

Sensory, Chemical and Histological Assessment of Meat Quality as Tenderized by Papain and Oryzae Proteases

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ABSTRACT

A tenderizer mixture consisting of partially purified papain and oryzae protease was used to tenderize two cuts of beef meat; namely, bottom round (*Biceps femoris*), and sirloin (*Longissimusdorsi*), taken from old aged animal after 24 hr of slaughter. Sensory and chemical methods were used for the assessment of meat quality. Highly significant difference was detected between the scores for the tenderized cuts and the control, with no detectable difference for flavour. Freezing the enzyme treated meat and thawing before cooking, reduced the cooking time. Positive correlation coefficient was obtained between tenderness scores and TCA-soluble nitrogen. Generally, TCA-soluble nitrogen increased by increasing the enzyme concentration. Yet, the results revealed that the amount and the source of the used enzyme, must be selected to avoid toughness or mushiness due to under or over tenderization of the meat. Each of the partially purified papain and oryzae protease, as well as a mixture of the two preparations was used for the histological study. The study necessitated the use of relatively high enzyme concentration (4%), than those used for proper tenderization being 0.0025 and 0.005%. That is to facilitate the demonstration of even the minor changes of the tissue muscle fibers and connective tissues. The muscle fibers, bundles of fibers, nuclei at periphery of the fibers, fat cells, connective tissues, perimysium collagen, sarcolemma which envelope the muscle were apparent in both of the stained cross and longitudinal sections. The muscle histological structures were markedly affected, to various degrees by the enzymatic tenderization. The effect of papain was more pronounced than that of oryzae proteases, particularly upon cooking.

Keywords: meat tenderization, papain, oryzae, proteases, quality, histology

INTRODUCTION

Consumers for their satisfaction, ask for good quality food products. Tenderness is one of the important sensory parameters, for assessing meat palatability and acceptance. Tenderness of meat is affected by many biological, physical, chemical, and mechanical factors. The biological effects are mainly enzymatic. Endogenous enzymes of meat play an important role in reducing meat toughness through proper ageing conditions. Besides that, in the meat industry, exogenous proteases of plant, bacterial, and fungal sources are used to achieve the desired tenderness in the cooked meat. Meat tenderizers are used at commercial scale by injection, either pre-slaughter of the animal or post-slaughter by injecting the whole carcasses or the quarters. Improper post-slaughter cold storage of the animals carcasses causes cold shortening, and accordingly non reversible meat toughness, Locker & Haggyard (1963). The extent of tenderization is proportional to the level of calpains which accounts for the toughness of meat (Pransfield, 1994). Ageing as a conventional technology for improving

tenderness can improve primal beef cuts, but not the relatively tough cuts of beef. Shand *et. al.* (2006) reported that the inconsistencies associated with beef tenderness potentially cost the Canadian beef industry 21\$ million annually. That is because the lack of convenience of relatively tough cuts of beef as round and chuck, which represent about half of the weight of the carcass and sold at low prices as roast or ground beef. Bolumar *et. al.* (2013) reported new developments in shockwave technology intended for meat tenderization. Paolo & Silvia (2017) also reported the use of electrical stimulation (ES) in meat production and improving meat quality. ES is a procedure that depends on electric current passing through hot carcass immediately after slaughtering. Shockwave technology, also known as hydrodynamic pressure processing, or underwater shockwave processing (USP), is carried out by the application of mechanical pressure pulses in a large water tank, find its way as an emerging new technology for improving food quality particularly for meat tenderization of tough cuts of meat, that technology is still facing some con-

strains for application in the meat industry (Thomas *et al.*, 2013, Isabella, 2018, Ying *et al.*, 2020).

Generally, meat tenderization is carried out mainly by either of a biological or a mechanical method. The mechanical tenderization depends on the breakage of the muscle structure by a mechanical mean, as grinding mincing or hammering. The biological methods are based on the effect of the endogenous and the exogenous proteases. Phyllis *et al.* (2006) reported that meat tenderness is generally associated with myofibrillar and connective tissue proteins. Connective tissue is dominated by collagen, of which the insoluble portion contributes to meat toughness. Elastin is also an extremely stable connective tissue. Therefore, commercially applicable methods to degrade collagen and elastin to an extent which produces the desired tenderness and texture would add value to cuts high in connective tissue. Proteolytic enzymes (or proteases) derived from tropical plants, such as papain, bromelain and ficin, have often been tested and used to break down connective tissues to enhance tenderness of meat and meat products. However, it is important that, upon reducing the amount of detectable connective tissues, extensive degradation of muscle fibers, should be avoided. Proteases play an important role in degrading the structural proteins in the connective tissues, thus reducing toughness of meat. Bacterial proteases are also used in meat tenderization (Arshad *et al.*, 2016). Bacterial proteases show effective proteolytic degradation of elastin and collagen, but have negligible or no effect in degrading myofibrillar proteins. The hydrolytic activity of bacterial enzymes in the collagen was found to be intermediate to the activities of papain and bromelain. Accordingly, Zhang *et al.* (2013) recommended that the combined use of plant and bacterial proteases may have synergistic effect on meat tenderization.

Konno *et al.* (2004) reported that papain as a cysteine protease in papaya latex, protects the trees from herbivorous insects. On the other hand; according to Bekhit *et al.* (2014) exogenous proteases as papain play an important role in meat tenderizers. Improving tenderness and accordingly the palatability of tough cuts of beef will increase the value of meat which will result in higher returns for producers. In the present study, partially purified papain, and partially purified oryzae protease preparations were prepared to investigate their use and effect as exogenous proteases on meat tenderi-

zation. That is to highlight the effect of these two proteases toward meat proteins and their impact on meat quality. Chemical, sensory, and histological assessments were suggested, using two cuts of meat muscles, to demonstrate the effect of the tested tenderizers. This enables the optimal conditions for tenderizing fresh meat, to be established on a small scale as in restaurants and even at home, with the elimination or reduction of any negative impacts on the other meat quality attributes.

