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CHANGES IN CYTOCHROME C OXIDASE (CCO) AND LACTATE DEHYDROGENASE (LDH) ENZYME ACTIVITY OF THE WHITE EPAXIAL MUSCLE TISSUE OF SEA BASS (*DICENTRARCHUS LABRAX* L.) AS SHELF-LIFE PREDICTIVE INDICES DURING ICE-STORAGE

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ABSTRACT

Fish freshness can be evaluated by sensory, physical and biochemical parameters. The purpose of the present work was to evaluate the potential utilisation of enzyme activity changes as indicators of shelf life in ice-stored sea bass. Changes in the enzyme content and organoleptic score of ungutted ice-stored sea bass were monitored over a two-week period. Organoleptic score and activity of CCO and LDH decreased gradually with a 50% reduction after 10 days of storage in ice. The results show that monitoring the activity of the mitochondrial and cytoplasmic enzymes of fish muscle can be used as an indicator of fish freshness.

- Key words: freshness, quality, refrigeration, seafood, spoilage -

INTRODUCTION

Fish and seafood are highly perishable foods. Spoilage of chilled, fresh and minimally processed fish is a function of endogenous enzyme and bacterial activity (GRAM and HUSS, 1996; SHIMADA *et al.*, 2000, EIE *et al.*, 2007). Compared to land animals (AGUIAR *et al.*, 2009), fish flesh has a higher water content, a high free amino acid pool and contains less connective tissue (BUCHTOVÁ *et al.*, 2009, DUMAS *et al.*, 2010). These lead to rapid biochemical changes during cold-storage and shorten the shelf-life of fish (HUSS, 1988). The development of unpleasant odours of raw fish correlates well with bacterial spoilage and storage time (PONS-SANCHEZ-CASCADO *et al.*, 2006).

The demand for sea bass in Europe constitutes a significant element of the European fish market; sea bass is widely cultivated in the Mediterranean Sea. Consumers of European sea bass frequently buy this product fresh, within a few days of ice-cold storage. The shelf-life of ice-stored fish varies according to several parameters and can be extended depending on the initial bacterial and lipid content, as well on the season, killing method and handling conditions (HUSS, 1988; GRIGORAKIS *et al.*, 2004; LAMBOOIJ *et al.*, 2008). An initial autolytic enzymatic process, followed by bacterial spoilage in the later stages of shelf-life, are the post-mortem changes that occur during ice-storage of sea bass (KYRANA and LOUGOVOIS, 2002; PAPADOPOULOS *et al.*, 2003).

Several methods exist to assess fish quality. Sensory, microbial, chemical and physical post-mortem changes of sea bass have been well reviewed (ALASALVAR *et al.*, 2002; KYRANA and LOUGOVOIS, 2002; KOUTSOUMANIS *et al.*, 2002; PAPADOPOULOS *et al.*, 2003; GRIGORAKIS *et al.*, 2004; OZOGUL *et al.*, 2005; CAKLI *et al.*, 2006; HOWGATE, 2006).

Recent developments of electronic devices such as the electronic nose systems offer new, rapid methodologies for assessing the quality of seafood. Using an electronic nose apparatus, LIMBO *et al.* (2009) reported a significant reduction of sea bass freshness after 8 days in cold storage at 2°C.

In general, the release of proteolytic enzymes from muscle cell lysosomes occurs immediately after death (HUSS, 1988). The decomposition of structural and enzymatic muscle proteins results in a progressive protein structural loss and degradation of metabolic enzymes. As a result, enzyme activity can influence the postharvest quality of seafood (HAARD, 2000). During storage, the rate of protein degradation is proportional to the storage time (BAUCHART *et al.*, 2007). Thus, the level of protein decomposition during ice-storage should be related to the shelf-life of fish during ice-cold storage. This is a well-known phenomenon observed during storage of terrestrial animal meat (COLLINS *et al.*, 1991; GODIKSEN and JESSEN, 2001). Among the various endogenous enzymes, the roles and post-mortem activities of specific proteinases such as caplain and cathepsins have been studied in sea bass (LADRAT *et al.*, 2003; DELBARRE-LADRAT *et al.*, 2004; CHÉRET *et al.,* 2006). However, there is a lack of data on the activity and changes in other endogenous enzymes that occur during ice-storage of sea bass. Such information would be useful in providing new alternative indexes for evaluating sea bass freshness.

