RESEARCH PAPER
AL-ANBAR JOURNAL OF VETERINARY SCIENCES
Vol. 12 issue: 1 2019 L- ISSN :1999-6527

Tenderization Efficiency of Cucumis Extract (*Cucumis Trigonus Rox-b)* Compared with Papain enzyme on the Aged Bull Meat by Injection Method

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Abstract

This study was carried out to develop a method for enhancing tenderness and overall qualities of Karadi tough aged bull meat (more than 5 years old) by using proteolytic extract from cucumis fruit (*Cucumis trigonus Rox-b*) compared to papain enzyme. After slaughter bull and dressing carcass, the three main muscle, longissimus dorsi (LD), semimembranosus (SM) and supraspinatus (SS) were evaluated by injecting brine supplied with extract solutions in different concentrations. (0.1, 0.2 and 0.3 % of cold cucumis extract solution and 0.02 % of Papain extract solution and distilled water as group) at average of injection of 10 % muscle weight. Overall, there were significant ($p<0.05$) reduction of muscle pH, water holding capacity and cooking loss and significant ($p<0.05$) increase in collagen solubility, nitrogen solubility in LD, SM and SS muscle samples treated with cucumis extract compared with control and Papain enzyme. The increased concentration of cucumis extract resulted in significant increase ($p<0.05$) in total and myofibrillar protein solubility with slightly increase of sarcoplasmic solubility in muscle samples that treated with cucumis extract compared to control and Papain enzyme. The electrophoresis pattern of muscle treated samples also revealed extensive proteolysis occurring in each muscle type. The results of sensory evaluation indicated that the tenderness, juiciness and overall acceptability of all treated muscle samples significantly ($p<0.05$) improved compared with control. In our experiment, generally cucumis extract tended to be more effective than papain extract for most of the studied traits. There for it can be summarized that cucumis extract is one of the best alternative source of proteolytic enzymes for the effective tenderizing of meat.

كفاءة التطرية لمستخلص النباتي المحللة للبروتين كوكوميس (*Cucumis trigonus Rox-b*) بالمقارنة مع انزيم الباباين في لحم الثور المسن بطريقة الحقن

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الخلاصة

أجريت هذه الدراسة لتحسين التطرية والصفات النوعية للحوم الثيران الخشنة المسنة باستخدام الانزيمات النباتية المحللة للبروتين من فاكهة كوكوميس (*Cucumis trigonus Rox-b*) مقارنة بانزيم الباباين. بعد الذبح وسلاخة الجلود للثيران تم تجهيز الذبائح، تم ثلاثة عضلات رئيسية وهي العضلة الطولية الظهرية Longissimus dorsi (LD) والعضلة نصف الغشائية Semimembranosus (SM) ،والعضلة فوق الشوكية Supraspinatus (SS) حيث تم تقييمها عن طريق حقنها مع المستخلص الانزيمي بتراكيز مختلفة (0.1 ، 0.2 ، 0.3 % من مستخلص محلول كوكوميس البارد و 0.02 % من محلول مستخلص الباباين و الماء المقطر كمجموعة السيطرة وبمعدل حقن 10% من وزن كل عضلة. بشكل عام اشارت النتائج بان درجة تركيز ايون الهيدروجين، قابلية اللحم على حمل الماء و نسبة الفقدان عند

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الطبخ انخفض معنويا (p<0.05) في العضلات المعاملة مع مستخلص الكوكوميس في نفس الوقت لوحظ ارتفاع معنوي (p<0.05) في ذائبية الكولاجين و النيتروجين في العضلات LD ، SM و SS مقارنة مع معاملة المقارنة ومعاملة مستخلص البابابن. اظهرت النتائج بان مع زيادة تركيز مستخلص الكوكوميس ازداد معنويا (p<0.05) قيمة بروتينات المايوفيبريل والبروتينات الكلية مع زيادة قليلة في قيمة بروتينات الساركوبلازم في العضلات المعاملة بمستخلص الكوكوميس بالمقارنة مع معاملة البابابن ومعاملة السيطرة. اشارت نتائج الترحيل الكهربائي لبروتينات العضلات المعاملة بمستخلص الانزيمات انحلال البروتينات على نطاق واسع في كل عضلة. كما اضهرت نتائج التقييم الحسي للعضلات المعاملة بمستخلص انزيم الكوكوميس والبابابن تحسينا معنويا (p<0.05) في طراوة ، عصيرية والتقبل العام بالمقارنة مع معاملة السيطرة. من خلال نتائج الدراسة المسحصلة عليها يوجد تفوق معنوي لمستخلص الكوكوميس مقارنة بانزيم البابابن في اغلبية الصفات المدروسة لذلك يمكن الاستنتاج باستخدام *Cucumis trigonus Rox-b* كمصدر بديل للانزيمات المحللة للبروتين لتطرية اللحوم بشكل فعال.

الكلمات المفتاحية : التطرية ، كوكوميس، البابابن، لحم الثور المسن

Key words: Tenderness, Cucumis extract, Bull meat, Injection, Sensory evaluation.