MATERIALS AND METHODS

Oryzae protease: *Aspergillus oryzae* NRRL 1988, obtained from Northern Utilization Research and Development Division, USDA, was used for the preparation of partially purified fungal protease, according to El-Zalaki *et al.* (1974a&b).

Partially purified papain: The latex of papaya (*Carica papaya*) fruits was used for the preparation of partially purified papain, according to El-Zalaki (2021).

Tenderizer mixture: Partially purified papain and Oryzae protease, containing 430 and 50 enzyme units/g, respectively were mixed in a ratio of 10 : 1 by weight. The mixture was then mixed, in a proportion of 4% by weight, with rice starch, as a filling material.

Meat cuts: Two different muscles of beef meat; namely, the bottom round (*Biceps femoris*), and the sirloin (*Longissimus dorsi*) were taken from old aged animal after 24 hr of slaughter.

Proteolytic activity: The proteolytic activity and the enzyme units was measured by Anson's method according to Greenberg (1955) and Petrova & Vintsyunaite (1966). Where, one enzyme unit being the amount of enzyme that produces in one minute, at pH 7.5 and a temperature of 35°C products of hydrolysis containing one microequivalent of tyrosine not perceptible with trichloroacetic acid.

Trichloroacetic acid (TCA) soluble nitrogen: The colour reaction of the digestion products (TCA- soluble nitrogen), expressed as tyrosine, was measured at a wave length of 650 millimicrone, using the Folin-Ciocalteu reagent, according to McDonald & Chen (1965).

Enzymatic meat tenderization: The present study suggested the use of meat slices of approximately 1cm thickness, 4 cm width, and 6 cm length, which were incised on each surface. The meat slices

were soaked for five minutes in a minimum amount of an aqueous enzyme solution, containing 0.0025 to 0.04% of the tenderizing enzymes mixture, then left to drain for few seconds. Control meat slices were dipped in water.

Meat freezing: Some slices of the enzyme treated bottom round were wrapped in aluminum foil, frozen at -15°C and stored for two weeks at -5°C . The slices were thawed for 1 hr at room temperature before cooking and then subjected to a taste testing panel. That treatment was suggested in the present study to investigate the effect of the freezing storage conditions can affect the tenderization of the cooked meat slices.

Meat cooking: The drained meat slices were wrapped in aluminum foil thinly greased with hydrogenated oil, cooked to a proper doneness in an electric oven at 150°C , for 45 and 60 minutes in case of the sirloin and the bottom round, respectively.

Taste panel: Five trained panelists were asked to score in a hedonic scale for flavour and tenderness, indicating whether the samples of the low scores are tough or mushy (Kramer & Bernard, 1962).

Histological sections: Each of the partially purified papain and oryzae protease, as well as a mixture of the two preparations were used for the histological study. Meat slices of 0.5cm thickness, of each of the two muscles, were soaked, in an aqueous enzyme solution of a concentration of 4%, for 30 minutes. The control samples were also prepared. The histological study was carried out on uncooked and cooked meat. Fixation, dehydration,

clearing, impregnation with paraffin wax embedding, section cutting, mounting on slides, staining with Weigert's iron Haematoxylin, and counter staining with Van- Gieson, were carried out according to Gatenby & Beams (1950) and Baker *et. al.* (1957).

RESULTS AND DISCUSSION

Enzyme concentration: The tenderized cooked meat samples were submitted to a taste panel for assessing tenderness and flavour of the two meat cuts. The ranks of acceptance as assigned by panelists scores are presented in Table (1), where the highest ranks of round cut (*B. femoris*) and sirloin (*L. dorsi*) were for the meat slices treated by tenderizer concentration of 0.005 and 0.0025%, respectively. The effect of enzymes was more pronounced for tenderness scores, with no bad impact for the flavour scores, as compared with the control. Generally, the results revealed that the amount and the source of the used enzyme, must be selected to avoid mushiness due to over tenderization of the meat. This is in accordance with Dransfield (1994). The proper enzyme concentration depends on the meat cut, the method of applying the enzyme, the method of cooking and consumer preference. Individual meat muscles responded differently to the used proteolytic enzymes. Shand *et. al.* (2006) reported that the enzyme-treated meat showed a gradual reduction in shear force with an increase of enzyme concentration. That was due to the effect of papain and proteases on improving the meat quality, as a result of the biochemical changes of the tenderized meat (Andersons *et. al.* 2012, Akpan *et. al.* 2015, Abdel-Naeem & Mohamed, 2016).

Table 1: Effect of enzyme concentration on tenderness and flavour scores* of panelists for two cuts of cooked tenderized meat

| Enzyme concentration % | B. femoris scores | | L. dorsi scores | |
|------------------------|-------------------|-------------|-----------------|-------------|
| | Tenderness** | Flavour** | Tenderness | Flavour |
| | Mean (Rank) | Mean (Rank) | Mean (Rank) | Mean (Rank) |
| 0.000 | 5.0 (3) | 6.7 (2) | 5.1(3) | 6.3 (2) |
| 0.0025 | 6.5 (2) | 6.9 (2) | 7.3(1) | 7.4(1) |
| 0.005 | 7.8(1) | 7.4(1) | 6.7 (2) | 7.1 (1) |
| 0.010 | 4.4 (4) | 5.0 (3) | 4.2 (4) | 3.7 (3) |
| 0.020 | 2.5 (5) | 2.1 (4) | 3.0 (5) | 3.2 (4) |
| 0.040 | 1.3 (6) | 1.1 (5) | 0.7 (6) | 0.4 (5) |

*Mean of 4 replicates.

