INTERACTION OF MALONALDEHYDE (A BYPRODUCT OF LIPID OXIDATION) WITH MYOFIBRILLAR PROTEINS OF SEA SALMON (*Pseudopercis semifasciata*).

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Background.

Lipid oxidation is one of the main processes responsible of lost of quality in foods because affects different constituents such as lipids, proteins, vitamins, etc. This oxidative deterioration is related to negative changes in flavor, texture, appearance, nutritional value and protein functionality. A breakdown product of lipid oxidation - malonaldehyde (MAL) - can react with proteins, phospholipids and nucleic acids, producing covalent links and cross-linking of large molecules *(Aubourg, 1993)*. It had been possible to register protein polymerization, insolubilization, polypeptide chain scission, amino acid destruction and form addition products with proteins *(Li and King, 1999)*.

Myofibrillar proteins are a relevant muscle components susceptible to oxidative reactions, exhibiting some of the effects previously mentioned like chemical, physical, functional and structural changes *(Li and King, 1996; Srinivasan and Hultin, 1997; Liu and Xiong, 2000)*. On the other hand, Differential Scanning Calorimetry (DSC) offers a direct method to study the thermal transitions of these proteins; it is important to determine and predict the final quality of meat products *(Paredi and col., 1998)*.

Objective.

To study the interaction of malonaldehyde with myofibrillar proteins of sea salmon (*Pseudopercis semifasciata*).

Methods.

Myofibrillar proteins from sea salmon (*Pseudopercis semifasciata*) were prepared according to the procedure of *Wagner and Añón (1985)* and suspended in 0.6 M KCl, 0.03 M Tris, pH = 7.0. Protein concentration was determined by a modified Biuret method *(Robson and col., 1968)*.

Malonaldehyde (MAL) solution was prepared by acid hydrolysis of 1,1,3,3-tetraetoxipropane (TEP) (Ke and col., 1984).

Myofibrillar proteins (5 mg/mL) were incubated with MAL 30 mM (protein: MAL ratio 2.3:1) at 27 ± 1 °C from t = 0 to 8 hours, moderate agitation. At each time, samples were centrifuged at 5000 g, 10 minutes, 4°C obtainig the corresponding pellets.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE): Proteins were dissolved in 0.6 M KCl, 0.03 M Tris, pH = 7.0 and buffer 8 M urea, 0.3 % SDS, pH = 9.0 (with or without β - mercaptoethanol ME) was additioned. Slab SDS-PAGE was carried out using the Laemmli discontinous buffer system *(Laemmli, 1970)*. A stacking gel of 3 % acrylamide and a gradient resolving gel with 3 - 15 % was used. Also, bidimensional SDS-PAGE of each samples were performed as follows: 1) without ME, 2) with ME. Silver dye was used to second dimension gels. Results were analyzed using a Molecular Analyst Software (BIO-RAD).

Differential Scanning Calorimetry (DSC): Differential scanning calorimetric studies were perform in a Polymer Rheometric Scientific. Samples (17 - 20 mg wet weight) were placed in DSC hermetic pans, ensuring a good contact between the sample and the capsule bottom. Triplicate samples were analyzed by scanning at 10 °C/min over the range of 15 - 100 °C. Total and partial denaturation enthalpies were estimated by measuring the area under the DSC transition curve.

Statistical Analysis: Analysis of variance was applied to the data using a SYSTAT statistical package ***(Wilkinson, 1990)***.

Results and discussion.

DSC thermograms of myofibrillar proteins control showed three endothermic transitions peaks with maximum temperature ($T_{máx}$) of 43.4, 52.6 °C and 68.7 °C associated to denaturation of principal components: myosin and actin. In the case of systems constitued by myofibrils incubated with MAL 30 mM can be observed a displacement to lower temperatures for the first transition. A significant change in the transition peaks, resulting in a flattened profile, was registered **(Figure 1)**. Also, the decrease in thermal stability was accompanied by significant modifications ($p < 0.05$) in the total and myosin area (ΔH_{total} and $\Delta H_{peak\ 1}$) **(Table 1)**. It was possible to suggest that MAL is related to the induction of structural changes being very notorious protein alteration developed along four hours of incubation.

Electrophoretic patterns of proteins treated with MAL 30 mM at different time of interaction, in ausence of ME, show a gradual decrease of intensity of some bands such as a very high molecular weight fraction (> 200) (top separator gel), ≈ 93 and (15-20) kDa **(Figure 2)**. In the presence of the disulfide splitting reagent, there were similar effects on myosin heavy chain (≈ 200 kDa) and myosin light chains (15 - 20 kDa) **(Figure 3)**. These changes could be related with a decrease in 0.6 M KCl solubility of modified proteins, associated to the formation of some grade insoluble pellets after centrifugation.

More information about the nature of bonds involved in protein species was obtained by bidimensional electrophoresis. Densitometric patterns of the second dimension (with ME) for two bands (band 1: > 200 kDa and band 2: ≈ 93 kDa) show for band 1 the appearence of species from 100 to 150 kDa and the increase of those of 50-100 and 15-20 kDa during the contact time with MAL. In case of band 2, could be registered a significant increment of molecular species of 50-100 kDa (data not shown). It suggest that MAL, a breakdown product of lipid oxidation, cause the formation of protein polymers by disulfide cross-linking.

