean±S.E)

Treatment	Collagen content			Collagen Solubility		
	LD	SM	SS	LD	SM	SS
Control	a C5.51±0.002	a A5.70±0.003	a B5.65±0.001	e A7.33±0.029	e C6.40±0.022	e B6.81±0.016
1% CE	b B5.20±0.002	b A5.63±0.001	d C5.17±0.002	d A10.07±0.021	d C7.49±0.037	d B9.70±0.026
2% CE	d C4.92±0.001	d B5.05±0.001	b A5.26±0.002	b A12.02±0.017	b C8.88±0.036	b B10.99±0.022
3% CE	e C4.01±0.002	e A4.67±0.001	e B4.48±0.001	a A15.77±0.058	a C10.05±0.042	a B13.49±0.033
0.2% Papain	c C5.01±0.002	c A5.44±0.001	c B5.20±0.001	c A11.07±0.058	c C7.99±0.038	c B9.95±0.028

Means having different small letters (abc.) among treatments for each muscle are significantly different ($P < 0.05$). Means having different capital letters (ABC) among muscles for each treatment are significantly different ($P < 0.05$).

higher collagen solubility followed by SS muscle, then SM muscle. It may be due to the differences in number of cross-links between collagen molecular and collagen content (Maiorano *et al.*, 2000).

SDS-PAGE

Effect of cucumis and papain enzyme extract on protein patterns an illustrative protein patterns by SDS-PAGE for the muscle’s samples (LD, SM and SS) started with different concentrations of cucumis extract and papain illustrated in Fig. 1. The two proteins myosin heavy chain (MHC) and actin (AC) are the major proteins in all muscles types. It was observed that muscles samples treated with cucumis extract and papain indicated to the reduction in number and intensity of the protein bands as result to proteolysis of the muscle proteins in all treated muscles samples with cucumis extract and papain compared to the control. In addition, it was observed from

the figure the breakdown of high molecular weight proteins into low molecular weight proteins of 36 KDa and below (Huff-Lonergan *et al.*, 1996 and Naveena *et al.*, 2004). The breakdown of proteins in high quantitative noticeable in the longissimus dorsi (LD) and Supraspinatus (SS) muscles treated with 2 and 3% of cucumis extract than those of semimembranosus (SM) muscle treated, papain and control. In addition, comparing the treatments (cucumis-treated) to the control, the myosin heavy chain band was clearly degraded into lower molecular weight products as seen at the bottom part of the gel. When comparing SS and LD muscle, the MHC band of this muscles were clearly degraded into lower molecular weight than those SM muscle. Moreover, breakdown of the AC from cucumis treated muscles samples where was degraded on SDS-PAGE, especially in LD and SS muscles. Previous reported by Wada *et al.*, (2002) that

