HISTOLOGICAL STRUCTURE OF BEEF SAUSAGE AND CHICKEN PATTIES MANUFACTURED WITH AND WITHOUT PROTEASE INHIBITOR.

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SUMMARY

Beef sausage and chicken patties were manufactured with or without protease inhibitor. The added level of protease inhibitor was 1,2 and 3% out of the recipe ingredients. Inspection of the samples by transmission electron microscope (TEM) was used to compare the hisotological structure of the investigated samples. On the other hand, changes that occur in the structure of beef sausage and chicken patties which manufactured with or without the addition of protease inhibitor were histologically studied by transmission electron microscpe during storage at 4°C for two weeks.

INTRODUCTION

It is well known that the fibres (unit of muscle structure) are long, narrow,multinucleated cells which may stretch from one end of the muscle to the other and may attain a length of 34 cm; although they are only 10-100 μ in diameter (Walls, 1960). Any given muscle contained fibres of varying diameter, the smaller ones being more peripheral and the large ones are more central in their distribution. Surrounding each fibre, and underneath the connective tissue of the endomysium, is a sheath of the sarcolemma, (Robertson, 1957), and the muscle cell nuclei are generally found just beneath the sarcolemma. Within the sarcolemma; the myofibrils are located and surrounded by a fluid phase known as a sarcoplasm; in which a certain structure is formed i.e. mitochondria or sarcosomes. The sarcoplasmic lipid bodies and the sarcoplasmic reticulum or sarcotubular system as well as a dissolved or suspended substances are also identified within the previous structure; (Bennett, 1960). An understanding of the chemical and physical structure of the muscles in meat and meat products as well as the changes that take place as a result of specific technological treatments could be based on histological analysis. Subsequently, and from such point of view, such study is carried out to answer the question; to what extent the histological structure based on transmission electron microscope of beef sausage and chicken patties that manufactured with or without protease inhibitor could be altered during storage at 4°C for two weeks.

MATERIALS AND METHODS

A. Materials:

** Beef meat, and chicken samples: Beef meat was obtained from "RAMADA"refrigerator of the "GERCO" Company; related to the Ministry of supplies, EGYPT. "EL SHARK EL AWSAT"Company that located in "KALYOBIAH" governorate (EGYPT) is the source of frozen chicken samples; with an individual weight that ranged between 850 up to 1100 gm.

** Potato tubers (alpha variety): The Horticulture Department, Agricultural Research Centre, Ministry of Agriculture is the source of potato tubers (alpha variety) that used for extracting of protease inhibitor.

B. Methods

* Technological methods: Extraction of protease inhibitor was performed by the method described by Belitz et al. (1971).

* Manufacturing of beef sausage and chicken patties: The formula of beef both sausage or chicken patties was based on using the previous meat sources which were grinded by an electrical meat mincer and the product was termed as a control sample (Free of protease inhibitor; P.I). On the other hand, other samples were manufactured in the presence of the crude P.I. to reach the level of 1,2 and 3% of the recipe formula as follows:

Ingredients	Control (Free of P.I)	Levels of protease inhibitor (P.I) in the tested samples		
		1%	2%	3%
Meat (Beef or chicken)	92	92	92	92
Protease inhibitor	0	1	2	3
Water	8	7	6	5

With respect to the beef sausage samples, tney were prepared by stuffing the responsed mixture in a natural casings (small intestine) after which they were packed in white foam dishes and kept in a refrigerator at 4°C for 15 days. On the other hand, the chicken patties were prepared by using the previous responsed formula after shapping in patties forms and the produced samples were packed in white foam dishes (6 pieces in each one)and kept at 4°C for 15 days.

* Analytical methods:

Transmission electron microscope (TEM): The histological structure of beef sausage and chicken patties as also achieved by using the transmission electron microscope with described in details by Coleman et al., (1986). Silver sections were prepared using an LKB ultramicrotome equipped with a diamond knife and the sections were mounted on uncoated copper grids (200-300 mesh) after which they were stained with uranlyl acetate followed by Reynolds lead citrate (Reynolds, 1963). A Zeiss Transmission 10 electron microscope, 80 kv, was used for inspection. Operations were carried out at the unit of Electron Microscope, National Institute of Cancer, Cairo University.