** The scores for the stored frozen *B. femoris* showed the same rank after cooking.

On the other hand, the storage period had no effect on the sensory attributes of the meat. The frozen stored bottom round enzyme treated meat slices, have the same ranks as the directly cooked slices, as shown in Table (1). Yet, freezing the enzyme-treated slices shortened the cooking time from 60 min to be 45 min, after thawing the steaks at room temperature. According to Weir *et. al.* (1958), papain effect on meat tenderness was more pronounced when the steaks were frozen and allowed to thaw at room temperature before cooking.

Trichloroacetic acid (TCA) soluble nitrogen: The results illustrated in Fig (1), indicate that the TCA- soluble nitrogen, of the two cooked meat cuts, increased as the enzyme concentration increased. That was due to the effect of papain and oryzae-protease, in the tenderizer mixture, on meat proteins. Papain has specificity for amino acids with aromatic side chains such as phenylalanine and tyrosine (Berger & Schechter, 1970).

The statistical analysis for the means of the TCA-soluble nitrogen of the control and the en-

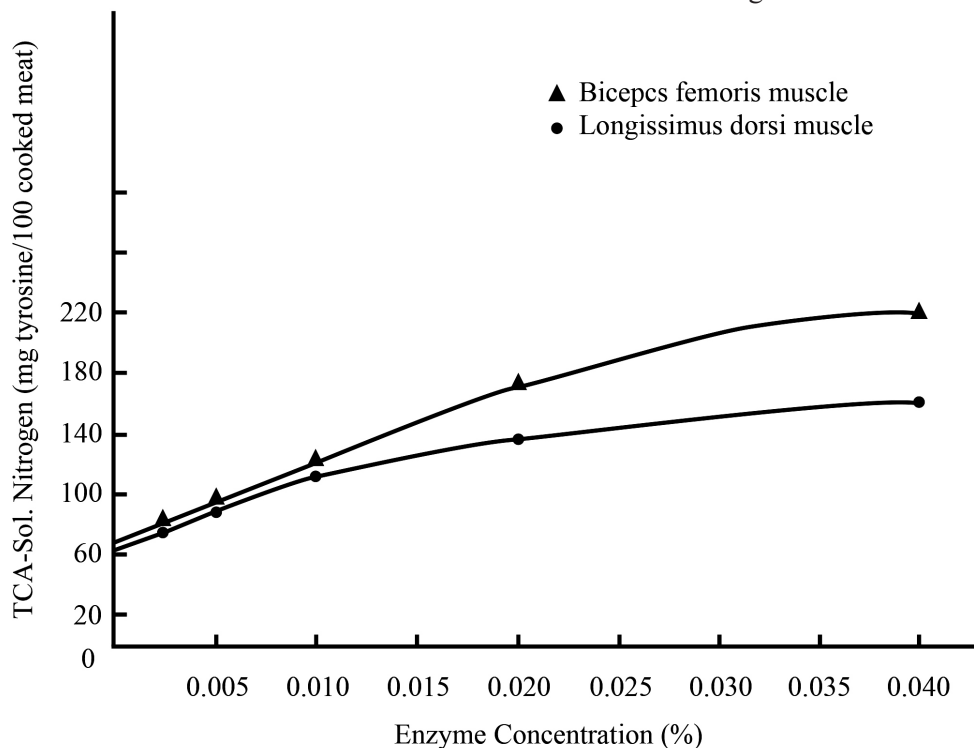


Fig. 1: TCA-soluble nitrogen in two cooked meat muscles treated with different concentrations of tenderizing enzymes

zyme treated two meat cuts using t-test in pairs (Lijerome 1957), indicated a highly significant difference between the two groups, as presented in Table (2). The increase of the TCA-soluble nitrogen, associated with the increment of the enzymes

concentration, is not an absolute indicator of good meat quality. That is obviously shown in the repulsive of meat, as indicated by the very low scores and least ranks as shown in Table (1) due to the mushiness of the cooked meat.

Table 2: TCA-soluble nitrogen (mg tyrosine/100) of two cooked meat cuts

| Cut | <i>B. femoris</i> | <i>L. dorsi</i> |
|----------------------|-------------------|-----------------|
| Control sample range | 58-62 | 52-66 |
| Mean | 59.8 | 58.8 |
| Enzyme treated range | 142-185 | 102-116 |
| Mean | 150.0 | 111.6 |
| T | 34.2** | 19.98** |

Degree of freedom 4

The results of the statistical analysis presented in Table (3) indicate that a positive correlation coefficient (r^*) exists between TCA-soluble nitrogen (mg tyrosine /100) and tenderness scores of cooked *B. femoris* meat cut, treated with the proper enzyme concentration of 0.005%.