Introduction

Increasing demand for high-quality meat, especially red meat, had been expected (33) being the eating quality is the most important factor in the consumers' choice of meat (49). Organoleptic attributes (Flavor, juiciness and tenderness) are the three main factors which influence meat quality (1),(14),(38). Traditionally, most of the bull meat in Iraq comes from aged or spent males. Generally, consumers view is that bull meat is undesirable toughness, course texture and undesirable palatability traits and are not appreciated by the consumers. This is mainly because bull meat usually obtained from old animals that have served other functions in other live and reproduction efficiency declines. Beef palatability is affected by many factors and tenderness is cited as one of the most important trait, and consumers are willing to pay more for tender meat (57),(32). Tenderness of meat mainly depends on the intramuscular connective tissue amount, sarcomere length and proteolytic potential of the muscle (24). Meat tenderness differ among bovine muscle from various anatomical locations largely because of the differences in structural, components, which influence tenderness namely the myofibrillar and connective tissues proteins (3), (51). The previous study found that the postmortem process is effective to reduce meat toughness. Postmortem processes such as physical, chemical and enzymatic treatment are widely used in the meat industry (19). One of the common post-mortem technique to tenderize meat is marination, which had been practiced for a long period of time. Marination, basically involves the infusion injection and tumbling of meat with marinades. The marination techniques effectively improved the tenderness, juiciness, flavour and colour of meat. Exogenous proteases is relatively progressive method, which can used for meat tenderization to improve meat quality. There are many proteolytic enzymes such as plant proteases (papain, bromelain and ficin), protease from *Aspergillus oryzae* and *Bacillus subtillis*, which have been approved as generally regarded as safe (GRAS) for use in the meat industry by the US Department of Agriculture (15),(25). These enzymes can degrade muscle proteins and dissolution collagen, which helps in meat tenderization (40). The use of enzymes breakdown the collagen protein in connective tissues and does not breakdown myofibrillar proteins. The enzymes from papain and bromelain are the widespread plant, which used for meat tenderization (30). As meat tenderizers, proteolytic enzymes are best proper for degradation of connective tissue in collagen at relatively low pH and temperature (42). One of promising enzyme, *Cucumis trigonus Rox-b* traditionally used as meat tenderizer (36). Cucumis fruit contains cucurbitacin as one of the main chemical constituents such as flavonoids, tannins, alkaloids, saponins and triterpenes. Cucumis fruit have a good potential in retarding the activity of the free radicals, thus possessing good composition antioxidants (13). Thus, this study aimed to

determine the effects of cucumis extract treatments on physico-chemical, biochemical and sensory properties of aged bull meat.

Materials and Methods

Collection and drying local cucumis

Fresh cucumis (*Cucumis trigonus Rox-b*) fruit was obtained from local farms in penjwen region of Iraqi Kurdistan. The collected fruit were washed with distilled water, cut into pieces and remove the seeds. The peels obtained were dried in an oven at 37^{o}C. The dried peels were ground in laboratory milling machine to a fine passed through a 30-mesh sieve, the powder stored in tight containers under refrigeration.

Preparation of cucumis fruit extract

Cucumis trigonus extract was prepared according to the method described by Balakrishnan and Kokilavani (2). The dried fruit powder (500 g) extracted with 2500 ml of 99% ethanol. The mixture was kept in the shaker for 48hr, and the suspension was filtered through two layers of muslin cloth. The residue was resuspended in the equal volume of 99% ethanol for 48 hr. Then filtered again. The two filtrates were pooled and the solvents were dried in oven at 37^{o}C. The yield of the dried extract was about 90 gram. The dried crude extract was used for further study.

Extract treatment of muscle 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 Science Engineering, University of Sulaimani. Longissimus dorsi (LD), Semimembranosus (SM) and Supraspinatus (SS) muscle were excited from loin, round and chuck cuts, the external fat and visible connective tissues trimmed from muscle, then packed in polyethylene bags and kept in refrigerator at 4^{o}C for 24 hr.

After chilling, muscle samples were taken out of the refrigerator, cut into equal pieces in the same size in length and thickness, having the approximate weight of 100g for each muscle pieces. The cutting of muscles pieces were made a long the muscular fibers. The pieces of muscle (LD, SM and SS) were separately divided into five (5) groups. Each group having at least 6 pieces (500g) for each muscle/treatment, and injected with different concentrations of crude cucumis extract solution (0.1, 0.2 and 0.3 %), (0.02 %) of Papain solution and distilled water as control group at a rate of injection of 10 % muscle weight. The distance between injection sites of the muscle was 2 cm. Thus, there are five (5) treatments as follows:

T1: Control muscle (injected with distilled water) was considered as a control treatment.

T2: Muscle samples injected with 10% with cold cucumis extract solution at concentration of 0.1 (v/w)

T3: Muscle samples injected with 10% with cold cucumis extract solution at concentration of 0.2% (v/w)

T4: Muscle samples injected with 10% with cold cucumis extract solution at concentration of 0.3% (v/w)

T5: Muscle samples injected with cold Papain solution at concentration of 0.02% (v/w)

After injection with enzyme treatment, the muscle were allowed to equilibrate for 30 min at room temperature then muscle samples were kept in polyethylene bags and stored in refrigeration at 4^{o}C for 48 hr. then kept in freezing at -18^{o}C. The muscle samples were evaluated for physico-chemical properties, stability of lipid oxidation and sensory traits of aged bull meat as described below.

Analysis of muscle samples

pH

At room temperature (27^{o}C). 50 ml of chilled distilled water mixed and homogenized with 10 gram of the treated

muscle samples and the pH values were measured by pH meter (W.T.W 2F40-114, Germany). The pH meter initially calibrated with pH 7 and pH 4 buffers before used in pH determination (52).

Water holding capacity (WHC)

Water holding capacity (WHC) of muscle samples were determined based on method in Ozalp and Karakaya (37). Eight (8) gm of grinded meat sample was put into a centrifuge tube after that 12 ml of 0.6M NaCl solution was added into the tube. The tube for 15 min subsequently was stored at 4±1^{o}C. Then, the tube was centrifuged at 3000 x g for 15 minutes at 5°C. By using measuring cylinder, the volume of supernatants was recorded and WHC was expressed as a percentage of initial volume. WHC was calculated according to Ketnawa and Rawdkuen (25). Moreover, expressed in percentage as the following equation:

WHC (%) = ((Initial volume -Volume of supernatant)/Initial volume) x 100.

