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Study of the Histamine Production in a Red Flesh Fish (*Sardina pilchardus*) and a White Flesh Fish (*Dicentrarchus punctatus*)

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Abstract

The histaminic poisoning, known since 1910, still raises relevant questions. Several authors have agreed that the red flesh fish is more susceptible to accumulate histamine during deterioration, than the white one. This is why this type of food is often involved in this kind of poisoning.

High values of histamine concentration (77.7 mg/100g) was observed in sardines having stored for 24 hours at 30°C, while the rate of histamine in white flesh fish, under the same conditions and at the stage of rejection remained non-significant (2.52 mg/100g).

The initial pH and its evolution during alteration remained distinct for the two fish species. The microbiological study of the process indicated that the evolution of the total mesophile microflora and that of the lactic flora are faster in the case of sardine. A clear difference was also observed in the yeast stains between the two fish species. Indeed, it should be noted that whereas a prevalence of species having a fermentative metabolism (*Pichia farinosa* and *Debaryomyces hansenii*) was observed in the case of sardines (red flesh fish), a prevalence of non fermentative species (*Candida versatilis*) was observed in the case of sea bass (white flesh fish). Hence, we can distinguish two possible scenarios: one being due to the desamination of amino acid contained in white flesh fish; and the second being due to the decarboxylation of amino acids yielding to the production of histamine in red flesh fish.

Key Words: Sardine, sea bass, alteration, pH, yeasts.

Introduction

Several authors have agreed that the red flesh fish is more likely to accumulate histamine during alteration; that is why this type of aliments is often implied in the histaminic intoxications. But the factors which do not imply the white fish in such intoxications are seldom studied and discussed (Stratton *et al.*, 1991). The amines found in food are mostly composed of histamine, cadaverine, tyramine, tryptamine and putrescine. Histamine is the most studied amine (Ababouch *et al.*, 1991; Berdy *et al.*, 2000; Ozugu *et al.*, 2002; Predy *et al.*, 2003) because of its presence in fish used for alimentation.

This study was conducted in order to complete studies reported on the production of biogenic amines in fish (Afilal, 1998; Ababouch *et al.*, 1991; Afilal *et al.*, 1995; Afilal and Zlaiji, 1997). Indeed, we report herein a comparative study between the alteration of red flesh fish such as sardines and a white flesh fish such as sea bass at 30°C.

Materials and methods

Origin of Fish and Sampling

The study was carried out on two fish species, red flesh fish, the sardines, of the Clupeidae family, *Sardina pilchardus* species which is of small size (25 to 35 unit in a kilogram), and white flesh fish, the sea bass, of Serranids family, *Dicentrarchus punctatus*, species with an average size of 2 to 3 fish in kilogram.

The analysis was carried out in February on sardines originated from the Mediterranean coast of Short-nap cloth Kebdana (100 km far from Oujda). The sea bass was sampled in March from Nador lagoon on the Mediterranean coast, then transported under ice to the laboratory.

Chemical Analysis

The pH measurement of the muscle was carried out according to the method described by Elmarrakchi *et al.* (1990), and the dosage of the TVB-N (total volatile basic nitrogen) by the Kjeldahl method. The histamine quantification in the muscle is carried out by spectrofluorimetry (Lerk and Bell, 1976).

Microbiological Analysis

Four microbial groups were counted during fish alteration: total aerobic mesophile flora on "Trypticase Soy Agar" (TSA) medium, Enterobacteria on the Mac Conkey medium, lactic bacteria on De Man Rogosa and Sharpe (MRS) medium and yeasts on PDA medium (potato dextrose agar) (Deak and Beuchat, 1987; Niven *et al.*, 1981). Plates seeded by bacteria were incubated in an incubator at 30°C

during 48 to 60 hours in the case of bacteria and 3 to 4 days in the case of yeasts and lactic bacteria.

Results and Discussions

During alteration, sardines showed a high accumulation of histamine (77.7 mg/100g) whereas in the case of the sea bass under the same conditions of alteration, this accumulation remained weak (2.52 mg/100g) as shown in Figure 1 below.

Among the factors explaining these results, one quotes the abundance of histidine in the proteins present in the red flesh fish. During alteration, amino acids were released following peptide degradation under the action of proteases and microbial peptidases. Then the free amino acids were decarboxylated into biogenic amines by bacterial decarboxylases. Thus the accumulation rate of various amines remained proportional to the rate of free amino acids. In sardines, the quantity of histamine was always higher compared to other biogenic amines, because the free histidine was the most abundant amino acid. Indeed, one found more than 280 mg/100 g of free histidine, and approximately 1 g/100 g of total histidine (Ababouch *et al.*, 1991; Morrow *et al.*, 1991).