RESULTS AND DISCUSSION

Surrounding the muscle as a whole is á sheath of connective tissue known as the epimysium; from the inner surface of the latter, septa of connective tissue penetrate into the muscle and separating the muscle fibres. The connective tissue round each fibre is called the endomysium and the sizes of these muscle fibre bundles determine the texture of the muscle (Walls, 1960). The relative proportions of connective tissue and muscle fibres are vary among muscles and, in part, account for the relative toughness of meat.

The electron diagrams of the unstored samples (control of beef sausage and chicken patties) which are free of protease inhibitor (P.I) are given in Figs. (1 and 2). The corresponding ultrastructure of the same samples containing 3% "P.I" are also given in the same figures. In both of the previous samples, the noticeable longitudinal straitions are mainly due to the presence of thin myofibrils and the available electron diagram of the aforementioned figure indicates the presence of alternating bands along the length of each myofibril. The "A" bands (dark areas) as well as the "I" bands (light areas) are sharply designated on each myofibril. These bands in either beef sausage or chicken patties are generally lie in an opposite form with respect to the adjacement myofibiril. The "Z" bands which appear as a dark narrow lines in each "I" band are also well seen.

The previous configurations of the control samples were slightly ultrered in the unstored samples as a result of the presence of the protease inhibitor (P.I). In other words, the added "P.I" has the capability to penetrate along the structure of the myofibrils and lead to a style wave structure within the characterized bands. These structural configurations may lead to other steric position which is not the best fit forms for maximal activity of protease enzymes. So, the presence of "P.I" strengthen the efficiency of the muscle fibers and so, being out of attack by the protease enzymes. Such statement is mainly due to the efficiency of "P.I" for diffusion and distribution along the gabs available between the structural bands; especially in parallel with sarcomeres. However, these findings are detected in the homogenous matrix surrounding the myofibrils known as sarcoplasm of either the beef sausage or in the chicken patties; but in a best fit forms in the former than the latter.

The differentiation of "P.I" behaviour in the two tested samples; namely beef sausage and chicken patties could be explained by the opinion of Trautmann and Fiebiger,(1952). They mentioned that there is an abundance of sarcoplasm in the muscle fibers which differed according to meat sources. Some fibres may contain a higher proportion of sarcoplasm than others, and in such case, the myofibrils are not distributed uniformly but are arranged in groups called fields of "Cohnheim". Subsequently, the higher amounts of sarcomplasm in these fibres are mainly accounted by the area of cytoplasm which separates the fields of fibrils.

Other possible reason of the differentiation between beef sausage and chicken patties that processed with or without "P.I" is that myofibril itself is composed of numerous parallel filaments, some of which are extended from the "Z-lines" to the edge of the "H-zone", while others traverse the entire width of the "A-band". These latter filaments(which traverse the "A-band") seem to be relatively thick; those which stop at the edge of the "H-zone" are relatively thin. The thick and thin filaments are now known to consist of molecules of the contractile proteins i.e., myosin and actin (Huxley, 1960). Electron micrographs have revealed the presence of fine structure of the "M-zone" in the centre of the myosin filaments. There are three to five parallel striations running perpendicular to the long axis. These "M-bridges" appear to link the myosin filaments to their six nearest neighbours. The "M-bridges" themselves are linked by thin filaments running between those of the myosin and parallel to the latter; knappeis and Carlsen; (1968). It would thus appear that the bridge-filament lattice of "M-substance" keeps the myosin filaments centrally aligned in the sarcomer.

The myosin molecule is differed in length from one source to another and each molecule consists of two apparently identical units. Each unit has a long "tail" (light meromyosin), a "collar" (heavy meromyosin S-2) and a "head" region (heavy meromyosin, S-1). It seems possible that there may be additional protein associated with the latter form (Gergely, 1970). The points of attachment of the light meromyosin shaft and that of the head of the myosin molecule (S_1) to the intermediate region (S_2) are susceptible in our opinion to be attacked by proteolytic enzymes. Such configurations suggest that these junction points could act as hinges, permitting S_1 and S_2 to swing out from the shaft towards the actin filaments (Lowey, 1968). On the other hand, myosin molecules (one of the myofibrillar protein) are not found in "Z" or "I" bands but present in "A-band"; while actin and tropomysin are localized in both "I and A" bands. So, it is of importance to emphasize on the relation between the presence of "P.I" and the activity of protease enzymes during storage of the tested samples; a trend may which lead to structural changes in the native configuration of the muscle fibers.