Cooking loss

Cooking loss was estimated by packing weighed samples of approximately 100g sealed in heat resistant plastic bags, kept in water bath at 75^{o}C for 50 min followed by cooling, dry blotting, and weighing (Honikel, 1998). Cooking loss was calculated as follows:

Cooking loss% = Row weight - Cooked weight /Row weight × 100

Protein solubility

Depending to the process which was stated by Joo et al. (21). Solubility of proteins were measured from 2 gm minced muscles sarcoplasmic proteins via using 0.025 M potassium phosphate buffer (pH 7.2) with 20 ml of ice-cold. All samples by frequent shaking were homogenized and stored at 4°C. Then for 20 min at 1500xg, the samples have been centrifuged. Next step extraction of total protein (myofibrillar, sarcoplasmic) was conducted by using 1.1 M potassium iodide, 40 ml ice-cold in 0.1 M phosphate buffer (pH 7.2) After that total proteins homogenization, centrifugation and determination have been done. Myofibrillar protein concentrations gained via differentiation between sarcoplasmic protein and total solubility.

Collagen solubility

Extraction of soluble and insoluble collagen for the treated and untreated muscle samples by modified Hill procedure (17) and method described by Wattanachant *et al*.,(55). The hydroxylproline concentrations of the diluted samples were determined by measuring the absorbance at 558 nm against a standard curve of hydroxylproline according to the method described by Bergman and Loxley, (4). The hydroxyproline (HOP) concentration was calculated as follows:

Mg (HOP)/gm tissue = [(HOP) ug/ml x dilution factor*]/ [Sample wt. (gram)]x1/1000.

* Delution 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 (6). Then reports total collagen (soluble + insoluble collagen) and collagen solubility (%) (Soluble collagen / total collagen x100).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE)

Based on Laemmli (26) SDS–PAGE have been done different treatment conditions applied on minced muscles (2 gm) at 85^{o}C. Muscles were mixed with 18 ml SDS solution 5% (w/v), then homogenization process conducted to the mixture with water bath incubation at 85 ^{o}C for 1 hr for melting the protein. After that centrifuge for 5 min at room temperature using a centrifuge 8000xg (Cooler centrifuge, Labnet, Germany) for separating the non-dissolved debris. Then by using a 1:1 (v/v) ratio the supernatants with the sample

buffer were mixed. Sample buffer consist (20% glycerol , pH 6.8 containing 4% SDS , 0.5 M Tris–HCl, , and 10% Beta-mercatoethanol (BME) , Also they were boiled during 3 min. Loading was started by using 20µg protein in to poly-acrylamide gel which consist (4% stacking gels and 10% running). In addition, electrophoresis set was used at stable current of 15 mA per gel via Mini Protean Tetra Cell unit (Bio-Rad Laboratories, Richmond, CA, USA). After this step, staining have been done overnight, using staining solution 50% (v/v) methanol, [0.02% (w/v) coomassie brilliant blue R-250)] and acetic acid 7.5% (v/v). After doing de-staining the gel the patterns of protein was made clear visible until achieving clear background**.**

Thiobarbituric acid (TBA) value

The TBA was determined by using solution containing 20% trichloroacetic acid (TCA) in 2M phosphoric acid according to the method described by Witte *et al*., (1970). The absorbance was read at 530 nm by using spectrophotometer (shimdzu, Japan). The TBA value was expressed as mg malondialdehyde (MDA)/kg muscle. The results calculated by multiplying the absorbance by 5.2 factor.

Sensory evaluation

From frozen storage, the pieces of muscle samples were removed and thawed under refrigeration at 4^{o}C for 24 hrs prior to sensory evaluation. Then for during 20 minutes the samples were cooked by using an oven at 180^{o}C to75±1^{o}C as internal temperature via probe thermometer monitored and served warm to 7 skilled panel members consisting of post-graduate students of the department of animal science and meat scientists with previous experience. The evaluation of sensory traits done via 8-unit descriptive scale were one and eight were the extremes of each trait (8- extremely desirable color, highly desirable flavor, overly desirable juicy, highly desirable tender and extremely desirable acceptable, respectively) was used for determine color, flavor and aroma, tenderness, juiciness and overall acceptability. Panelists were required to cleanse their palate between samples with drinking water (23).

Statistical analysis

The obtained data were statistically analyzed 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 (8) was used to compare significant differences among means with each factor on all studied traits.

Results and Discussion

pH value

The pH values for the control and treated muscle samples are presented in table 1. The pH value significantly (P <0.05) reduced for LD, SM and SS muscle respectively after treated with cucumis extract (CE) and papain enzyme extract solution, however, 0.3% cucumis extract significantly (P <0.05) recorded a lower value of pH for the three muscles. This result may be due to low pH of local cucumis extract, which was probably caused the lower pH of the treated muscle samples. Moreover, hydrolysis of muscle by extract of cucumis may be release amino acid that can decrease the pH for treated samples. The pH values in the current study are in agreement with Naveena (36) and Verma (50) who reported that treatment with cucumis extract reduced the pH of buffalo and emu meat chunck. Ketnawa and Rawdkuen (25) had reported similar results for the tenderization of different meats by bromelain. From the results revealed, that the higher pH value was recorded by papain enzyme treated samples were probably due to higher pH of the papain extract. There was a significant

difference (P<0.05) in pH value between muscle in all treatments, the lowest pH value was found in SM muscle followed by LD and SS muscle. This result it may be due to the differences in the rate of glycolysis process as well as the differences in muscle fiber structure (54). The rate of glycolysis process affected by several factors including animal type, age, muscle type and pre-post slaughter of animal condition (27). In addition, it may be probably due to the differences in glycogen content for each muscle.