The difference in the histamine accumulation between the two studied species, was not a unique phenomenon, but seemed to be a consequence of a scenario of alteration, based on several other differences between the two types of fish.

Evolution Differences of Organoleptic Characters

In the study of the sensory variations between the two species, during alteration, we noticed that the limit sensory acceptance for sardine arouse following 7 hours of storage at 30°C. Indeed, the phase of putrefaction (release of bad smells) started after 20h of storage. In the case of the lagoon sea bass, produced by pisciculture under controlled feeding, no putrid odor was noticed even after 36 hours.

In general, fish species with red flesh contained more lipids in their muscles compared to fish having white flesh. Some micro organisms had lipoxydases, which catalyzed the reaction between the accumulated free fatty acids and molecular oxygen; the resulting products, were degraded into aldehydes and ketones. The increased putrefaction in certain cases was related to the relative abundance of lipids and the rancidity was related to the formation of propanol, pentanol, ethanol, produced by peroxidation (Bennour *et al.*, 1991). In addition, deterioration at ambient temperature was characterized by an important micro-organisms proliferation which produced H_2S (Gram, 1989; Huss, 1988; Jorgensen *et al.*, 2000).

Sardina pilchardus, which has a fast growth and short longevity (6 years on average), is primarily planctophage (first level) and also zoo-planctophage (second level of the food chain), whereas the basic food of *Dicentrarchus punctatus*, which are a perciforme and erratic coastal, is constituted of shellfish and fish.

pH Variation

The pH variations during alteration of the two species showed in the beginning a light fall due to the accumulation of lactic acid, followed in both cases by an increase in pH which was due to the accumulation of nitric compounds as a result of the alteration of proteins (Figure 2).

It must also be noted that the initial pH was more acid in sardines (pH = 6.2) which would have consequences explaining the difference of the alteration scenario between the two kinds of fish. This observation suggested that microbiological and biochemical reactions were different. Thus, several studies showed that the conjunctive fish tissue was less resistant to pH acid which enhanced microbial invasion of the muscle (Koutsoumanis *et al.*, 2000; Koutsoumanis, 2001). In the same way, the histamine production was more active at pH acid and the optimum pH for the histidine decarboxylase seemed

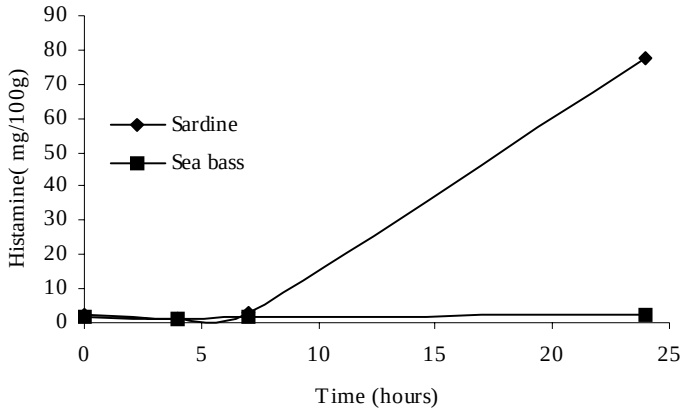

Figure 1. Histamine evolution in the muscles of sardine and sea bass during storage at 30°C.

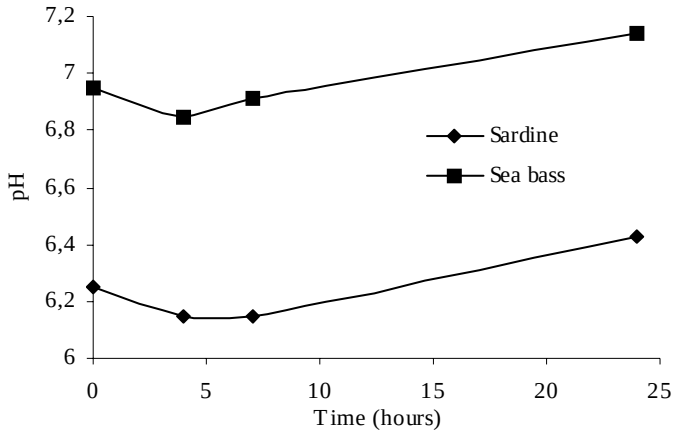

Figure 2. pH evolution of the muscles of sardines and sea bass during storage at 30°C.

to be within the range of 2.5 to 6.5 depending on micro-organisms (Afilal and Zlaiji, 1997). The pH values from 6.0 to 6.3 were also favorable for the oligopeptidases which degraded peptides and made amino acids more accessible to the decarboxylases.

The red muscle contained more carbohydrates than the white one. The alteration of the sugar in anaerobes made the pH of sardine, after catching, "between" 5.5 to 6.5. This created more favourable conditions for the histamine production.

