EFFICACY OF TARIN FROM COLOCASIA ESCULENTA (L.) SCHOTT ON THE HISTOLOGICAL CHANGES OF BUFFALO MEAT (BUBALUS BUBALIS L)

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ABSTRACT

The protease activity of tarin from corms of taro or 'gabi' was studied using the semitendinosus muscle from three different ages of buffalo. Corms of Colocasia were reported to contain tarin or G1 globulin, a second major storage protein fraction, which accounts for the proteolytic activity. The experiment was conducted to determine histological changes in muscle tissue of tarin-treated fresh buffalo meat and the specific site of action of crude tarin on fresh buffalo meat. Crude tarin extract has noticeably higher proteolytic activity ($8.5988x10^{-03}$) than pronase ($6.4060x10^{-03}$) per ml substrate. The optimum amount of crude tarin extract that can exhibit proteolytic activity was observed to be about 25 ml per 100 g of fresh meat.

Scanning electron microscopy revealed that the microstructure of tarin-treated fresh buffalo meat was affected by the proteolytic activity of tarin. Evidently, myofibril degradation and collagen denaturation occurred as early as 30 minutes incubation of meat at room temperature. The breakdown of the connective tissue protein was most effective at extended incubation period using the optimum amount of crude tarin extract. The results of this investigation showed the effectiveness of this alternative microbial protease.

Keywords: buffalo meat, tarin, histological changes, *semitendinosus* muscle.

INTRODUCTION:

Proteases used as meat tenderizers are found in tropical plants such as papaya and pineapple. Commercial proteases are often in the form of freeze-dried powder (Hagar, 1998). Enzymes occur naturally in foods and many of the traditional food processing technologies involve the use of enzymes in bread making, as meat tenderizers and in cheese manufacture. With the advances in food science today, these enzymes can be extracted, concentrated and added to foods during processing. In fact, Enzymes that are important in meat tenderization are now widely accepted by the food industry as well as by housewives.

Taro[*Colocasia esculenta* (L.) Schott] locally known as Gabi, is known for its edible corms and leaves, as well as for its traditional uses. Studies revealed that the enlarged, starchy underground stems which are properly designated as corms contain proteolytic enzymes. The elucidation of this plant material toward protein degradation is of interest because of possible biotechnology applications. Researches have shown that the enzymes important in the tenderization of meat are those having the ability to breakdown the connective tissue proteins as well as the proteins of the muscle fibers rendering the meat tender.

As an alternative to the existing commercial meat tenderizers hence, this study was conducted to discover the efficacy of tarin from corms of Gabi on the histological changes on tough muscles of buffalo meat and the specific site of action of crude tarin on fresh buffalo meat. Consequently, the potential of tarin as tenderizer and trypsin inhibitor is deemed beneficial to smallhold farmers and processors. Taro extract was utilized as the source of proteolytic enzyme in this experiment.

MATERIALS AND METHODS:

PLANT MATERIAL AS SOURCE OF ENZYME:

In this experiment, locally grown variety of taro or gabi (Fig. 7), popularly known as “binting dalaga” in Camarines Norte was utilized as the plant source of proteolytic enzymes. Crude taro enzymes were extracted from the enlarged stem designated as corm.

Figure 7. Taro plant with the fleshy corm and stem

PROTEIN EXTRACTION FROM CORMS OF TARO:

Enlarged stem or corm of taro was peeled, shredded and weighed. The tissue was suspended in a homogenization buffer using 100 g tissue per 200 ml phosphate buffer in a Waring blender at a medium speed for 20 seconds and then strained using cheesecloth. The crude taro extract was centrifuged for 30 minutes at 3000 rpm to separate the starch. It was further filtered to get a clear supernatant which served as the source of crude enzyme (tarin) used in this experiment. Protein extraction methods were adapted and modified from that illustrated by Lu and Levin (1978); Necessary et al., (1985); Srisung (2005)

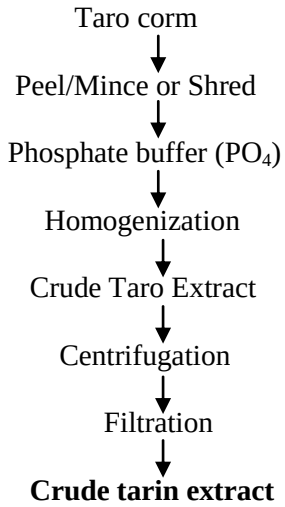


Figure 2. Flow diagram for extraction of tarin from corms of taro

DETERMINATION OF PROTEASE ACTIVITY OF CRUDE TARIN EXTRACT:

