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Cathepsin enzymes, part 1

2 July 2014

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Contributors to post-mortem autolysis

Catheptin enzymes have a potential role in the acceleration of post-mortem degradation of fish subjected to pre-slaughter stress.

There are two primary types of quality deterioration in fresh fish and shellfish: endogenous and exogenous. Much attention has been devoted to exogenous deterioration, since this is the primary cause of reduced quality in fresh products. The quality deterioration is primarily caused by proteolytic microorganisms that contaminate the fish as a consequence of post-harvesting handling and processing. Some of the major microorganisms responsible for reduced quality are those belonging to the *Pseudomonas* genus.

Endogenous decomposition can also serve as a significant contributor to quality loss. Some of the undesirable endogenous textural and other physical and biochemical changes are due to a group of enzymes referred to as catheptic enzymes. These enzymes are also a major cause of gel softening in the production of surimi from various fish species.

Post-mortem tenderization

Post-mortem tenderization is one of the most unfavorable quality changes in fish muscle. Therefore, the biochemical processes that cause it have been extensively studied in order to identify potential quality indicators or control post-mortem degradation.

Post-mortem muscle tissue evolution is characterized by successive biochemical reactions and autolytic modifications that result in disorganization of the molecular structure. A proteolytic degradation of myofibrillar and connective tissue components is observed in the tissue. There has been considerable debate about the specific protease responsible for post-mortem changes.

(https://link.chtbl.com/aquapod)

The participation of various proteinases in autolytic processes of ice-stored fish depends on the location of the enzymes in cytosol and/or factors affecting tissue compartmentalization, the presence of activators or inhibitors, and the susceptibility of the proteins responsible for muscle integrity to in situ cleavage by the respective enzymes. Two major intracellular degradative pathways are involved in these degradations: a lysosomal pathway, including cathepsic proteases, and a cytosolic calcium-dependent pathway with calpains.

Cathepsin enzymes

Cathepsins are proteolytic enzymes that occur in animal tissues – especially the liver, kidneys and intestine – that catalyze autolysis after death. There are approximately 13 members of this family, which are distinguished by their structure, catalytic mechanism and which proteins they cleave.

Most of the members become activated at the low pH found in lysosomes. Thus, the activity of this family lies almost entirely within those organelles. There are, however, exceptions such as cathepsin K, which works extracellularly after secretion by osteoclasts.

Cathepsins degrade polypeptides and are distinguished by their substrate specificities (Table 1). According to the type of active site, the endogenous proteinases of seafood can be classified as serine, cysteine, aspartic and metalloproteinase. Their activities are controlled by specific and endogenous inhibitors, activators, pH and temperature of the environment. However, the proteolytic activity varies greatly among species and with harvesting season, gender maturation, spawning and other variables. Cathepsic enzymes are active from pH 3 to 8 and under temperatures as high as 60 degrees-C.

Flick, Classification of cathartic enzymes, Table 1

Cathepsin	Specificity (Protease)
A	Serine
B	Cysteine
C	Cysteine
D	Aspartyl
E	Aspartyl
F	Cysteine
G	Serine
H	Cysteine
K	Cysteine
L1	Cysteine
L2 (or V)	Cysteine
O	Cysteine
S	Cysteine
W	Cysteine
Z (or X)	Cysteine

Table 1. Classification of catheptic enzymes.

Lysozymes are known to harbor about 13 cathepsins, which play a key role in protein turnover in vivo and in the post-mortem rheological changes of fish muscle. Among these lysozymal enzymes, cathepsins B, D, H, L, L-like and X have been purified and characterized from fish and shellfish muscles.

Among the four classes of proteinases, more recent attention has been focused on the cysteine proteinases. Cathepsins B, D and L are considered critical in fish muscle post-mortem modifications or in gel softening during setting of surimi. Cathepsins A and C contribute to the hydrolysis of muscle protein in a concerted action with other cathepsins.

Product quality

A potential role of catheptic enzymes in the acceleration of post-mortem degradation of fish when subjected to pre-slaughter stress has also been suggested. During stress and exhausting exercise, fish use anaerobic energy, leading to the production of lactic acid and resulting in low initial post-mortem pH in muscle. It has been suggested that this low pH could be responsible for deteriorated final muscle quality, since the optimal pH for cathepsins lies between 5.0 and 6.0.

Today, much farmed salmon is filleted post-rigor after three to five days of storage on ice. This period of storage is required for easier removal of pin bones and to avoid processing while the fish are still in rigor. Reducing pre-slaughter stress can increase the potential of pre-rigor filleting, thus providing greater freshness and higher-quality products on the market. Pre-rigor fillets of Atlantic salmon are thicker and firmer, and offer more intense coloration and less gaping.

Most studies on pre-slaughter stress in fish of commerce have so far focused on parameters like adenosine triphosphate, lactate and cortisol levels as primary stress responses, the onset of rigor mortis, texture, color and drip loss. The impacts of pre-slaughter stress on muscle structure and the biological factors responsible for the quality deterioration process have been less identified.

High-pressure processing

High-pressure processing (HPP) is a technology of growing interest for food preservation due to its ability to control the activity of some degradative enzymes. The effects of HPP variables at pressure levels of 100, 200 and 400 megapascals and holding times up to 30 minutes on the enzyme activities of cathepsins B and D were studied by sodium dodecyl sulfate polyacrylamide gel electrophoresis and isoelectric focusing electrophoresis.

Increases in pressure and holding times decreased the activity of both cathepsins. In contrast, cathepsis B was less affected by pressure than cathepsin D, which had lower activities at 100 and 400 megapascals. Since HPP causes lysozyme disruption and also denaturation, aggregation and fragmentation of sarcoplasmic proteins, these changes could be related to the decrease in cathepsin activity.

(Editor's Note: This article was originally published in the May/June 2014 print edition of the Global Aquaculture Advocate*.)*

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