Variation of TVB-N

The total volatile basic nitrogen TVB-N for the two fish species was the same (Figure 3). It evolved in the same direction during the first hours following storage to reach after 7 hours a value of 23.52 $mgNH_3$/100g in sardine and 25.52 $mgNH_3$/100g in the sea bass. After 24 hours at the same temperature, the rate of the TVB-N increased in the case of the latter to reach a value of 161.28 $mgNH_3$/100g, whereas for sardine it reached a value of about 84.00 $mgNH_3$/100g.

The changes of the TVB-N values were attributed partly to the desamination of the free amino acids (Elmarrakchi *et al.*, 1990). Thus one can say that in white fish, the degradation of the amino acids during alteration followed the way of desamination and none the decarboxylation. This explained the weak rate of accumulated histamine in the case of the sea bass, which would be a consequence of evolution of particular microflora characteristic to this species after a certain stage of latency.

Thus, the scenario of sequences of alteration stages in sardines started with an initial pH acid, which would support fast invasion of the muscle by both, the contaminant microflora and the commensal one, from which amino acids were released along with stressing fermentation reaction. The tendency to a more acidic pH would be unfavorable for the growth of many micro-organisms, which started a tendency to the regulation by decarboxylation of the amino acids releasing alkaline amines. In the case of the sea bass, the scenario of alteration would rather lead to the desamination, since the pH remained around 7.

Quantitative and Qualitative Variation of the Microflora

The microbiological analysis is summarized in Table 1, Figure 4 and 5 also showed clear differences between the two fish species. Indeed, the initial SPC (standard plate count) value in sardine was about 7.7 10^5 cfu/g, whereas in the case of the sea bass this value was about 4.4 10^5 cfu/g. The speed of growth of SPC was accentuated in the case of sardine, leading after 24 hours of storage, to a microbial value higher than 30 10^6 cfu/g. This bacterial growth was accompanied by a qualitative evolution of the micro flora which appeared owing to a prevalence of mesophiles bacteria which have a bacilli Gram negative shape (Table 2). Thus, the deterioration of stored Moroccan sardines at 30°C was mainly due to the multiplication of mesophiles species. Indeed, in the cold seas, there was prevalence of the *Enterobacteria psychrophilus* or psychrotrophes bacteria. On the contrary, in hot seas there was the prevalence of Gram positive mesophiles bacteria (Gram, 1989; Huss, 1988; Koutsoumanis, 2000). And, in the case of the moderated zones such as Morocco, the average temperature allowed the cohabitation of a diversified and heterogeneous flora although dominated by mesophiles.

A qualitative difference was also observed in the case of yeasts (Table 2). The identified yeasts were: *Candida versatilis* (Asporogéne) and, *Pichia anomala, Saccharomyces cerevisiae* (Sporogénes). It is worth to note that a predominance of stains with the ability to ferment sugar (*Pichia farinosa* and *Debarypmyces hansenii*) was observed in sardines. These results were also reported by Deak and Beuchat (1987).

Lactic bacteria were not present in the initial sardine microflora, whereas in the case of the sea bass

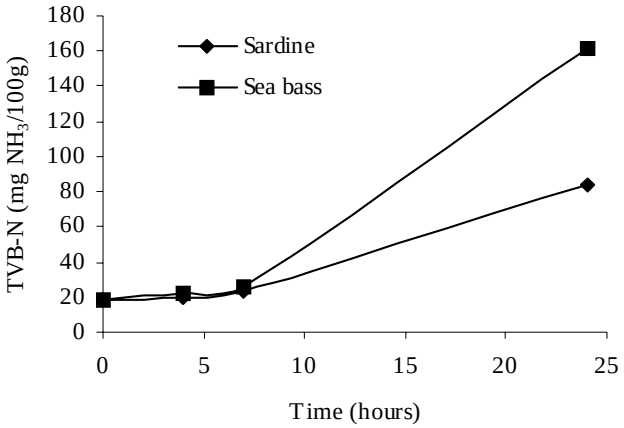

Figure 3. TVB-N evolution of the muscles of sardines and sea bass during storage at 30°C.