The assay methods used to determine the protease activity of crude tarin extract was modified from Jayaraman (1987) and EDC (1979). The technique was based on the science of absorption of light known as spectrophotometry. To determine the activity of tarin, the crude tarin extract, was subjected to a test to determine its protease activity as described by Dixon and Webb (1979) and Jayaraman (1987). Pronase is a protease enzyme from *Streptomyces griseus* that is commonly used in as reference material assay methods. The pronase activity served as the control from which the tarin activity from crude tarin extract was compared to. The two enzyme sources (crude extract and pronase) contain .5 ml sample mixed with .75 ml Hammerstein casein solution, and .75 ml phosphate buffer. This mixture was incubated for 1 hour at 37 ^{0}C. Then, 2.0 ml of .44 M trichloroacetic acid (TCA) was added to each treatment to stop the reaction. Samples were centrifuged, then, filtered to separate the undigested casein. The amino acids liberated in the supernatant were then measured using a spectrophotometer where optical density at 280 nm was read. The enzyme activity (EA) was defined as the amount of enzyme (International Unit, IU) which transform the reading at 280 nm and is equivalent to 1µg tyrosine per minute at 37 ^{0}C.

DETERMINATION OF HISTOLOGICAL CHANGES ON FRESH: BUFFALO MEAT AS AFFECTED BY CRUDE TARIN EXTRACT:

Preparation of meat samples. The dressed carcass from a male Bulgarian Murrah with an age of 7 years and body weight of 590 kilograms was chilled at a temperature of 2-4 ^{0}C for 36-48 hours to let rigor mortis pass prior to experiment. The *semimembranosus* of the round muscle from both sides of the chilled carcass were taken as samples. Extra fat, bone dust and connective tissue were removed from meat samples. Three (3) meat slices with 38.1 mm thickness were assigned to treatment 1 (control or untreated), treatment 2 (30 minutes incubation) and treatment 3 (60 minutes incubation), respectively. The control treatment used only distilled water, while the other two treatments were injected with crude tarin extract..

Experimental treatment: Each treatment has 3 replicates.

Treatment 1 Control
Treatment 2 30 minutes incubation with crude tarin
Treatment 3 60 minutes incubation with crude tarin

The amount of crude tarin extract injected was based from the results of the preliminary study which was 25% of the fresh weight of buffalo meat. The application of crude tarin extract was done by injecting on both sides of the meat sample using a syringe (Figure 3).

Figure 3. Injection of crude tarin extract in fresh buffalo mea

Electron microscopy examination was done using the following preparations: the treated meat samples were held at room temperature for 30 and 60 minutes incubation periods, respectively (Figure 11). The control treatment was also incubated for 60 minutes. The samples were then washed with phosphate buffer and fixed with 3% glutaraldehyde for two days. Preparation of meat samples for histological analyses was based on the standard procedures set by Electron Microscopy Service Laboratory (ESML). The crude tarin-treated meat samples were examined using the SEM Hitachi Model S-510 at the Electron Microscopy Service Laboratory at the UPLB-BIOTECH.

RESULTS AND DISCUSSION

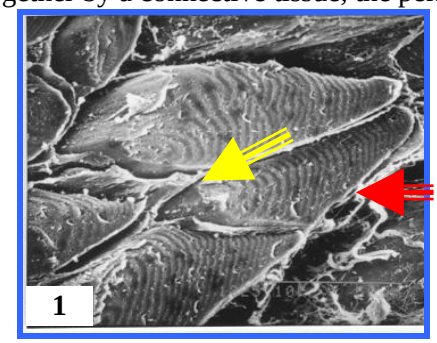
DETERMINATION OF PROTEASE ACTIVITY OF CRUDE TARIN EXTRACT:

The results of the spectrophotometric assay showed that, crude tarin activity (8.5 unit per ml) was higher than that of the enzyme standard used, pronase (6.4 unit per ml). Related studies on plant proteases showed that these proteolytic enzymes digest the suspended particles of proteins making the mixture transparent. The amount of ultraviolet light absorbed by the solution is related to the number of tyrosine units produced by the enzymes from crude extracts. Hence, the greater this number, the greater is the activity of the enzymes in crude extracts as described by Dixon and Webb (1979). Crude tarin extract also manifested greater sensitivity at lower pH.

HISTOLOGICAL CHANGES IN FRESH MEAT: TREATED WITH CRUDE TARIN EXTRACT:

There were three (3) sample images recorded, each at magnification 800 x in order to obtain a more precise observation of the microstructure of every sample. Results of the scanning electron microscopy (SEM) on the histological changes in the microstructure of muscle fiber of buffalo meat were presented in Figures 4, 5, and 6**.** The integral muscle bundle is surrounded by connective tissue sheaths called the perimysium enveloping each fascicle and the endomysium which envelopes the myofibril. The representative electron micrograph images of the cross and longitudinal section of *semitendinosus* muscle showed the effects of crude tarin activity on the muscle tissue of fresh buffalo meat.