Ashie *et. al.* (2002) used papain and microbial proteases for meat tenderization. Calkin & Sullivan (2007) stated that papain, at optimum temperature of 65–75°C, was found to be excellent in hydrolysis of myofibrillar proteins, but moderate in hydrolysis

Table 3: Correlation between TCA- soluble nitrogen (mg tyrosine/100) and tenderness scores of cooked *B. femoris* meat cuts

| Control | <i>B. femoris</i> | <i>L. dorsi</i> |
|------------------------------|-------------------|-----------------|
| TCA- soluble nitrogen (mean) | 58-62 | (59.8) |
| Tenderness score (mean) | 3.8-4.7 | (4.2) |
| Correlation coefficient r* | 0.320 | |
| Treated Enzyme | | |
| TCA- soluble nitrogen (mean) | 142-158 | (150) |
| Tenderness score (mean) | 6.5 -8.0 | (7.5) |
| Correlation coefficient r* | 0.784 | |

* Degree of freedom 4.

of collagen. On the other hand *Aspergillus* and *Bacillus* proteases at temperature of 55– 60°C, were poor and excellent, respectively, for hydrolysis of collagen. They recommended that the combined use of plant and microbial proteases may have synergistic effect on meat tenderization. Exogenous enzymatic tenderization, is considered a progressive advanced mean to improve meat tenderness and overall quality. Five proteolytic enzymes, of various sources, have been approved by the USDA as GRAS for use in the meat industry (FDA, 2001).

Histological effects: Each of the partially purified papain and oryzae protease, as well as a

mixture of the two preparations was used for the histological study. A high concentration of 4 % of the papain-oryzae protease tenderizer mixer was used, in order to demonstrate even the minor effect of the enzymatic tenderization on the histological structure of the two cuts of meat. The longitudinal and the cross sections of the *B. femoris* muscle of the raw uncooked meat, are illustrated in Fig (2) and (3), respectively, showing the muscle nuclei as black dots, and a thin net collagenous fibers of the sarcolemma which envelop fibers of the longitudinal section.

Treatment of the same cut with oryzae protease followed by cooking, resulted in cracks, breaks, and degradation of the muscle structure, particularly the muscle fibers, rather than the connective tissues, as illustrated in Fig (4).

The effect of papain treatment on the structure of the raw cut of the bottom round, illustrated in Fig (5), indicates that, the effect of papain was more pronounced than that of the fungal protease, as most of the connective tissues degraded, with the remainder appearing in the form of black spots. That was associated with degradation of the muscle fibers, the sarcolemma and the muscle nuclei.

The effect of papain treatment was more obviously, on both of the muscle fibers and the connective tissues, upon cooking as shown in Fig (6).

