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EFFECT OF STORAGE ON THE CHANGES IN CATHEPSIN D ACT I VITY, NUCLEOTIDE CONTENTS , PEPTIDE PROFILES AND MUSCLE ULTRASTRUCTURE OF ARISTICHTHYS NOBILIS, R.

JAMILAH BAKAR

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BY

JAMILAH BAKAR

Thesis submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy in the Faculty of Food Science and Biotechnology

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Dedicated to my husband and

all my children

last but not least my mum

and in memory of

my late father.

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by

JAMILAH BAKAR

MAY, 1993

Supervisor : Yu Swee Yean, PhD

Faculty : Food Science and Biotechnology

Cathepsin D from the muscle of bighead carp *(Aristichthys nobilis*, R.) was extracted, purified and partially characterized. The extraction and purification of the enzyme was achieved by autolysis of the muscle, acetone precipitation, gel filtration on Sephadex G100-120 and on ion-exchange carboxymethyl cellulose (CMC) column chromatography. It had a molecular weight (m.w) of 37,500 - 38,000 dalton (D) with a pH optimum at 3.2 and temperature optimum of 50°C. Myofibril was also optimally digested at pH 3.2. The purified enzyme had a single major peptide band on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and was completely inhibited by pepstatin.

Storage studies at 28° (ambient), 0° and -20°C were carried out to qualify and quantify changes in catheptic activity, nucleotide concentrations from ATP catabolism, K_1-value, SDS-PAGE profile and the ultrastructure of white, red and belly muscles of bighead carp (*A. nobilis*, R). Red muscle had the highest initial catheptic activity and K_1-value (34.01 ± 1.0%) as compared to white and belly muscles. The rate of change of K_1-value was temperature dependent - being faster at higher temperature. Different patterns and rate of change of K_1-value were observed among the muscle. Inosine -5'-monophosphate (IMP) was the most abundant nucleotide in all fresh muscles. It decreased rapidly during storage and had an inverse relationship with time ($r = -.91$) for red; $r = -.83$ for belly and $r = -.72$ for white). Inosine (HxR) accummulated in all muscles during the three storage temperatures studied.

Degradation of connective tissues (perimysium, endomysium/ plasmalemma) was the most conspicuous change in ambient temperature storage. However, progressive detachment of the muscle fiber ends from the myocommata were observed in samples stored at 0°C. Minimal degradation of ultrastructure of muscles was observed for frozen stored muscle kept less than 4 months. Changes in peptide patterns of muscles were only observed after prolonged storage at ambient (> 16 hr) and at 0°C (> 5 days). No obvious change of peptide bands was observed during frozen storage up to 5 months.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi syarat keperluan untuk Ijazah Doktor Falsafah.

EFFECT OF STORAGE ON THE CHANGES IN CATHEPSIN D ACTIVITY, NUCLEOTIDE CONTENTS, PEPTIDE PROFILES AND MUSCLE ULTRASTRUCTURE OF *ARISTICHTHYS NOBILIS*,R.

Oleh

JAMILAH BAKAR

MEI, 1993

Penyelia : Yu Swee Yean, Ph.D

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Cathepsin D dari otot ikan kap kepala besar (*Aristichthys nobilis*, R.) telah diekstrak, ditulinkan dan disepara cirikan. Pengekstrakan dan penulinan enzim telah berjaya dilakukan dengan autolisis otot, pemendakan aseton, penurasan gel di atas Sephadex G100-120 dan kromatograpi penukaran ion-lajur di atas carboxymethyl cellulose (CMC). Ia mempunyai berat molikul sebanyak 37,500 - 38,000 dalton dengan pH optima di 3.2 dan suhu optima pada 50°C. Myofibril juga dihadamkan pada pH optima 3.2. Enzim yang ditulinkan mempunyai satu jalur utama peptid di atas elektroporesis natrium dodecyl sulfate (SDS-PAGE) dan dihalang sepenuhnya oleh pepstatin.

