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Research of the Main Indexes of Freshness Anchovy (Engraulis engrasicolus Linnaeus, 1758) and Sardines (Sardina pilchardus Walbaum 1792) of Mediterranean

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***Abstract*—**Anchovy (Engraulis Encrasicholus) and sardine (Sardina Pilchardus) are blue fishes linked to our alimentary tradition of Mediterranean. In our work, particularly, we tested for the first time physical and enzymatic methods to verify the freshness of species of blue fish, anchovy and sardine of Mediterranean. In connection with to the lowering of the pH after post-mortem stage we assisted to a increase in proteolytic activity of calpaine and catpsine. Already after 2 h in post-mortem there was a significant increase.

***Keywords*—**Engraulis *encrasicholus, Sardina pilchardus*, freshness, index rigor.

I. Introduction

ANCHOVY *(*Engraulis *Encrasicholus)* and sardine (*Sardina Pilchardus)* are always considered the food of the poor, but, slowly, they have been revalued for their high nutritional value. In fact they are highly nutritional products, for their contents in water and protein, rich in vitamins and poor in fat and saturated fat when compared with other protein-rich animal food. It is well known that fish oil is the major and the best source of polyunsaturated fatty acids (PUFA), called omega-3 fatty acids, especially eicosapentaeonic acid (EPA), and docosahexaenoic acid (DHA) [1]. Nowadays blue fishes are used to preparate typical traditional products, generally marinated and salted and are parts of exquisite dishes. In fact their richness in water and protein make them suitable to the preparation of ready to eat like live, fresh, chilled, frozen, chopped, dried, salted, pickled, cooked, powder, etc. As seasonal products they must be preserved. One of the main problems related to the trade in fish products not preserved it is given from their easy perishability. After the death, the fish encounters rapidly numerous alterations because of the unstable structure and of the special chemical composition of their tissues [2]. The freshness is the distinctive element of a not damaged product that does not show marks of alterations and maintains the property of the species unchanged. A fish product is defined fresh when it was caught up to 4 days before, was not damaged and was kept on ice in flakes .It is generally accepted that fresh fish (or fillets/portions) and frozen-thawed fish are types of products which should be differentiated [3]. Fresh fish is understood as being fish freshly caught or which has been chilled and stored for the short period at normal refrigeration temperature prior to purchase or use. For storage over longer periods freezing is normally utilized. However, while frozen storage is effective in protecting against microbiological deterioration of fish meat, its physicochemical and sensorial properties suffer [4]. The methods developed for differentiating between fresh and thawed fish are evaluated by sensory methods, chemical, physical, biochemical and microbiological processes. In the available literature, there are various methods attempting to distinguish between chilled and thawed fish, used with variable success. In a comparative study with fish using organoleptic parameters, it was demonstrated that the distinction between frozen-thawed and fresh fish from the *Gadidae* family cod and whiting could be very difficult [5]. The microbiological methods of differentiation are based on the fact that thawed fish tissue is a more appropriate medium for growth of some microbial species. In respective comparative investigations, it was observed that after thawing, the number of microorganisms was higher compared to fresh chilled cod and this resulted in a shorter shelf life of thawed products [6]. The biochemical methods evaluate the enzymes released from the organelles contained in the cells of the fish product after freezings and defrostings. The test produced and optimized are about the search for cytochrome oxidase and glutamate aspartate aminotransferase (GO both present in the mitochondria), succinate dehydrogenase and lysosomal enzymes. The aim of our research is to obtain the best methods for assessing the freshness of fresh bluefish. In our work, particularly, we tested for the first time physical and enzymatic methods to verify the freshness of species of blue fish, anchovy and sardine of Mediterranean.

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II. Material and Methods

Along this work selected blue fishes of Mediterranian species were used from anchovy Engraulis encrasicholus and sardine Sardina pilchardus given by “Cooperativa Ittica “of Catania. The samples of fish come from night fishing with seine (purse seine) in the marine areas of the Mediterranean. The samples were divided into n. 8 lots, identified by the letters S (sardines) and A (anchovies). Immediately after fishing, the samples were placed in tanks with salt water and ice until the death to "thermal shock". Monitoring physical indices was carried out on board. The transport to the laboratory was performed using polystyrene boxes containing ice flakes in a ratio of 2:1. In the laboratory, samples of fish have different destinations. The samples have been washed, decapitated, eviscerated, and fillets deliscati obtained, again

washed and then dried. According to the method developed by Ho et al. (1999)[7] has been carried out the determination of calpain and cathepsin. In order to verify the freshness of samples was carried out the determination of rigor index in accordance with the method [8]. Statistical processing of data was performed using the program STATISTICA (ver. 6.1).