During storage of the investigated samples (beef sausage and chicken patties), the protease enzymes (pepsin, chymosin, trypsin and chymotrypsin) act on the muscle fibre and breaking down or sometime dissolving the sarcolemma and the muclei. Such trend was accompanied by the disintegration of endomyosial collagen, connective tissue collagen and elastin with the complete disappearance of crosse-striation as seen in Figs.(1 and 2). Some of the protease enzymes attack muscle fibre protein, nuclei of muscle fibres and cells located in the endomysia but are inactive towards collagenous and elastic fibers. These changes are usually associated with the swelling of the fibres, and lately the distruction of the connective fibres with their conversion to a homogenous substances. By extending the storage periods up to two weeks, the protease enzyme lead to one of the following trends:

- Presence of a week cross-striation as well as the appearance of transverse breakds.
- Disappearance of kinks or waves that characterise muscle fibers.

- Decrement of collagen tissue, but elastin is not affected.

The histological changes of beef sausage and chicken patties are more pronounced in the control samples (free of *P.I*) after about two weeks of storage at 4°C. These changes are mainly accompanied with the presence of a network(empty spaces)containing wide meshes of gabs areas a pattern which is out of detection in the samples containing 3% "P.I".

CONCLUSION

Electron microscopic inspection was carried out to shed more intensive light upon the activity of protease inhibitor for preventing the activity of protease enzyme in both beef sausage and chicken patties samples. Experimental work by transmission and scanning electron microscope proved that "P.I" has the capability to penetrate along the structure of the myofibrils and lead to a style wave structure within the characterized bands. These structural configruations may lead to other steric position which is not the best fit forms for maximal activity of protease enzymes. So, the presence of "P.I" strenthen the efficiency of the muscle fibers and being out of attack, i.e. by the protease enzymes. Such statement is mainly due to the capability of "P.I" for diffusion and distribution along the gabs available between the structural bands; especially in parallel with sarcomeres. The histological changes of beef sausage and chicken patties are more pronounced after two weeks of storage at 4°C of the control samples (free of "P.I") in relation to the others which containing protease inhibitor. These changes are mainly accompanied by the presence of a net work (empty spaces) containing wide meshes of gabs areas which was out of detection in the samples containing 3% "P.I".

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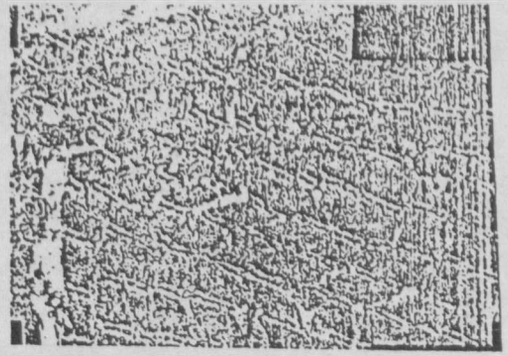
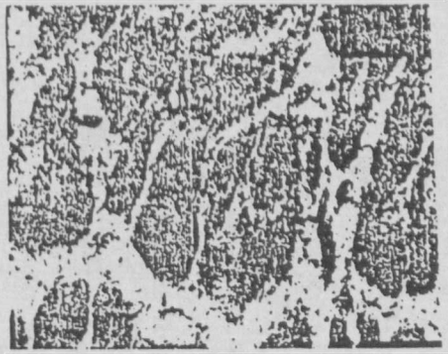