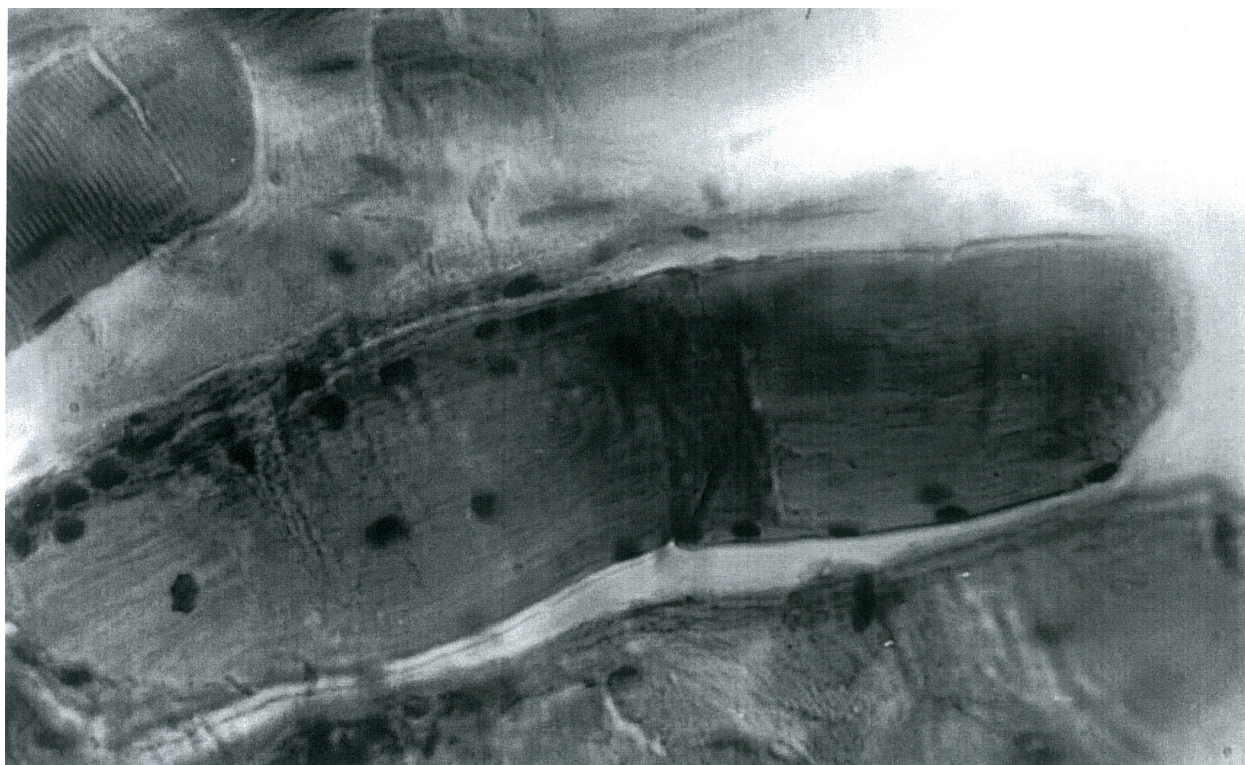


Fig. 2: Longitudinal section of *B. femoris* muscle of the raw uncooked meat

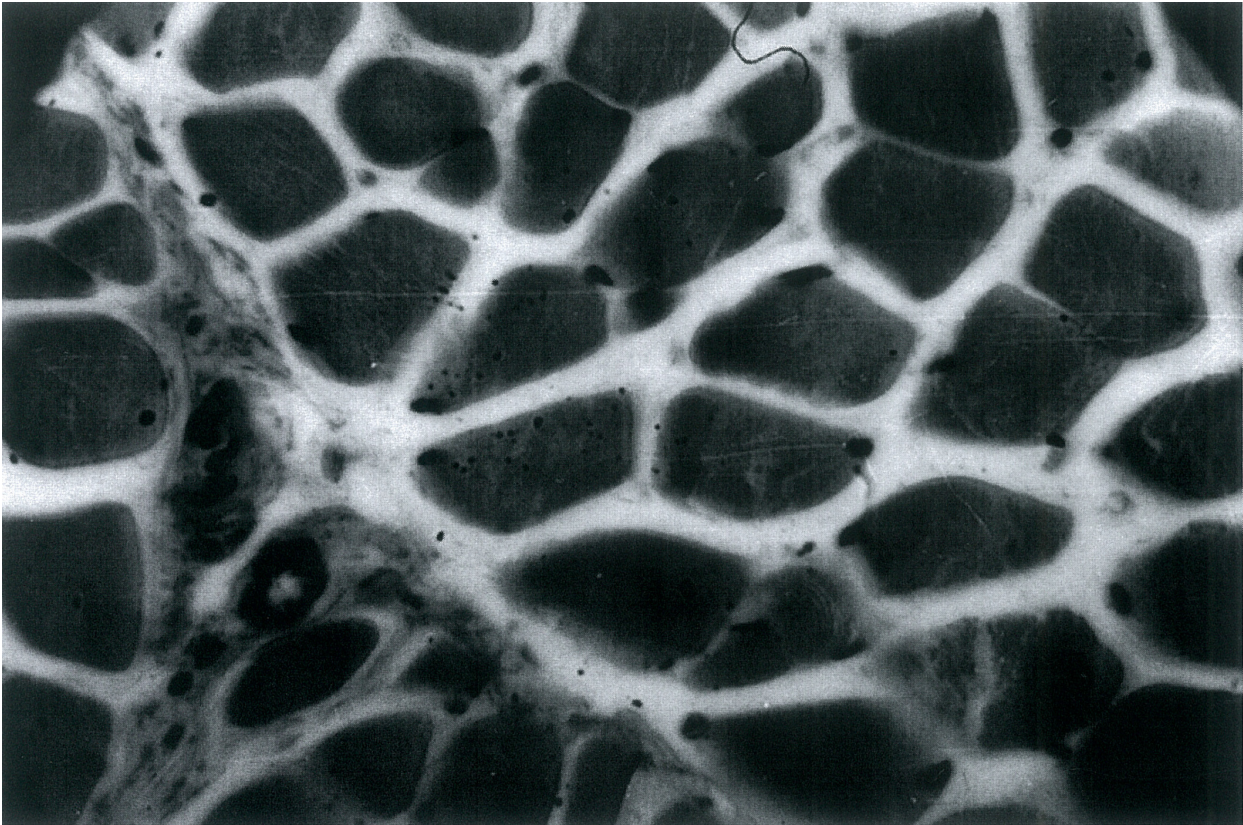


Fig. 3: Cross section of *B. femoris* muscle of the raw uncooked meat



Fig. 4: Cross section of oryzae protease treated cooked *Biceps femoris* muscle.