Kajian penyimpanan di suhu 28°C (bilik), 0° dan -20°C juga dijalankan untuk menilai jumlah dan mutu perubahan di dalam aktiviti kateptik, kepekatan nukleotid dari katabolisma ATP, nilai K_1, profil SDS-PAGE dan ultrastruktur otot putih, merah dan perut ikan kap kepala besar (*A. nobilis*, R). Otot merah mempunyai nilai awalan aktiviti kateptik dan K_1 (34.01 ± 1.0%) yang tertinggi berbanding dengan otot putih atau perut. Kadar perubahan nilai K_1 bergantung kepada suhu - lebih cepat di suhu yang tinggi. Corak dan kadar perubahan nilai K_1 yang berlainan didapati didalam otot-otot. Inosine -5'-monofosfat (IMP) adalah nukleotid yang terbanyak sekali di dalam semua otot segar. Ia menurun dengan cepat semasa pengstoran dan menunjukkan kaitan yang songsang dengan masa ($r = -.91$ bagi otot merah; $r = -.83$ bagi otot perut dan $r = -.72$ bagi otot putih). Inosine (HxR) didapati terkumpul di dalam semua otot semasa penyimpan di ketiga-tiga suhu pengstoran yang dikaji.

Degradasi tisu penyambung (perimysium, endomysium/plasma-lemma) didapati sangat nyata berlaku ketika pengstoran di suhu bilik. Perkembangan kearah pencabutan hujung gentian otot daripada myocommata dilihat di dalam sampel yang disimpan di suhu 0°C. Degradasi ultrastruktur yang minimum didapati bagi otot yang disimpan sejukbeku yang tidak melebihi 4 bulan. Perubahan jalur peptid otot hanya ketara selepas pengstoran yang lama (> 16 j) pada suhu bilik dan >5 hari pada 0°C. Tiada perubahan yang nyata diperolehi bagi jalur peptid otot yang disejukbekukan sepanjang masa kajian.

CHAPTER 1

INTRODUCTION

In the year 2000, an estimated 104 million tons of fish will be needed for human consumption of which 90% will be consumed in developing countries (Pedrosa - Menabrito and Regenstein, 1988). To meet this demand, they suggested that the postharvest losses of fish be reduced by increased utilization of available resources and underutilized fish, increased activity in the aquaculture sector and finally to diversify fishing efforts.

Fish is a very perishable commodity and it has been estimated that 25 percent of the catch for human consumption is lost during postharvest (Santos, 1991). A better knowledge of spoilage mechanism will provide possibilities in devising techniques to reduce this postharvest loss.

Spoilage of fish can be defined as unacceptable changes occurring in fish muscle postmortem which inherently will affect the quality (Makundan *et al* ., 1982). Fish spoilage is triggered by the activity of endoenzymes of fish muscle (proteases, cathepsins, peptidases, etc.) on peptides and proteins; hence, establishing a favourable environment for the propagation of spoilage microoganisms (Pedrosa-Menabrito and Regenstein, 1988).

The processing and storage characteristics of cold and temperate water fish are well established but relatively little is known about tropical fish. Poulter *et al* . (1982) also found that proteins of some tropical fish are more stable during processing than proteins of species from colder waters.

Very little is known about enzymes which participate in the deterioration of fish. Investigations on cathepsins indicate that fundamental differences exist between mammalian and fish enzyme specificities which in turn are completely different from those occurring in meat or any other protein rich food (Siebert and Schmitt, 1980).

Most of the information available on catheptic enzymes were obtained from work done on spleen, kidney and liver terrestrial animal while little information is available on muscle which could be due to the relatively low activity of the enzyme in it (Fruton, 1960). Siebert (1958) found that the catheptic activity of fish muscle is ten times greater than mammalian muscle and also observed that fish muscle cathepsins played a role in the spoilage of fish prior to processing (Siebert, 1962). The work of Siebert triggered research activities in the scientific community whose main interest is in fish deterioration. Groninger (1964) published work on proteinase from albacore followed by Ting *et al*. (1968) who found that the crude catheptic activity in Chinook salmon (*Oncorhynchus tshawytscha*) muscle was forty times higher than