Water holding capacity (WHC)

The percent of WHC of meat samples exposed to different level of cucumis extract and papain enzyme is illustrate in table 2. A significant declined (P<0.05) in WHC was observed by added of extract. Papain treated sample had the highest WHC. Whereas, the lowest value was observed in the 0.3% cucumis treated samples for each muscle. The reduction of WHC in cucumis treated muscle samples might be due to the lower pH of cucumis extract. This decline in pH value may be responsible for overall reduction of reactive group of proteins available for water binding (9). This result is an agreement with Naveena (36), Ketnawa, and Rawdkuen, (25) who reported the reduction of WHC in buffalo and beef meat when treated with cucumis and bromelain extract. In our experiment, it was observed that the reduction of WHC percentages in cucumis treated samples of LD, SM and SS might be due to decline of WHC of aged animal (48). Furthermore, it may be due to slight denaturation of sarcoplasmic proteins, which play an important role in determining WHC could be the reason for decreased WHC (21). Besides the degree of WHC was due to the myofilament space into the extra-cellular spaces (25). It was observed from results in table (2) higher WHC in papain treated samples of LD, SM and SS might be due to higher pH of papain extract (6.22). This result was agreement with Naveena (36) who reported higher WHC in papain treated meat chunk of buffalo meat. From the data in table 2, it can be seen SS muscle gave the higher WHC as compared with other muscle. This may be due to higher pH of SS muscle (6.21). Such result is due to the differences in muscle function, fiber type and pre-post glycolysis process (16).

Cooking loss

There was a significant (P<0.05) increase in cooking loss percent in cucumis treated muscle samples compared to others in all muscle was shown table 2. However, in papain treated muscle sample there was a significant (P<0.05) decrease in the cooking loss. The highest loss of cooked sample was observed in 0.3% cucumis treaded sample. While, the lowest loss was found in papain treatment. Increasing cooking loss percent in our experiment by adding cucumis and papain extract may be related to the heating process, thus led to change of water content within the myofibrils in the narrow channels between the filaments, which caused the shrinkage of tissue matrices. (34).

Sanchez (43) stated that three main processes cause cooking loss increment of heated meat. First, evaporate of water with the increase of heating temperature. Second, shrinkage of myofibrillar proteins with increased temperature during heating. Which, starts at 40ºC and becomes more intense with the increase of heating temperature. As a result, a parallel decrease occurs in the interfibrillar volume and thus leads to a reduction in the myofibril's ability to hold water. Therefore, a part of water retained by capillarity is lost. Finally, at temperatures between 56 and 62ºC, a contraction of perimysial connective tissue seems to take place causing the compression of muscle fiber bundles and thus encourages the water to be released from the beef muscle. SS muscle significantly (p<0.05) had the lowest loss of cooked meat in all treatments as compared with LD and SM muscle except 0.3% cucumis treatment (table 2).

Table 1. pH value in longissimus dorsi (LD), semimembranosus (SM) and Suprapinatus (SS) muscles of aged bull meat treated with different concentration of extract from cucumis fruit and papain (mean±S.E)

Treatment	pH values		
	LD	SM	SP
(T1) Control	a B 5.84±0.005	a C 5.74±0.008	a A 5.92±0.006
(T2) 0.1% CE	c B 5.76±0.009	c C 5.68±0.006	b A 5.85±0.006
(T3) 0.2% CE	d B 5.72±0.004	d C 5.64±0.004	c A 5.82±0.006
(T4) 0.3% CE	e B 5.70±0.006	e C 5.62±0.004	d A 5.79±0.009
(T5) 0.02% Papain	b B 5.82±0.006	b C 5.70±0.004	b A 5.87±0.006

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

Table 2. Water holding capacity (WHC) and cooking loss percent in longissimus dorsi (LD), semimembranosus (SM) and Suprapinatus (SS) muscles of aged bull meat treated with different concentration of extract from cucumis and papain (mean±S.E)

Treatment	WHC %			Cooking loss %		
	LD	SM	SP	LD	LD	LD
(T1) Control	b B 27.46±0.015	b C 24.13±0.048	b A 27.89±0.009	d B 36.34±0.009	d B 36.34±0.009	d B 36.34±0.009
(T2) 0.1% CE	c B 26.67±0.006	c C 22.64±0.009	c A 26.82±0.009	b B 36.90±0.009	b B 36.90±0.009	b B 36.90±0.009
(T3) 0.2% CE	d B 26.17±0.017	d C 21.19±0.013	d A 26.33±0.006	c C 36.69±0.009	c C 36.69±0.009	c C 36.69±0.009
(T4) 0.3% CE	e B 25.65±0.011	e C 20.54±0.009	e A 25.82±0.006	a B 37.32±0.009	a B 37.32±0.009	a B 37.32±0.009
(T5) 0.02% Papain	a B 27.77±0.011	a C 24.62±0.008	a A 28.13±0.045	e B 35.74±0.006	e B 35.74±0.006	e B 35.74±0.006

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

Table 3. Protein solubility (mg/g) in LD, SM and SS muscles of aged bull meat treated with different concentrations of extract from cucumis fruit (CE) and papain (Mean±S.E)