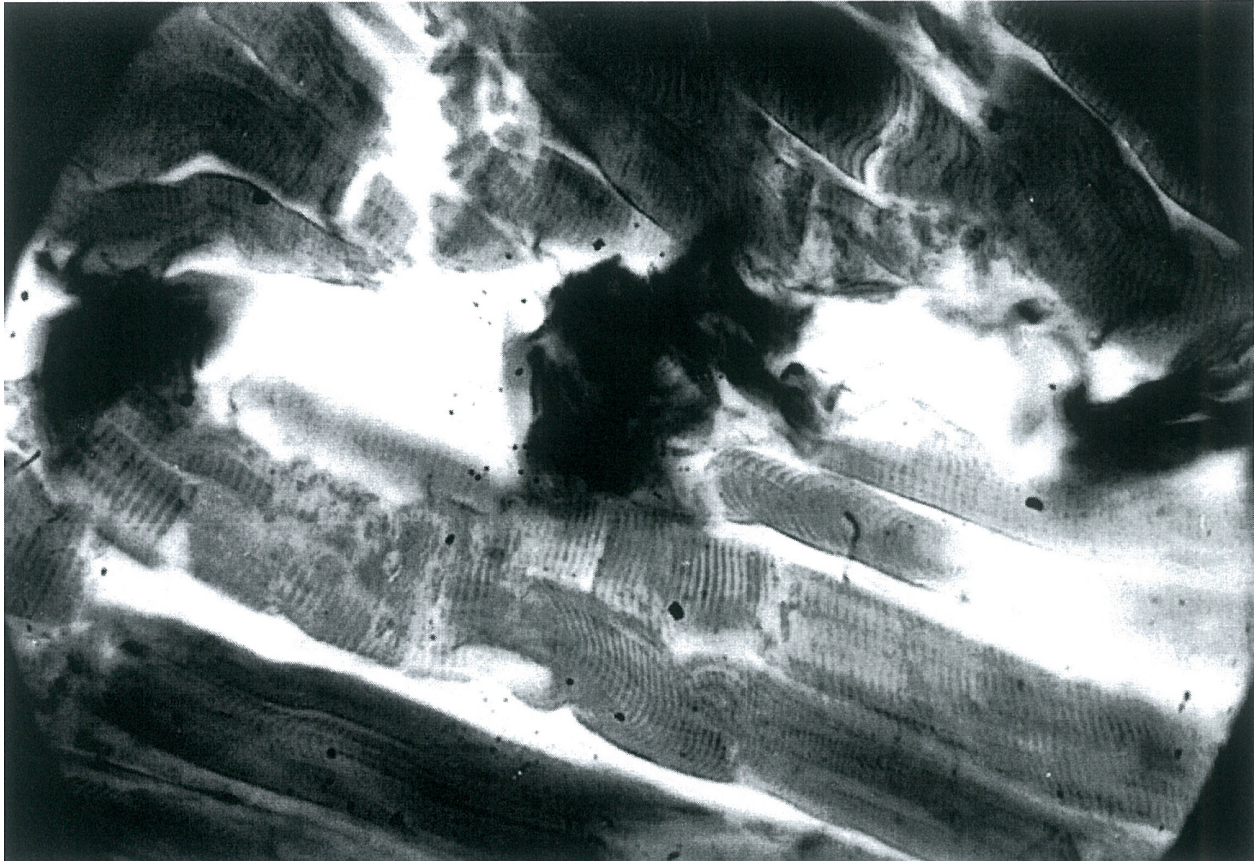


Fig. 5: Enlargement of longitudinal section of papain treated raw *Biceps femoris* muscle

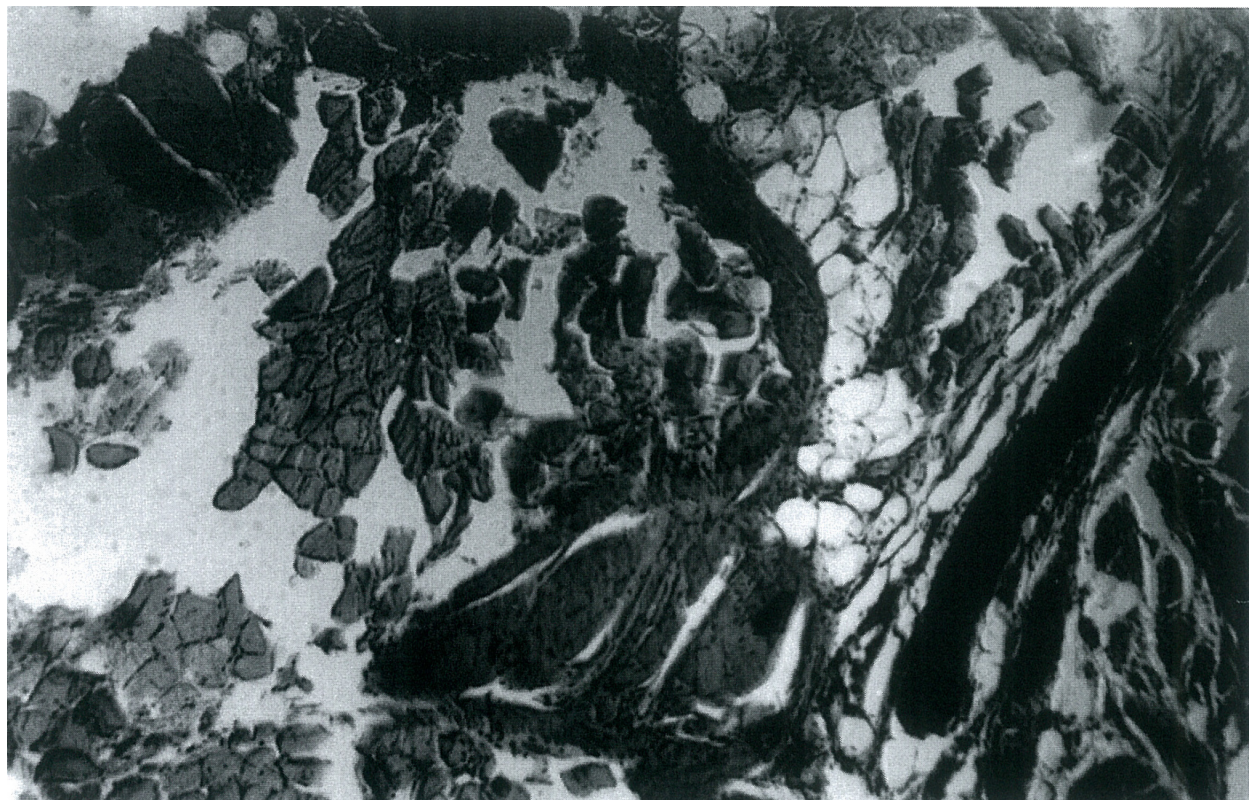


Fig. 6: Cross section of papain treated cooked *Biceps femoris* muscle

It is worthy to indicate that the *L. dorsi* as a relatively more tender cut as compared with the *B. moris*, and accordingly the muscle fibers, but not the connective tissues, are markedly affected by cooking, without the enzymatic treatment, as illustrated in Fig (7).

On the other hand, the effect of the enzyme mixture on the cooked two cuts of meat, resulted in extensive effects. Segmentation and degradation of both of the muscle fibers and the connective tissues were achieved as shown in Fig (8) and Fig (9). The Cross section of papain and oryzae protease treated