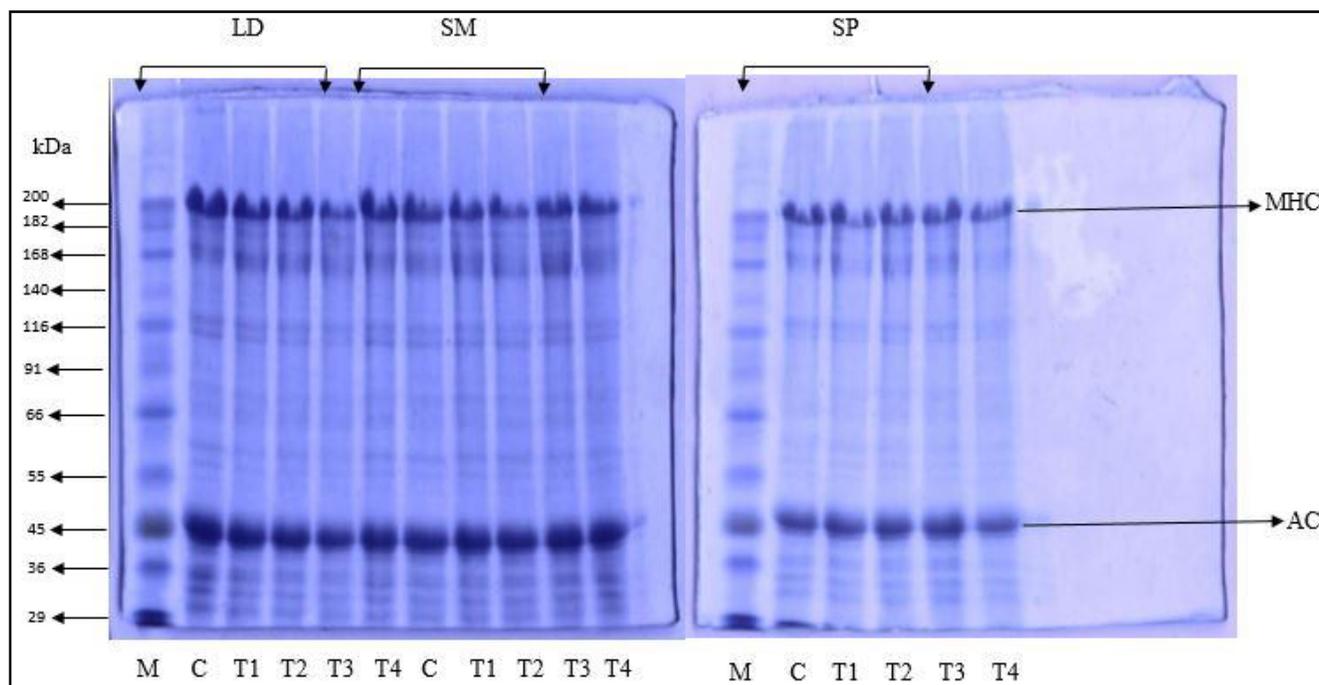


Fig. 1: SDS-PAGE patterns of muscles samples (LD, SM and SS) muscles treated with different concentrations of enzyme extract from cucumis and papain.

Table 4: Sensory evaluation scores in LD, SM and SS muscles of aged bull meat treated with different concentrations of enzyme extract from cucumis extract fruit (CE) and papain enzyme (Mean±S.E).

Treatment	Colour			Flavor			Tenderness			Juiciness			Overall acceptability		
	LD	SM	SS	LD	SM	SS	LD	SM	SS	LD	SM	SS	LD	SM	SS
Control	b A4.80 ±0.26	b A4.80 ±0.26	a A5.44 ±0.20	ab A5.44 ±0.20	c A4.48 ±0.31	a A4.80 ±0.26	b A5.12 ±0.16	ab A4.16 ±0.33	b A4.84 ±0.33	ab A4.80 ±0.26	c B3.52 ±0.16	b AB4.48 ±0.16	bc A4.80 ±0.00	c A4.16 ±0.20	b A4.48 ±0.16
1% CE	a A5.76 ±0.20	ab A5.76 ±0.33	a A6.08 ±0.31	a A6.08 ±0.16	bc A5.12 ±0.31	a A5.44 ±0.20	b A5.44 ±0.20	b B3.52 ±0.40	a A5.12 ±0.16	ab A5.12 ±0.31	bc A4.48 ±0.31	a A5.44 ±0.20	ab A5.44 ±0.20	ab A5.12 ±0.16	a A5.44 ±0.20
2% CE	a A6.08 ±0.16	ab A5.44 ±0.20	a A6.08 ±0.16	a A6.08 ±0.16	a A6.40 ±0.26	a A5.76 ±0.20	ab A5.76 ±0.20	a A5.12 ±0.16	a A5.44 ±0.20	a A6.08 ±0.16	ab A5.44 ±0.20	a A5.44 ±0.20	a A5.76 ±0.20	a A5.44 ±0.20	a A5.76 ±0.20
3% CE	a A6.08 ±0.16	a A6.08 ±0.16	a A6.08 ±0.16	a A6.08 ±0.16	ab A5.76 ±0.20	a A5.76 ±0.20	a A6.72 ±0.16	ab B4.80 ±0.00	a A6.08 ±0.16	ab A5.76 ±0.20	a A5.76 ±0.20	a A5.76 ±0.20	a A5.76 ±0.20	a A5.76 ±0.20	a A5.76 ±0.20
0.2% Papain	b A4.80 ±0.00	ab A5.44 ±0.20	a A5.12 ±0.16	b A4.80 ±0.26	bc A4.80 ±0.00	a A5.44 ±0.20	b A5.12 ±0.31	ab A4.48 ±0.31	a A5.44 ±0.20	b A4.48 ±0.40	ab A5.12 ±0.16	ab A5.12 ±0.16	c B4.16 ±0.20	bc AB4.48 ±0.16	ab A5.12 ±0.16