Treatment	Total Protein			Sarcoplasmic Protein			Myofibril protein		
	LD	SM	SP	LD	SM	SP	LD	SM	SP
(T1) Control	e A 92.30± 0.204	e C 79.40± 0.041	e B 87.15± 0.21	e A 29.38± 0.17	e C 26.85± 0.104	e B 27.65± 0.155	e A 62.93± 0.085	e C 52.55± 0.132	d B 59.50± 0.227
(T2) 0.1% CE	d A 96.68± 0.063	d C 82.20± 0.147	d B 91.95± 0.096	d A 31.25± 0.132	d C 27.95± 0.119	d B 29.43± 0.048	d A 65.43± 0.085	d C 54.25± 0.065	c B 62.55± 0.065
(T3) 0.2% CE	b A 109.68± 0.138	b C 96.40± 0.178	b B 106.08± 0.063	c A 35.23± 0.075	c C 30.53± 0.18	c B 32.50± 0.071	b A 74.45± 0.119	b C 66.10± 0.147	b B 73.58± 0.085
(T4) 0.3% CE	a A 117.83± 0.111	a C 101.83± 0.048	a B 113.58± 0.144	a A 40.53± 0.155	a C 34.30± 0.178	a B 37.30± 0.108	a A 77.38± 0.085	a C 67.53± 0.149	a B 76.28± 0.085
(T5) 0.02% Papain	c A 102.50± 0.082	c C 90.00± 0.0408	c B 99.10± 0.071	b A 36.25± 0.065	b C 32.40± 0.108	b B 34.35± 0.132	c A 66.25± 0.065	c C 57.60± 0.091	c B 64.75± 0.065

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

Table 4. Thiobarbituric acid (TBA) mg malondialdehyde/kg muscle of LD, SM and SS muscles of aged bull meat treated with different concentrations of extract from cucumis and papain (Mean±S.E)

Treatment	TBA values		
	LD	SM	SS
(T1) Control	a C 1.11±0.01	a A 1.44±0.010	a B 1.21±0.006
(T2) 0.1% CE	c C 0.80±0.013	c A 1.25±0.014	c B 0.95±0.004
(T3) 0.2% CE	d C 0.56±0.009	d A 1.10±0.009	d B 0.90±0.006
(T4) 0.3% CE	e C 0.51±0.013	e A 0.89±0.005	e B 0.80±0.013
(T5) 0.02% Papain	b C 0.93±0.011	b A 1.35±0.012	b B 1.14±0.013

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

Protein solubility

The data in (table 3) expressed the solubility of proteins in muscle samples exposed to different levels of cucumis extract and papain enzyme solution. Cucumis and papain extract significantly ($p<0.05$) affected protein solubility. All treated muscle samples significantly ($p<0.05$) had the highest sarcoplasmic, myofibrillar and total protein solubility in compared to the control treatment. The lowest protein solubility was recorded by control treatment, while the highest value was found in 0.3% cucumis extract.

However, papain treatment gave the moderate value for protein solubility. The regularly aligned filaments of myofibrils may have helped to prevent cucumis extract penetration, thus making the action seemingly resistant to extraction (7). The alteration of protein solubility in our experiment were due to myofibrillar protein degradation. The rise in the solubility of cucumis treated samples for LD, SM and SS muscle may be related to permeability increase of myofibrils, which will then disintegrate easily. Besides, sarcoplasmic protein solubility values of cucumis extract treated muscle samples for LD, SM and SS muscle obtained slightly increase compared to the control. Increase in protein solubility with cucumis extract also observed by Naveena (36) and verma (50) who reported that the protein solubility was increased in buffalo and emu meat chunk by adding cucumis extract. Less solubility of sarcoplasmic proteins in LD, SM and SS treated samples muscle was in agreement with Kang and Bice (22) 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 the weakest correlation. It seems from results the papain treatment of LD, SM and SS muscle had moderate increase in protein solubility values compared to the control. Increase in protein solubility with papain extract also reported by Naveena (36) in buffalo meat. It was observed from the results protein solubility values for cucumis treated samples were higher compared to papain treated samples for LD, SM and SS muscle. In our experiment, the higher protein solubility may be related to the lower pH of cucumis treated muscle samples, thus led to higher proteolysis. Significantly ($p<0.05$) higher sarcoplasmic, myofibrillar and total protein solubility values were observed in LD, SS and SM muscle respectively. Differences in protein solubility may be related to the difference in muscle structure and pH values for each muscle (53).

Nitrogen solubility (NS)

Nitrogen solubility percentages of aged bull meat an illustrative in Figure 1. There was significant differences ($p<0.05$) among cucumis treated muscle samples with an increasing trend when the concentration of cucumis was increased. Nitrogen solubility showed the highest percentage at 0.3% cucumis extract concentration of SM and SS muscle while, in LD muscle the highest percentage of nitrogen solubility was found in papain treated sample. At the control treatment, only a small amount of peptides was produced resulting in a low nitrogen solubility percentage. Increasing cucumis extract concentration to 0.3% allowed the occurrence of hydrolysis at a higher degree thus led to a higher nitrogen solubility. It was reported that increased proteolysis resulted in an increase in the content of soluble forms of nitrogen in hydrolysates during hydrolysis (20). Nitrogen solubility increased when the percentage of cucumis was increased. This observation was due to release of some peptides were hydrolyzed by the enzymes into amino acids and smaller peptides as the increased of cucumis concentration. In relation to the muscle differences it can be seen from the results LD muscle significantly ($p<0.05$) had a higher NS percent when compared with two other muscle.