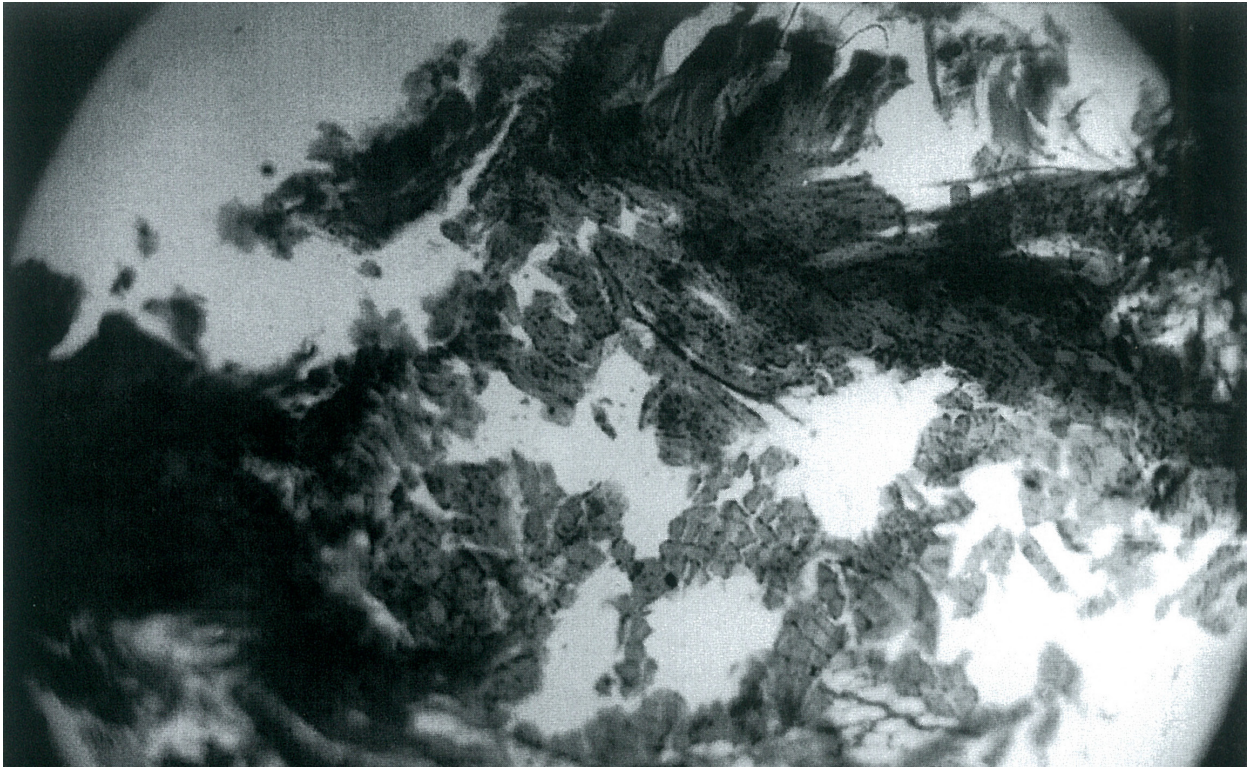


Fig. 7: Cross section of non-enzymatically treated cooked beef *L. dorsi*

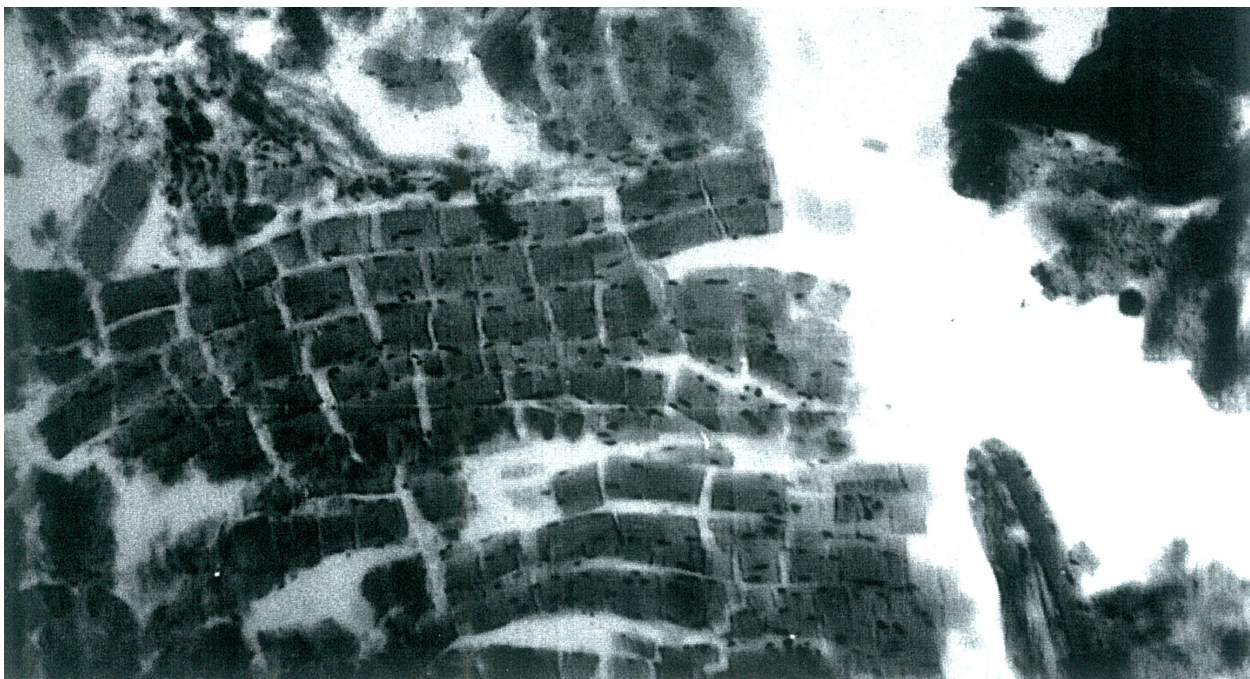


Fig. 8: Longitudinal section of papain and oryzae protease treated cooked beef *B. femoris*