The aim of the present work was to monitor post-mortem changes in a cytoplasmic and a mitochondrial enzyme during ice-storage of sea bass. Lactate dehydrogenase (LDH) is located in the cytoplasm and catalyses the interconversion of lactate and pyruvate. Muscle tissue is rich in this enzyme and its presence is related to the capacity for anaerobic glycolysis. Cytochrome *c* oxidase (CCO) is a membrane-bound mitochondrial enzyme which catalyses the reduction of molecular oxygen to water in the last step of the mitochondrial respiratory chain. The post-mortem changes of these two basic metabolic enzymes have been studied in ice- or frozen-stored meat of terrestrial origin (COLLINS *et al.*, 1991; GODIKSEN and JESSEN, 2001) and frozen fish (DAMODARAN NAMBUDIRI and GOPAKUMAR, 1992), but not ice-stored fish.

MATERIALS AND METHODS

Fish samples

Maricultured sea bass of commercial size (average weight 364.06±19.07 g) were obtained from a commercial fish farm (Sagiada, NW Greece) in April 2008. Standard killing methodology by immersion in ice / water (at 2/1 ratio) was followed, and ice-stored fish were transported to the laboratory within a day after killing. Fish were washed, and whole, ungutted samples were stored in insulated polystyrene boxes filled with ice in cold storage at 3°±1°C. Boxes had perforated bottoms to allow melting ice to drain. Samples of the epaxial muscle tissue were obtained for analysis (three fish per sampling day, on the 1st, 3rd, 7th, 10th and 15th day of ice-storage).

Assay of enzymes

All enzyme assays were performed in duplicate according to methods previously described (TYLER and NATHANAILIDES, 1995; NATHANAILIDES *et al.*, 2009). Duplication in analysis was chosen to ensure repeatability of the method for each fish. Each enzyme assay was performed on three fish on each sampling day. Homogenates were prepared and the CCO activity was assayed at 20°C by monitoring the reduction of ferrocytochrome c absorbance at 550nm, in a

medium containing 0.075 M potassium phosphate buffer, pH 6.8 and 0.025 mM ferrocytochrome c. The activity of the glycolytic enzyme lactate dehydrogenase (LDH) was assayed (by the rate of NADH oxidation at 340 nm when LDH catalyses the reversible oxidation of pyruvate to lactate) in a medium of 50 mM potassium phosphate buffer (pH 7.0), containing 0.15 mM NADH and 0.60 mM sodium pyruvate (omitted from the control). Enzyme activity is reported in units per mg protein. Protein content was estimated using the Folin-Lowry method (LOWRY *et al.*, 1951). All reagents were obtained from Sigma-Aldrich (St Louis, MO, USA). The use of Triton X-100 in the homogenisation buffer together with the absence of high-speed centrifugation of the muscle homogenates ensured that the total CCO activity was assayed (TYLER and NATHANAILIDES, 1995; GODIKSEN and JESSEN, 2001).

Sensory evaluation of fish quality

A trained sensory panel of four persons evaluated the odour of raw fish. Organoleptic evaluation included gill and vent odours. These two characteristics were scored according to the modified TFRU scheme according to ALASALVAR *et al.* (2002) using a four-point scale (0= fresh, 3= spoiled).

Statistics

One Way Analysis of Variance (ANOVA), and Tuckey test were used to make multiple comparisons of the means for the enzyme studied and odour scores (ZAR, 1984). In all cases, the confidence level was 95%. A two-tailed Pearson correlation was used to correlate the enzyme changes with the respective values previously found (GRIGORAKIS *et al.*, 2004) K-values of ice-stored sea bass. The SPSS 10.0 software was used for the statistical analysis.