Collagen solubility

The results in (Figure 2) demonstration percentages of collagen solubility of muscle

samples exposed to different sources of plant proteases. Both type and concentration of enzyme significantly ($p < 0.05$) affected collagen solubility. Significantly, all treated muscle samples had higher collagen solubility percent as compared to control ($p<0.05$). 0.3% cucumis extract obtained the highest collagen solubility for LD, SM and SS muscle. While, control treatment had the lowest value for LD, SM and SS muscle. It was observed that papain treatment surpassed control and 0.1% cucumis extract in collagen solubility. These results indicated that muscle 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, the rise of collagen solubility of cucumis treated muscle 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 (39). The solubility of connective tissue further than the total amount of connective tissue and is more highly associated with sensory traits Naveena (*35*). Higher collagen solubility in our experiment is agreement with Naveena (36) and Verma (50) who reported significantly higher collagen solubility in buffalo and emu meat chunk treated with cucumis extract and papain respectively compared to the control. In this study a significant ($P <0.05$) increase of collagen solubility was observed for LD muscle compared with other muscle. It may be due to the differences in number of cross-links between collagen molecular and collagen content (31). In addition, collagen is actually the determining factor in the textural differences among various muscle (39).

Thiobarbituric acid (TBA) value

The results in table (4) revealed that muscle samples injected by distilled water (control) resulted in the highest ($p<0.05$) TBA values. The injected process with distilled water led to increase the exudative loss, caused adverse effect on muscle cell membranes stability. This effect is presumable due to an increase of lipid oxidation in these membranes by action of lipolysis enzymes such as lipase and phospholipase (5). The results in table (4) showed that LD, SM and SS muscle injected with cucumis extract solutions at concentration of 0.3% had lower ($p<0.05$) TBA values, followed by 0.2% and 0.1% of cucumis extract treatments. It was observed from results in table (4) muscle injected by 0.3% of cucumis extract solutions was more effective in retarding lipid oxidation than those of the control, papain and other treatments. This result may due to its natural antioxidant content mainly cucurbitacin as one of the main chemical constituents such as flavonoids, tannin, alkaloids, saponins and triterpenes. cucumis fruit have a good potential in retarding the activity of the free radicals, thus possessing good antioxidant properties (12). The results in table (4) indicated the presence of significant differences ($p<0.05$) in TBA values among muscle. LD muscle had lower ($p<0.05$) TBA values than both SM and SS muscle. This result may be due to the lower fat content in LD muscle than both SM and SS muscle. Furthermore, oxidative process is depend on muscle types, therefore SM and SS muscle were more suitable locations for lipid oxidation than the LD muscle (28).

SDS-page

Impact of cucumis extract and papain on patterns of protein an illustrative protein figure by using SDS-PAGE to muscle's samples (LD, SM and SS) started with different concentrations of cucumis extract and papain are showed in Figure 3. Similar protein patterns in original bull meat was observed in lane 1 (control). In all muscles types two proteins actin (AC) and myosin heavy chain (MHC) are the major proteins. They were observed that muscle samples

treated with cucumis extract and papain indicated for the reduction intensity of the protein bands with reduction of its numbers as result for muscle proteins proteolysis in all treated muscles samples with cucumis extract and papain contrasted with the control. It was revealed from the figure cucumis treated sample had a higher range of protein breakdown than control and papain treated sample. Which, indicating more pronounced proteolysis. Proteolysis increased in cucumis treated sample can be connected with significantly higher protein solubility. This result was consistent with Rawdkuen (41) who observed that the intensity of high molecular weight proteins and number of bands decreased in calotropis procerain protease tenderized beef, squid and giant catfish meat when compared to control. Shin et al., (2008) stated degradation of MHC and actin proteins in beef meat when treated with papain and *Sarcodon aspratus* extract (a plant protease). Also Gerelt (11) reported the degradation of MHC protein into small molecular weight proteins of 140 and 90 kDa, in myofibrils treated with papain. Many researchers have been reported a significant correlation between MHC fragments and tenderness of meat. The higher fragments will be more tenderness of meat (45), (47). The breakdown of proteins in high amount was more visible in LD muscle sample than other muscle.

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Conclusion

The results of this study shows that there were general improvement in the physicochemical and sensory properties of bull meat samples injected with natural extracts of *Cucumis trigonus Rox-b* and papain. By adding large amounts of crude extract, the quality characteristics of the treated meat samples were improved. The tenderness, protein solubility, collagen solubility and nitrogen solubility of muscle samples were significantly improved through the use of cucumis extract. Technology for applying this enzyme is easily and cheaply available and can be exploited at the household or industrial level for tenderizing tough meat, and it can be used as a better alternative to chemical tenderizers or other plant proteases.

Table 5. Sensory evaluation scores in LD, SM and SS muscles of aged bull meat treated with different concentrations of extract from cucumis and papain (Mean±S.E).

Treatment	Colour			Falvour			Tenderness			juicness			Overall acceptance		
	LD	SM	SP	LD	SM	SP	LD	SM	SP	LD	SM	SP	LD	SM	SP
(T1) Control	c A 4.40± 0.400	c A 4.00± 0.462	c A 4.00± 0.462	b A 4.80± 0.000	c B 3.60± 0.400	b AB 4.40± 0.400	b A 4.00± 0.462	c A 3.60± 0.400	b A 4.00± 0.800	b A 4.00± 0.462	b A 3.60± 0.400	b A 3.60± 0.400	b A 4.40± 0.400	b A 4.00± 0.462	c A 4.40± 0.400
(T2) 0.1% CE	abc A 5.60± 0.462	bc A 5.20± 0.400	ab A 5.60± 0.462	ab A 6.00± 0.400	bc A 4.80± 0.653	a A 5.60± 0.462	a A 6.00± 0.400	b A 5.20± 0.400	a A 5.60± 0.462	ab A 5.60± 0.800	ab A 5.20± 0.766	a A 5.20± 0.400	a A 5.60± 0.462	ab A 4.80± 0.000	bc A 4.80± 0.653
(T3) 0.2% CE	ab A 6.00± 0.400	ab A 6.00± 0.766	ab A 6.00± 0.400	a A 6.80± 0.400	ab A 6.00± 0.400	a A 6.00± 0.400	a A 6.40± 0.000	ab A 5.60± 0.462	a A 6.00± 0.400	a A 6.00± 0.4.00	a A 5.60± 0.800	a A 6.00± 0.400	a A 6.00± 0.400	a A 6.00± 0.400	a A 6.00± 0.400
(T4) 0.3% CE	a A 6.80± 0.400	a A 6.80± 0.400	a A 6.80± 0.400	a A 6.80± 0.400	a A 6.80± 0.400	a A 6.40± 0.000	a A 6.80± 0.400	a A 6.80± 0.400	a A 6.40± 0.000	a A 6.40± 0.000	a A 6.00± 0.400	a A 6.00± 0.400	a A 6.40± 0.000	a A 5.60± 0.462	a A 6.40± 0.000
(T5) 0.02% Papain	bc A 5.20± 0.400	bc A 4.80± 0.000	bc A 4.80± 0.000	b A 4.80± 0.653	bc A 4.80± 0.000	ab A 5.20± 0.400	a A 5.60± 0.462	b A 5.20± 0.400	ab A 5.20± 0.400	ab A 4.80± 0.924	ab A 4.80± 0.000	a A 5.60± 0.462	a A 6.00± 0.400	ab A 5.20± 0.400	abc A 5.20± 0.400