Means having different small letters (abc.) among treatments for each muscle are significantly different ($P<0.05$). Means having different capital letters (ABC) among muscles for each treatment are significantly different ($P<0.05$).

protease from kiwifruit affect the structure of the MHC and the AC filaments of myofibrillar protein. The breakdown of muscle proteins play major role in determining the tenderness during the post-mortem storage (Melody *et al.*, 2004). Naveena *et al.*, (2004) showed that protease from cucumis improved the tenderness and the overall quality of tough buffalo meat. The results from the present study seems to confirm this. It was observed from Fig. 1, that presence breakdown of muscles proteins was in cucumis-treated samples for LD, SM and SS muscles can be related with higher ($P<0.05$) protein solubility. In addition, MHC and AC are stable and don't degrade under normal post-mortem conditions (without treated with enzyme) (Bandman and Zdanis, 1988). The cucumis treatment with 2 and 3% for muscles affected the MHC degradation into small molecular weight protein bands. Therefore, the cucumis-treated samples for LD, SM and SS muscles were tender and showed more induced degradation of MHC compared to the control. Based on the results, it can be determined that cucumis treatment has high proteolytic activity making the muscles more tender. Sawdy *et al.*, (2004) showed a significant correlation between MHC fragment and meat tenderness in bovine muscle. Other reported by Naveena *et al.*, (2004) who observed of lower numbers of protein bands in buffalo meat treated with *Cucumis trigonus* extract.

M: marker; C: control; numbers indicated the concentration of cucumis extract (1, 2 and 3% v/w) and papain (0.2% w/w); MHC: myosin heavy chains; AC: actin

Sensory evaluation

Table 4 showed the LD muscle treated with 2 and 3% cucumis extract recorded significantly ($P<0.05$) increase in color and flavor scores as compared with scores of the control and papain enzyme. SM muscle treated with 3% of cucumis extract had significantly ($P<0.05$) scores for color than those of control. LD, SM and SS muscles treated with 2 and 3% cucumis extract were resulted a higher scores for tenderness, juiciness and overall acceptability traits compared with papain, control and 1% of cucumis extract. The improvement in meat tenderness of the treated muscles with 2 and 3% cucumis extract may be due to proteolytic activity of the cucumis protease. The improvement of tenderness scores of cucumis treated meat samples reported by Naveena *et al.*, (2004). The improvement of tenderness scores of 2 and 3% of cucumis extract for LD, SM and SS muscles in our experiment might be due to increase proteolytic and collagen degradation, which is evident from higher collagen solubility and protein solubility and similar results were found for collagen solubility and protein solubility (Table 2 and 3). The improvement in the color and flavor scores for LD, SM and SS muscles treated with 2 and 3% of cucumis extract may be due to the presence of phenols compound in the cucumis extract, which retarded lipid oxidation and maintains the desired meat color in muscles. Previous reported by Gill *et al.*, (2015) who showed that cucumis fruit have a good potential in retarding the activity of the free radicals. It was observed from results in Table 4 showed

that the mean scores for all sensory traits did not significant differences among control, papain and cucumis treated samples for SS muscles. The results in Table 4 revealed that LD muscle were more tenderness than SM and SS muscles for each treatment; this may be due to the considerable differences in the myofibrils and collagen content of these muscles, thus LD muscle had a lower collagen content (Lawrie, 2002; Sazili *et al.*, 2004 and Li *et al.*, 2007). There were significant differences among muscles in the mean tenderness for treatment of 3% of cucumis extract. Therefore, muscles samples treated with 3% cucumis extract was rated superior and most preferred by the panelists and appears to be the desirable level to achieve the best tenderization effect. The improvement in the color, flavor, tenderness and overall acceptability scores with 2% and 3% cucumis extract in our experiment is consistent with reports by (Verma *et al.*, 2018).

Conclusion

Base on the results obtained in this experiment shows there was significant reduction of pH and WHC in muscles samples immersed with cucumis extract compared to control and papain. There was significant increase in collagen solubility and protein solubility in muscles samples treated with cucumis compared to papain. Electrophoresis pattern of treated muscles protein revealed the effective utilization of enzyme extract from cucumis fruit peels for tenderizing tough meat without adversely effecting other sensory evaluation. Therefore, technology applying for cucumis enzyme extract is easily to use, cheaply and can be exploited at the household or industrial for tenderizing tough meat and it can be used as a better alternative to chemical tenderizers and papain enzyme.

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