The size of the muscle fiber determines the texture of the muscle (Hammond, 1932 a; Walls 1960). Similarly, the relative proportions of muscle fiber and connective tissue account for a relative toughness of meat. The cross section of the control or untreated buffalo meat sample (Figure 4-1) showed that there was no alteration on the microstructure of muscle fiber. The longitudinal section (Figure 4-2) on the otherhand, showed the myofibrils still being intact or held together by a connective tissue, the perimysium.



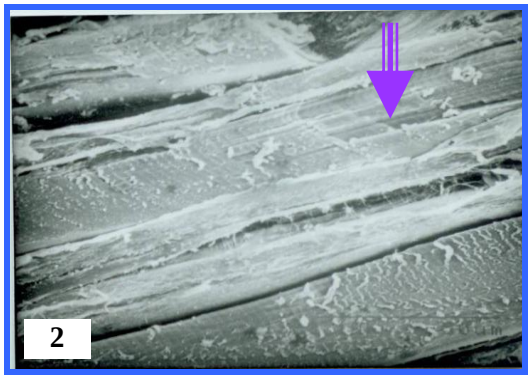


Figure 4. Scanning electron microscope images of untreated

buffalo meat (*semitendinosus* muscle); (1) cross section and (2) longitudinal section 800x. Shown were intact fascicle ( ) and visible endomysium ( ) and perimysium ( )

The results of the scanning electron micrograph of crude tarin-treated buffalo muscle (*semitendinosus*) which were incubated for 30 minutes at room temperature are reflected in Figures 5-1,2) . Some disintegration on the lateral attachment of myofibril to the sarcolemma and the gaps between fascicle due to degradation of perimysial connective tissue were very noticeable in the cross section view. This gap demonstrated a very evident lost of perimysial connective tissue. It was also observed that the enzyme tarin present in crude extract had partly disintegrated the myofibrils as shown in the longitudinal section of the electron microscope image. This observation was not explainable on the basis of the distribution of crude tarin extract on muscles, since there may not be evenly distributed across the muscle fibers, although, it was consistent with other electron microscopic studies.

Studies of Srisung (2005) confirmed that crude papain and crude bromelain treated samples have also partly degraded perimysium and endomysium. Moreover, the muscle fibers of buffalo meat treated with papain and bromelain have partly broken the myofibrils. Moreso, the study of Kim and Taub (1991) revealed that papain digested both myosin and actin with almost equal efficiency unlike bromelain which only breakdown the myosin with actin remaining intact.

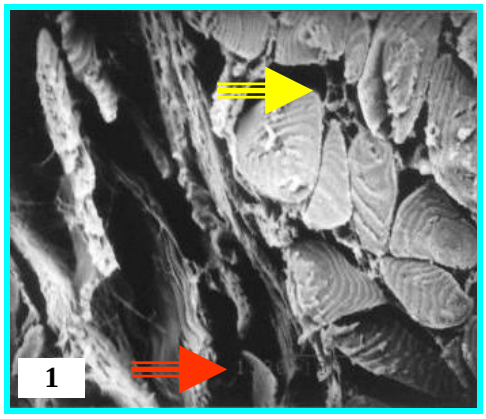
Similarly, bacterial and fungal enzymes have been used in tenderizing meat. According to study, these proteolytic enzymes act only on the proteins of the muscle fiber. They first digest the sarcolemma, causing the disappearance of muscle nuclei, and then degrade the muscle fiber, and eventually causing the loss of cross-striations.

On the otherhand, the action of the proteolytic enzymes of plant origin was preferentially acted against the connective tissue fiber. They first break up the mucopolysaccharide of the ground substance matrix, and then, progressively reduce the connective tissue fibers to an amorphous mass as disclosed by Partridge (1959). He further stressed that these enzymes do not attack native collagen: they act upon the collagen as it is denatured by heat during cooking.

Unlike the tenderizing changes during conditioning, the artificial tenderizing enzymes breakdown connective tissue proteins to soluble substance, hydroxyproline-containing molecule.

Sodium chloride (NaCl) itself, and other salts, have a tenderizing action on meat but not considerable (Wang et al., 1958). Some of these effects are due to an enhanced waterholding capacity, either direct or, as in the case of phosphate, through a concomitant raising of the pH (Bendall, 1954).

The different studies that were cited corroborates with the results of the earlier experiment on crude tarin activity. That tarin present in crude taro extracts also acts on the connective tissue and myofibrils as well as observed in the electron microscopy images.