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

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AL-ANBAR JOURNAL OF VETERINARY SCIENCES
Vol. 12 issue: 1 2019 L- ISSN :1999-6527

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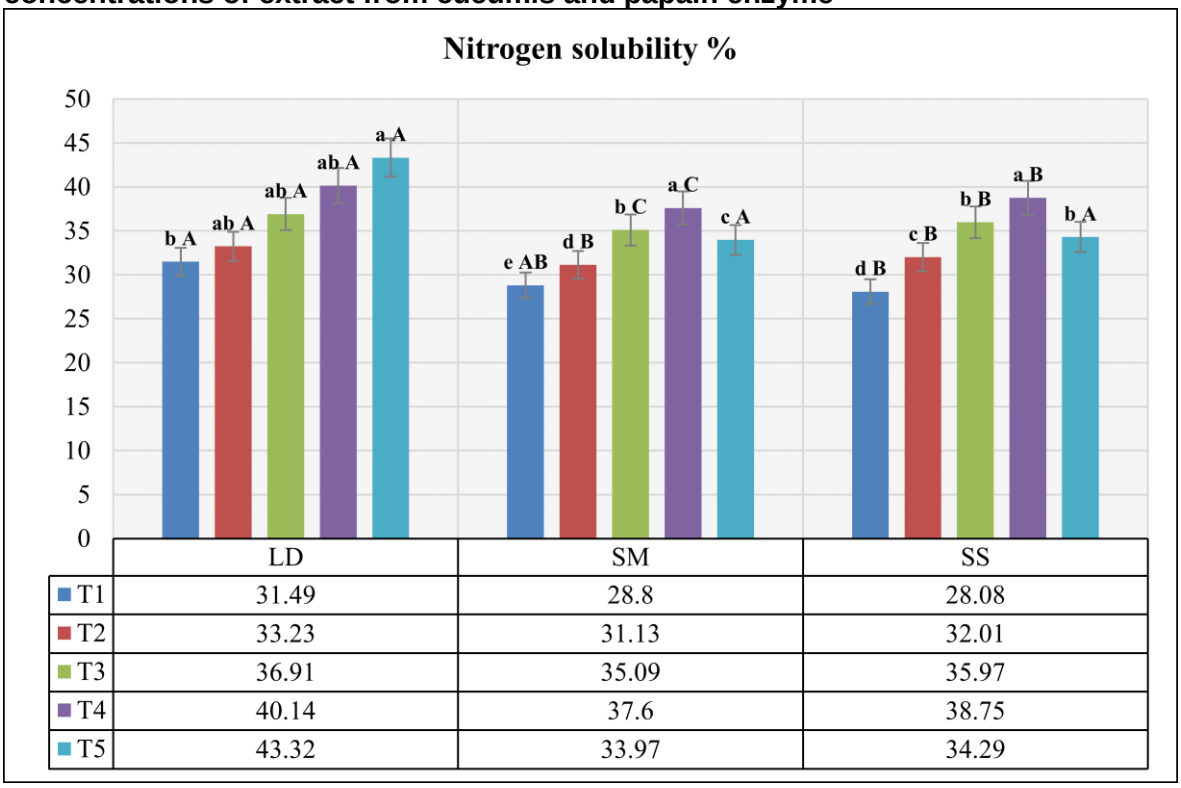
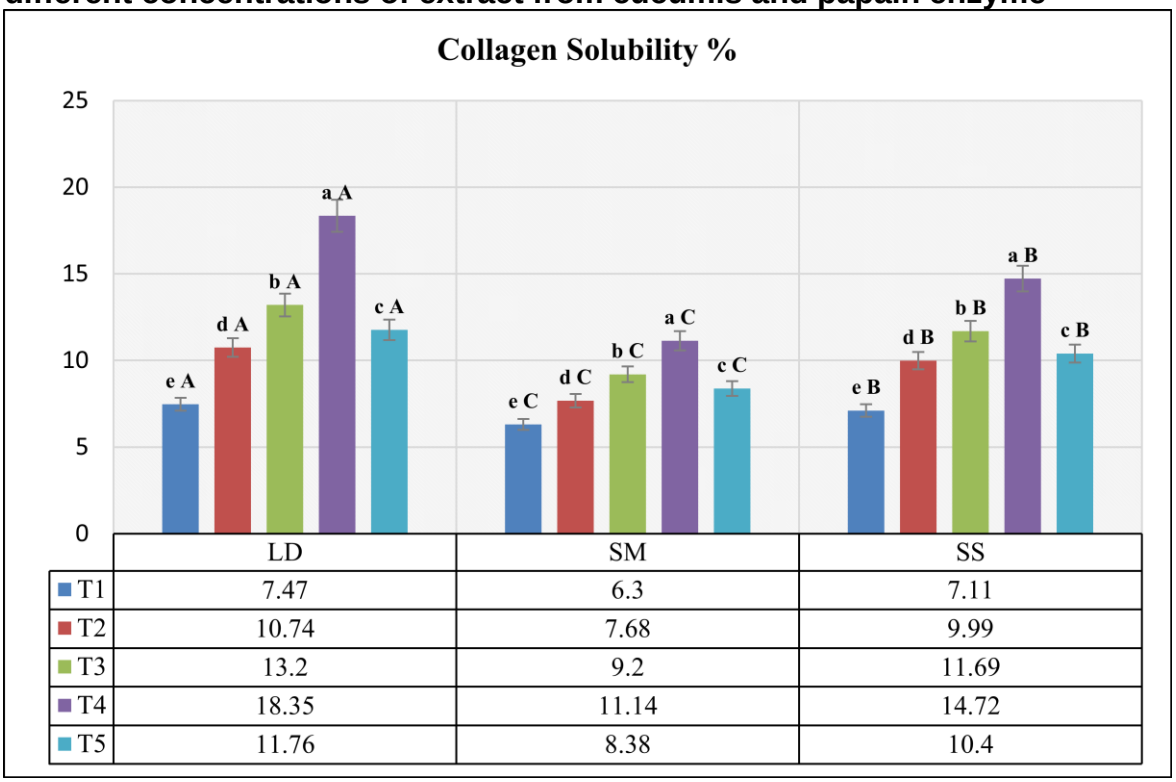
Figure 1. Nitrogen solubility of muscle samples (LD, SM and SS) treated with different concentrations of extract from cucumis and papain enzyme