Table 1. Evolution of sardines and sea bass microflora during incubation at 30°C (n.d: not determined)

	Initial Flora		Flora after 24h at 30°C	
	Sardines	Sea bass	Sardines	Sea bass
SPC 10^5cfu/g	7.7	4.4	300	159
Enterobacteria 10^5cfu/g	1.26	1.91	26.45	20.9
Lactic Bacteria 10^5cfu/g	n.d	0.133	1.35	7.05
Yeasts and moulds 10^5cfu/g	1.05	0.54	155	50.05

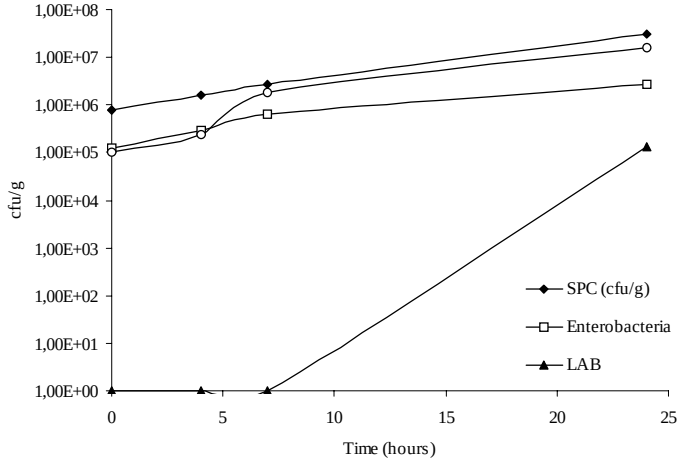

Figure 4. Micro flora evolution of the sardines during storage at 30°C.

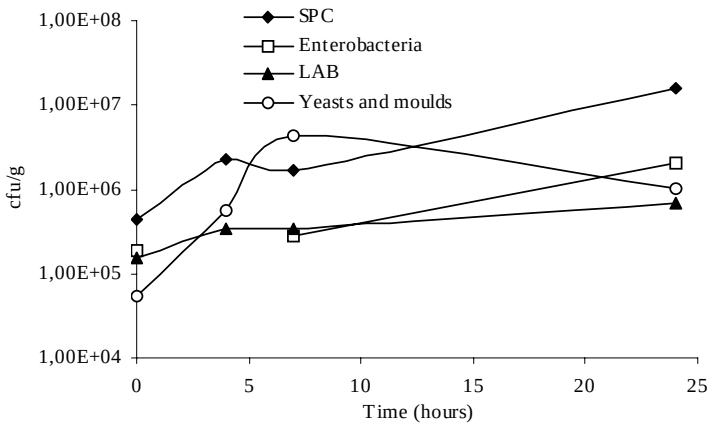

Figure 5. Sea bass microflora evolution during stockage at 30°C.

Table 2. Distribution of the yeast species isolated from sea bass and sardines during alteration at 30°C

	Sardines		Sea bass	
	number	%	number	%
Pichia anomala	13	34.2	8	57,14
Debaryomyces hansenii	11	28.9	2	14.28
Candida versatilis	3	7.9	2	14.28
Pichia farinose	5	13.1	0	0
Saccharomyces cervisiae	6	15.9	2	14.28
Total	38	100	14	100

the SPC was about 3.1 10^4 cfu/g (Figure 4, Table 2). This made it unlikely that the two fishes were contaminated quantitatively and qualitatively in the same way. Researches are undertaken to confirm this result.

The studied lactic bacteria were Gram positive, motionless and negative catalase, which were nutritional exigent. This explained the latency time observed in their evolution during alteration of sardines (Figure 4). This result was in agreement with the fact that intense enzymatic activities were taking place in fish immediately after death, implying the degradation of ATP, glycogen and phosphocreatine (Elmarrakchi *et al.*, 1990; Leroi and Courcoux, 1996). These reactions often occurred in a very short time and yielded a lot of products with low molecular weight such as inosine, ribose, lactic acid and creatin which were added to other extractable compounds from muscle. The mixture would constitute a substrate for bacterial growth and particularly for the most demanding ones such as certain strains of Lactobacillus (Leroi and Courcoux, 1996; Leroi and Pidoux, 1993a).

The yeasts did not seem to be implied directly in the formation of histamine (Leroi and Pidoux, 1993b); this did not exclude a possible indirect contribution of certain strains to the production of amines, by stimulation of the growth and bacterial metabolism (Standara *et al.*, 2000; Kaak and Krizek, 2003). Thus the rate of accumulated histamine during fish alteration would not depend solely on the presence of the strains having histidine decarboxylase, but would depend on the whole micro flora. Hence, the fast accumulation of histamine in sardines would be related to a more important proliferation of certain yeasts.

Conclusion

The implication of red fleshed fish in the histaminic intoxications would be due to the biochemical composition and the nature of the contaminant and commensal flora. The red muscle contained more lipids, sugars and histidine, which promoted a particular flora activated by acidic pH during alteration, and favourable to the release of amino acids such as histidine which became accessible to decarboxylation rather than desamination.

Other studies are necessary on different species in order to check all these assumptions on the alteration of red fleshed fish. These studies will make it possible to understand the role of the chemical composition in the selection of the commensal and contaminant flora, and to possibly propose adapted diets, in fish breeding, in order to minimize the consequences of fish alteration. These studies will surely contribute to preserve the public health by the knowledge of biochemical and microbiological mechanisms of fish deterioration.

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