Conclusions

Different thermal behavior and polymerization were observed when fish myofibrillar proteins were incubated with MAL. Aggregates of high molecular weight would involve disulfide bridges. This fact correlates with the appearance of new molecular species registered by differential calorimetric studies. In conclusion , MAL constitutes a promotor agent which action have important impacts in nutritional and technological aspects.

Pertinent literature.
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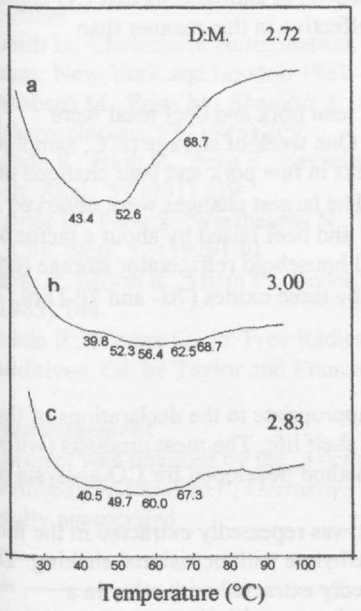

Table 1. Denaturation Enthalpies (ΔH_{total} and $\Delta H_{peak\ 1}$) corresponding to DSC thermograms of Sea Salmon myofibrillar proteins incubated with MAL 30 mM.

Time of incubation (h)	ΔH_{total} (J/g)	$\Delta H_{peak\ 1}$ (J/g)
0	40.3 ± 1.6	33.3 ± 1.1
4	20.5 ± 1.9 *	15.4 ± 1.3 *
8	16.4 ± 3.0 *	12.5 ± 2.9 *

Each value is represented as mean ± SD (n=3). An asterisk indicates that the values are significantly different from others in the same column ($p < 0.05$).

Figure 1. DSC thermograms of Sea Salmon myofibrillar proteins incubated with MAL 30 mM for different periods of time: a) control, b) 4 h and c) 8h. Heating rate: 10 °C/min. D.M.: dry matter.

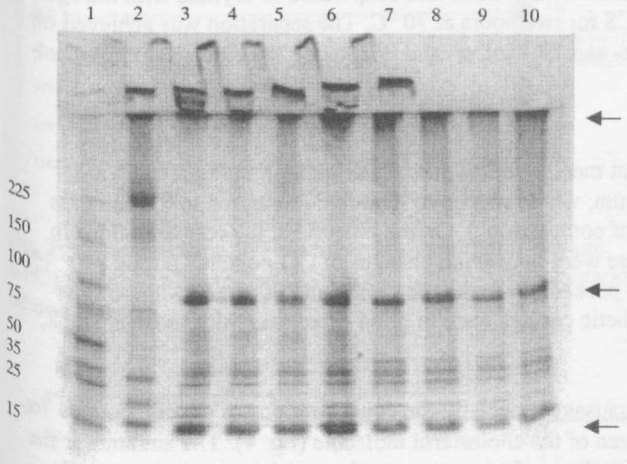

Figure 2. SDS-PAGE pattern of Sea Salmon myofibrillar protein (M) incubated with MAL 30 mM for different periods of time. Electrophoresis was performed on a 3 – 15 % gradient gel in ausence of ME. Lane 1: molecular mass standard; lane 2: rabbit myosin standard; lanes 3 – 5: control system (t = 0, 4 and 8 h respectively); lanes 6 – 10: M + MAL 30 mM (t = 0, 3, 4, 5 and 8 h respectively)

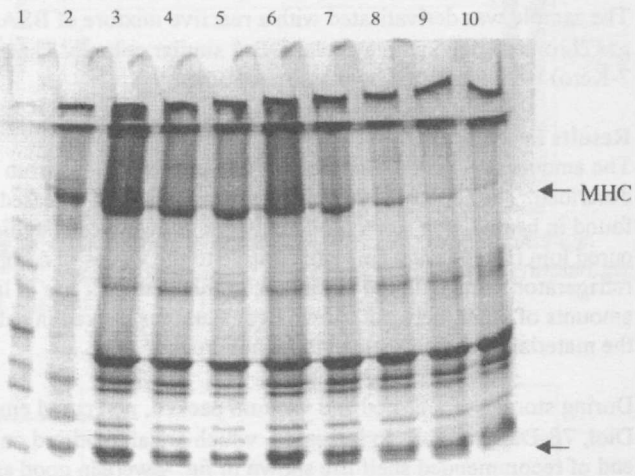

Figure 3. SDS-PAGE pattern of Sea Salmon myofibrillar protein (M) incubated with MAL 30 mM for different periods of time. Electrophoresis was performed on a 3 – 15 % gradient gel in the presence of 5% ME. Lane 1: molecular mass standard; lane 2: rabbit myosin standard; lanes 3 – 5: control system (t = 0, 4 and 8 h respectively); lanes 6 – 10: M + MAL 30 mM (t = 0, 3, 4, 5 and 8 h respectively). MHC: Myosin heavy chain.