	LD	SM	SS
T1	31.49	28.8	28.08
T2	33.23	31.13	32.01
T3	36.91	35.09	35.97
T4	40.14	37.6	38.75
T5	43.32	33.97	34.29

Figure 2. Collagen solubility of muscle samples (LD, SM and SS) treated with different concentrations of extract from cucumis and papain enzyme



	LD	SM	SS
T1	7.47	6.3	7.11
T2	10.74	7.68	9.99
T3	13.2	9.2	11.69
T4	18.35	11.14	14.72
T5	11.76	8.38	10.4

Small letter (abc..) among treatments for each muscle are significantly different ($p<0.05$).
Capital letter (ABC...) among muscle for each treatment are significantly different ($p<0.05$).

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AL-ANBAR JOURNAL OF VETERINARY SCIENCES
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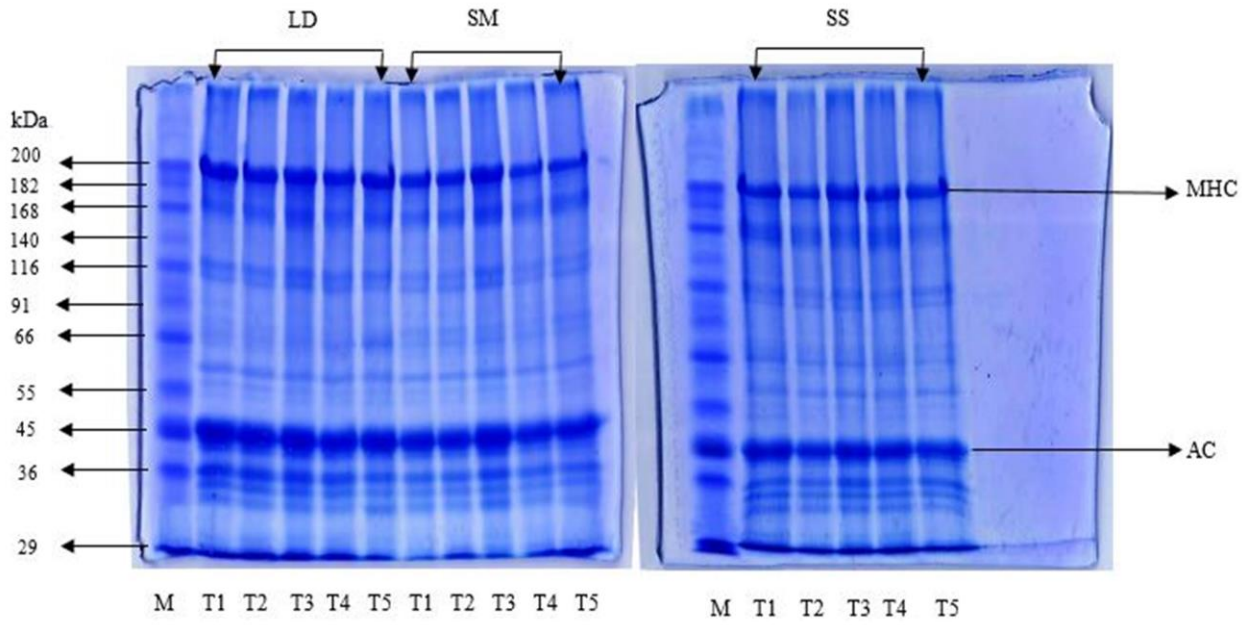
Figure 3. SDS-PAGE patterns of muscle samples (LD, SM and SS) treated with different concentrations of extract from cucumis and papain enzyme



M: marker; C: control; numbers indicated the concentration of cucumis extract (0.1, 0.2 and 0.3% v/w) and papain (0.02% w/w); MHC: myosin heavy chains; AC: actin

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