Ch.P.without P.I.; 6000X

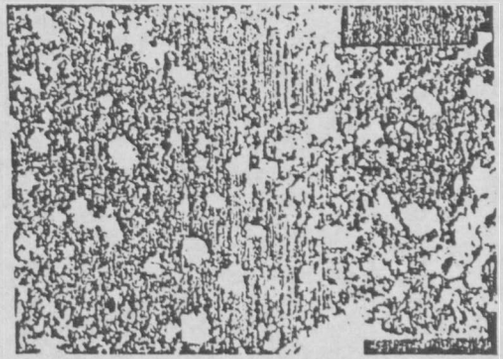


Ch.P. + 3% P.I.; 8000X

S.S. without P.I.: 6000X

S.S. + 3% P.I., 6000X

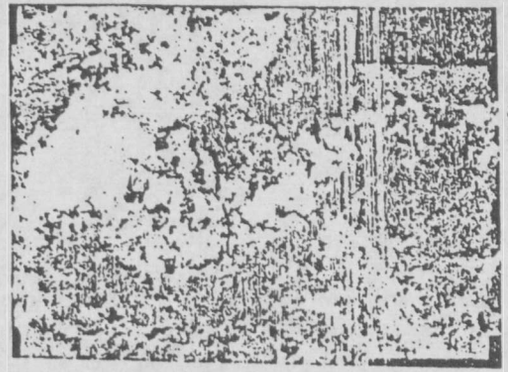
Unstored



Ch.P. without P.I.;10,000X

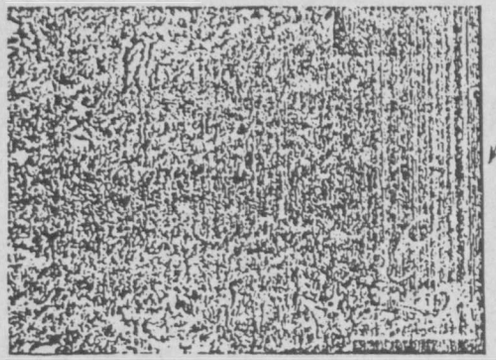
Two weeks storage at $4^{O}C$

Unstored

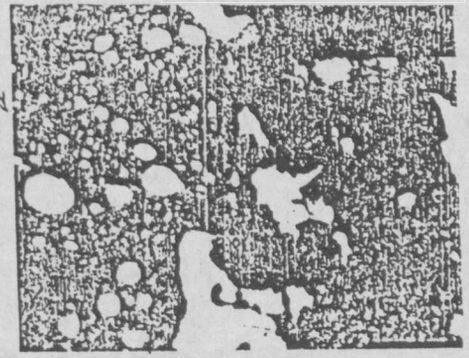


S.S. without P.I.; 10,000X

Two weeks storage at $4^{O}C$

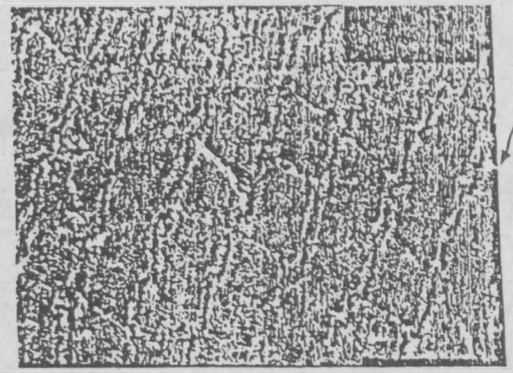


Ch.P. + 1% P.I.; 10,000X

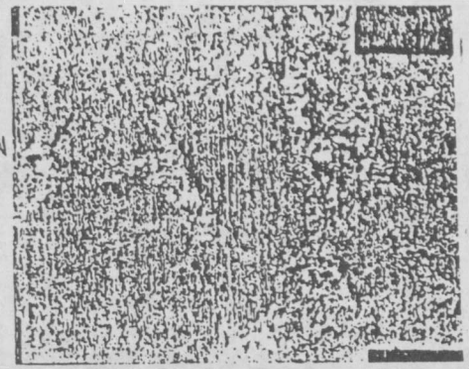


Ch.P + 3% P.I.: 10,000X

Fig.(2): Structure of chicken muscles in patties samples (Ch.P.) processed with and without protease inhibitor (P.I.) under transmition electron microscope at different magnifications.



$S_7S.$ + 1% P.I., 6000X



S.S. + 3% P.I.: 10,000X

Fig.(1): Structure of beef muscles in sausage samples (S.S.) processed with and without protease inhibitor (P.I.)under transmition electron microscope of different magnification.