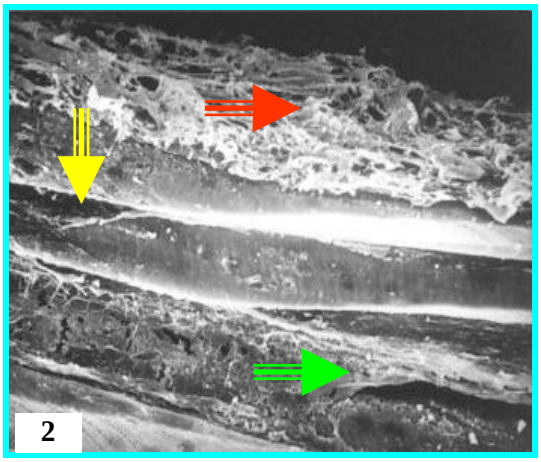


Figure 5. Cross - (1) and longitudinal (2) sections (800x)

of electron microscope images of crude tarin-treated (30 mins incubation) *semitendinosus* muscle of buffalo meat. Degraded endomysium( ), perimysium ( ) and fragmented myofibril ( )

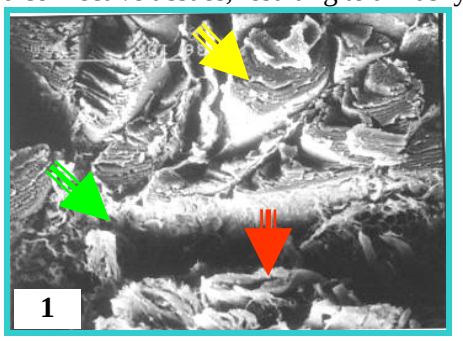
Shown in Figure 6-1,2 were the cross section and longitudinal section 800x of crude tarin-treated fresh buffalo meat incubated for 60 minutes. A very wide gap between and within fascicle in the cross section view was very visible. Likewise, crude tarin-treated sample shows fragmented and slightly ruptured myofibril. Similarly, the longitudinal section (Fig. 6-2) of the electron micrograph image attested that crude tarin caused complete degradation of myofibril. As incubation time increased from 30 to 60 minutes, gaps also increased considerably which is evidence of the degradation of epimysial and endomysial connective tissues and myofibrils as well. In general, gaps created by the action of crude tarin had a significant correlation with shear force value ($p < 0.05$) based on the results of this study.

Some discontinues areas were also detected and the architecture of sarcolemma had deteriorated in both 30 and 60 minutes incubation period. These results only confirmed that crude tarin binds and interacts in the muscle proteins to bind water which affect meat tenderness and juiciness.

These histological changes in the muscle fibers were consistent with the results of the several experiments on aging and in using papain and bromelain for tenderization. Degradation of muscle fibers by crude taro is to some extent similar to the findings of Srisung (2005) on the effects of crude papain and bromelain on the tenderness of loin muscle of buffalo meat.

Results of the electron microscope images showed that the structural disintegration of the myofibril protein and connective tissue (collagen) of *semitendinosus* muscle of buffalo meat were affected by proteolytic activity of crude tarin. This suggests that tarin in the crude extracts had the ability to hydrolyze and breakdown the soluble beef proteins as well as the connective tissue, similar to the action of commercial tenderizing enzymes thereby rendering the meat more tender.

Researches have shown that the enzymes important in the tenderization of meat are those having the ability to breakdown the collagen and muscle fiber proteins. As a result, the meat becomes tender (Tappel et al., 1956) which corroborates to the findings in this experiment A very noteworthy observation in this study was that, tarin, can tenderize muscle proteins even at room temperature. This breakdown of muscle protein is most effective at an extended time of incubation with a known amount of crude tarin extract. Based from the preliminary results of the SEM, the myofibrils were not greatly damaged, distinctly different from the action of papain, wherein papain completely degraded the myofibrils and connective tissues, resulting to a mushy texture of treated meat.



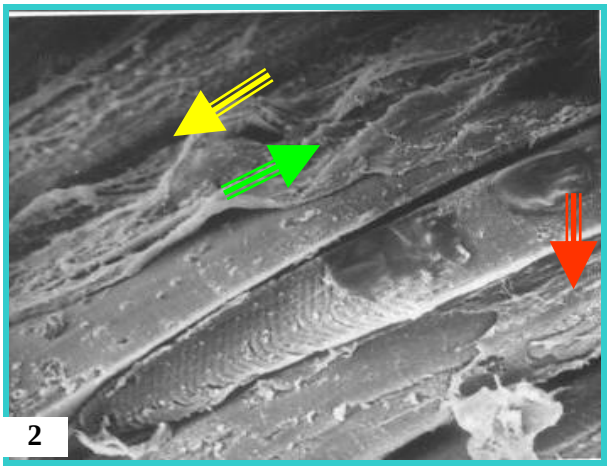


Figure 6. Cross - (1) and longitudinal (2) sections of electron microscope images of crude taro-treated (60 mins incubation) *semitendinosus* muscle of buffalo meat (800x). Shown were the disintegration of the attachment between and within the myofibrils (cross section), disintegrated myofibril (↓), degraded endomysial connective tissue (↓) and perimysial connective tissue (↓).

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