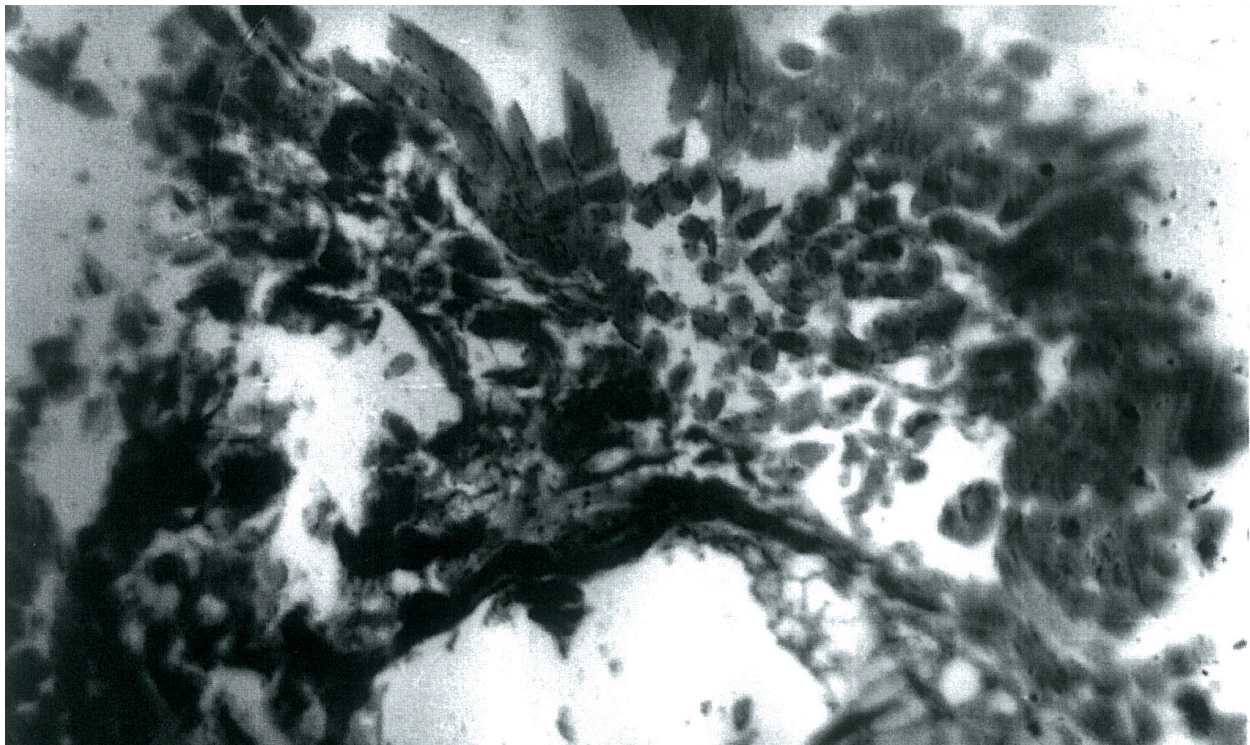


Fig. 9: Cross section of papain and oryzae protease treated cooked beef *L. dors*

cooked beef *L. dors* illustrated in Fig (9) showed that the effect of the enzyme followed by cooking markedly affect the muscle structure as compared with the effect of cooking , without the enzymes treatment, previously presented in Fig (7).

In conclusion, the histological results of the present study, revealed that the degradation of the muscle fibers was a common property associated with treating the two cuts of meat with either of papain or oryzae proteases. The effect was more pronounced upon the treatment with a mixture of the two partially purified preparations; namely, papain and oryzae proteases. The effect was markedly obvious in case of papain particularly after cooking. The sarcolemma was attacked and disintegrated, the nuclei were also faded away and disappeared in most areas.

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تقييم حسي وكيمائي وتشريحي لجودة لحم مطري بالبابيين وبروتيز الأوريزا

عصمت صابر الزلاقي

قسم علوم وتقنية الأغذية، كلية الزراعة (الشاطبي)، جامعة الإسكندرية.

تم تحضير مخلوط تطرية يتكون من خلط مستحضر منقي جزئياً من البابيين وبروتيز الأوريزا، اللذين يحتويان على ٤٣٠، ٥٠ وحدة إنزيم/جم على التوالي، بنسبة ١: ١٠. استخدم هذا المخلوط في تطرية قطعتين من اللحم البقري هما التليبيانكو (Bicepsfemoris) و bottom round (الإنتر كوت - Longissimus) Sirloin (dorsi) لحيوان كبير العمر بعد ٢٤ من الذبح. أظهرت نتائج التقييم الحسي تحسناً معنوياً إحصائياً في الطراوة مع عدم وجود تغيير في النكهة. كانت هناك علاقة طردية بين زيادة تركيز الإنزيمات ومحتوى النيتروجين الذائب في ثلاثي حامض الخليك TCA-soluble nitrogen. ذلك مع التأكيد على تحديد التركيز المناسب من المطري الإنزيمي لتلافي الخشونة toughness أو الطراوة الزائدة mushiness للحم حيث أن كلا منهما غير مرغوب. لم يكن لعملية التجميد قبل الطبخ تأثير يذكر فيما عدا خفض زمن الطبخ. تطلبت دراسة التركيب النسيجي استخدام تركيز عال من الإنزيم المطري وهو ٤٪ عن ذلك اللازم للتطرية المرغوبة (وهو ٠,٠٠٢٥، ٠,٠٠٥)، حتى يتسنى إظهار تأثير الإنزيم على تركيب النسيج، بما في ذلك أقل تأثير يمكن رصده في الألياف، الحزم، الأنسجة الضامة. واتضح تباين تأثير المستحضر المطري الإنزيمي، خاصة البابيين مع عملية الطبخ.