III. Results and Discussion

This assessment is also reflected by the enzymatic data obtained by determining the pH. In fact after the death of the cell we assist to a considerable decrease of the pH of the samples around 4,0 in the citosol that activates such enzymes. To confirm the usefulness of the pH measurement in association to the enzymatic analysis, some authors report lower values than the normal one at the time of death, as a stress index in a lot of species: salmon, tuna, gleans and rumble. In literature, the average pH of sardines and anchovies are respectively 6,11 and 6,18 .When the fish undergoes a stressful death, with prolonged agony, values of lower pH are obtained, due to a greater accumulation of lactic acid. Low values of pH (inferior to 7) measured to the death and in the first hours after the death (until the rigor mortis), indicate that animal has undergone a significant stress. In contrast, pH values higher than 7.6 and superior to those observed to the death generally indicate an animal "rested". After the rigor mortis the pH normally tends to decline rapidly by the first day of storage [9].

This decrease is linked to the accumulation of lactic acid produced by anaerobic glycolysis in post-mortem, the only way to produce ATP in such a situation. An eccessive decrease of the pH would an intense denaturation of the proteins that tend to insolubilize and lose some capacity for water retention that will be released by the tissue. In connection with to the lowering of the pH after post-mortem stage we assisted to an increase in proteolytic activity of calpaine and catpsine. Already after 2 h in post-mortem there was a significant increase. In Figs. 1 and 2 the proteolytic activity was monitored over 12h post mortem

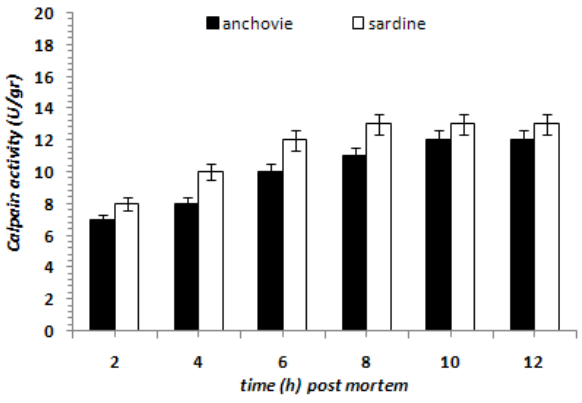

Fig. 1 Calpain activity monitored 12 hours after capture (post mortem)

We observed a maximum activity after 8h in the sardines, then that value is stabilized. Sardines are in a range of proteolytic activity more than anchovies (Fig. 1). The mechanism of proteolysis post-mortem of calpain determined the separation of intact filaments of actin and myosin, possible substrates of the proteasome and cathepsins (Fig. 2).

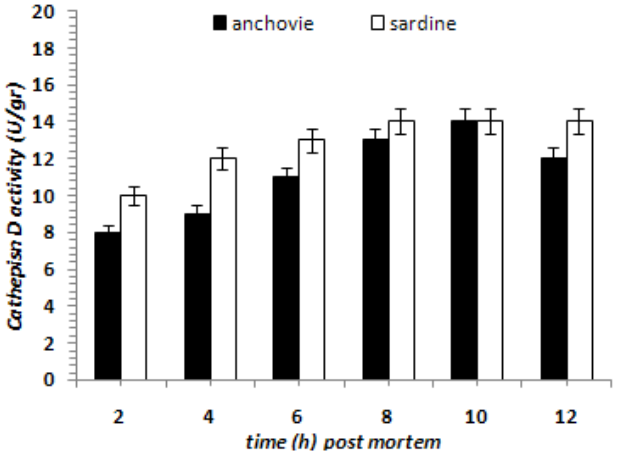

Fig. 2 Cathepsin D activity monitored 12 hours after capture (post mortem)

This determined a consequent softening of meat and the consequent lowering of the index of freshness. Different authors have studied this phenomenon but in different species as tilapia[10], the salmon[11.12],the mackerel[13.14], the carp[15,16]. After the step of post-mortem we observed an increase in the index of rigor mortis (Fig.3). This increase was observed [17] in different species as plaice, parrot bass, yellowtail, carp, red sea-bream, striped grunt, tiger puffer and rainbow trout. The sardines had a rigor index less than anchovie.

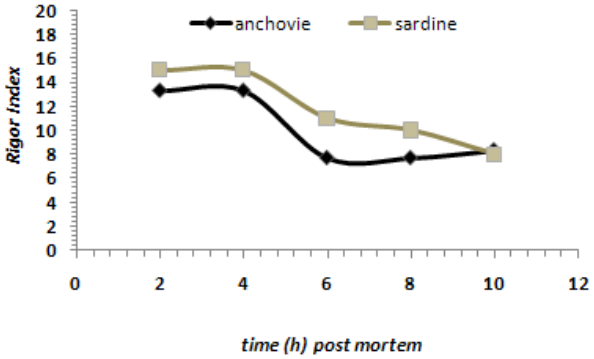

Fig. 3 Rigor Index monitored 12 hours after capture (post mortem)

IV. Conclusion

The analyzes show the use of indices of freshness applied for the first time in a species of blue fish. It could be a valid alternative to traditional methods. In particular the increase in the proteolytic activity of cathepsin D confirms the data in the bibliography of the biochemical mechanism of post-mortem. This process results in an increase of cathepsins and reduced

activity of calpain, leading the greater calcium concentration in the tissues. The index of rigor mortis determined to those species of small size for the first time was very good. This could be used directly in the fish markets of the Mediterranean to check the freshness of the blue fish.

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