RESULTS AND CONCLUSIONS

During ice-storage, significant changes were observed in the lactate dehydrogenase (LDH) and cytochrome c oxidase (CCO) activities in the white epaxial muscle of sea bass (Figs. 1 and 2, respectively). The LDH activity decreased significantly already after day 3 of ice-storage. On day 3, the average value was 767 μmoles min^{-1} mg $protein^{-1}$, compared to an initial value of 858 μmoles min^{-1} mg $protein^{-1}$ on day 1 of storage. The value continued to decrease until it reached a value of 503 μmoles min^{-1} mg $protein^{-1}$ on the 10^{th} day of ice-storage (Fig. 1). The CCO activity remained almost unchanged (no significant changes) until the 7^{th} day of storage and decreased thereafter. Thus, the non-significant change from 6.86 to 5.57 μmoles min^{-1} mg $protein^{-1}$ in the first seven days was followed by a rapid decrease to 2.61 μmoles min^{-1} mg $protein^{-1}$ on the 15^{th} day of storage (Fig. 2). The differences in the loss of activity between the two enzymes may be the result of different post-mortem conditions in the cytoplasm and inner-mitochondrial membrane environment. Nevertheless, both enzymes exhibited an initial reduction after 3 (LDH) or 7 (CCO) days of storage in ice, this reduction was more marked after 10 days of storage.

The panellists reported a significant reduction in the organoleptic quality of odour in ice-stored sea bass. On the 15^{th} day of ice-storage, the fish exhibited deterioration in odour and the smell was characterized as "unpleasant" to "unacceptable" by the panellists. The odour changes observed during ice storage are presented in Fig. 3.

There was a very good correlation between the LDH and CCO activities and the K-values, according to GRIGORAKIS *et al.* (2004). The respective two-tailed Pearson correlations for LDH and CCO were -0.977 ($p<0.001$) and -0.846 ($p<0.001$), respectively.

Similar to the present results, the LDH activi-

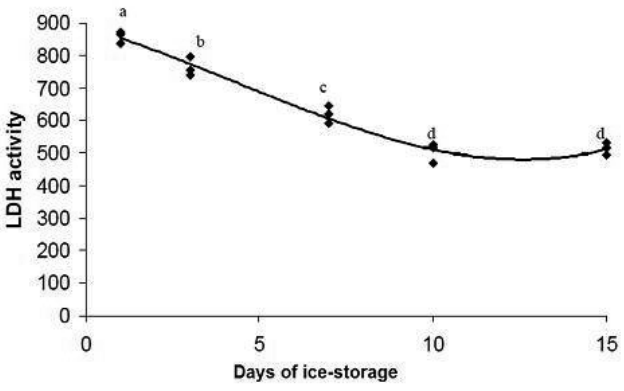

Fig. 1 - Changes of LDH activity (μmoles min^{-1} mg $protein^{-1}$) of white epaxial muscle tissue of sea bass during ice-cold storage. Best-fit curve is third order polynomial, with regression value 0.97. Different letters (a,b,c,d) denote statistically significant changes of activity over storage time.

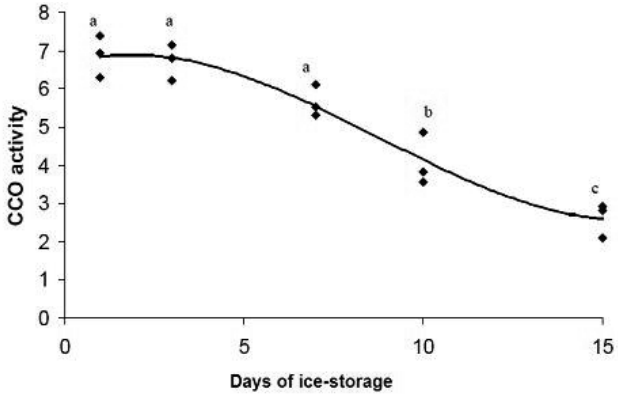

Fig. 2 - Changes of CCO activity (μmoles min^{-1} mg $protein^{-1}$) of white epaxial muscle tissue of sea bass during ice-cold storage. Best-fit curve is third order polynomial, with regression value 0.93. Different letters (a,b,c) denote statistically significant changes of activity over storage time.

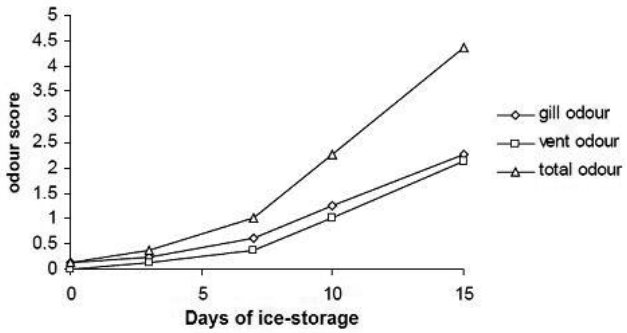

Fig. 3 - Organoleptic changes in sea bass during ice storage, evaluated by a four-member panel, as represented by the sum of the average gill (0= fresh, 3= spoiled) and vent (0= fresh, 3= spoiled) odour scores (total odour score).

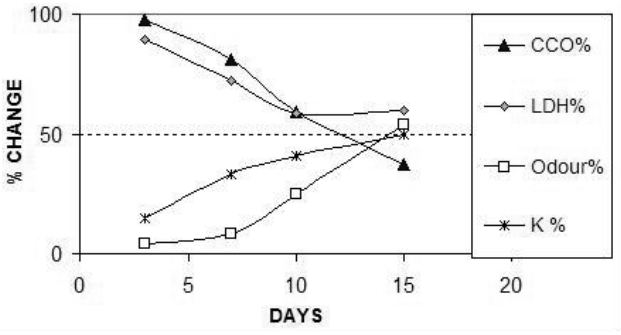

Fig. 4 - Changes (%) in the activity of CCO and LDH, total odour scores and K value (K-value data obtained from GRIGORAKIS *et al.*, 2004) of white epaxial muscle tissue of sea bass during ice-cold storage.

ty decreased in frozen-stored fish muscle in five fish species (DAMODARAN NAMBUDIRI and GOPAKUMAR, 1992); these results indicate that this enzyme is suitable for evaluating freshness in ice-stored or frozen-stored fish. The LDH activity was significantly reduced even after the 3rd day of ice storage, and this indicates that the respective enzyme can be a very good freshness indicator for sea bass. The correlation of LDH activity with initial quality changes, and its suitability as a freshness index has been previously proposed for frozen mullet, pearlspot milkfish and tilapia (DAMODARAN NAMBUDIRI and GOPAKUMAR, 1992).

On the other hand, CCO activity seems to be a more suitable spoilage indicator, because it indicates the degree of spoilage rather than the freshness stage of the fish. It remains rather stable during the first seven days of ice-storage and then decreases significantly. Both enzymes can be useful indexes for assessing the shelf-life of ice-stored sea bass. This is confirmed by the strong negative correlation of both enzymes with the K-value and organoleptic scores.

The spoilage rate during ice-storage of sea bass varies according to the initial handling and storage conditions and the killing method used (OZOGUL *et al.*, 2005; CAKLI *et al.*, 2006). In the present work, after 10 days of storage, sea bass received lower organoleptic scores due to the development of unpleasant odours and after 15 days the fish were characterized by strong unpleasant odours (Fig. 3); this period also coincides with the acceptability limit provided in the literature (KYRANA and LOUGOVOIS, 2002; TALIADOUROU *et al.*, 2003; GRIGORAKIS *et al.*, 2004; PALEOLOGOS *et al.*, 2004; CASTRO *et al.*, 2006). These researchers reported the initial development of an off-flavour and moderate sensory scores after 9 days in ice-storage. The results did not vary between gutted and ungutted ice-cold stored sea bass (ÖZOGUL *et al.*, 2005; POLI *et al.*, 2006). In agreement with the aforementioned literature, the present results also indicate that unpleasant odours develop within 10 days. This is a critical period for the quality of ice-stored sea bass, and can be characterized as the high quality shelf-life of the fish, since significant quality reduction occurs thereafter. A 50% reduction in CCO and LDH activity, coincides with the previously reported (GRIGORAKIS *et al.*, 2004) 50% increase in the respective K value in ice-stored sea bass (Fig. 4).

The results of the present work show that proteolytic activity in the white muscle of sea bass flesh during ice-cold storage coincides with a reduction of muscle CCO and LDH contents. In conclusion, the monitoring of CCO and LDH activities during ice-cold storage provides reliable indexes of the shelf-life of this product. The changes in these two enzymes indicate that LDH may be a useful index of freshness, since it exhibited alterations even at the early stages of ice-storage, while CCO appears to be more suitable for determining the degree of spoilage, since the levels changed significantly in the later stage of shelf-life.

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