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Santiago P. Aubourg

Influence of formaldehyde in the formation of fluorescence related to fish deterioration

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Abstract In previous studies fluorescence detection at different excitation/emission maxima during common fish processing has been used. A bathochromic shift towards higher wavelength maxima was observed and measured as the ratio between absorption at two of the maxima tested. This fluorescence ratio (δF) value correlates positively with fish damage. In the present work, the influence of formaldehyde (FA) on the value δF was studied. A model system was set up in which FA reacted at 30 °C for 25 days with propylamine and fish muscle. It was observed that FA was less able to produce fluorescent compounds compared with common fish oxidation products that were also tested, i.e. propanal and hexanal. However, in the presence of both lipid oxidation aldehydes, the FA-containing mixtures led to a higher δF value. Model systems consisting of FA and fatty fish (sardine) muscle produced more fluorescence than FA and lean fish (cod), because of the formation of lipid oxidation compounds under the reaction conditions of the former systems. It is thus concluded that the presence of FA in a reacting medium enhances fluorescence formation, such that δF be can used as an accurate measure of fish damage. It is thought that measurement of δF in processes such as the freezing of gadoid fish, in which both FA and lipid oxidation are produced, could be of benefit.

Key words Formaldehyde · Amines · Fish · Fluorescence · Lipid oxidation

Introduction

Rancidity plays a significant role in fish damage that occurs during processing [1, 2] because marine life lipids contain a high proportion of polyunsaturated fatty acids (PUFA) [3]. It has been proven that primary and secondary lipid oxidation products may react with biological amino constituents (proteins, peptides, free amino acids and phospholipids) to produce interaction compounds [4–7], resulting in browning [8], flavour changes [9, 10] and a loss of essential nutrients [11, 12].

The analysis of these interaction products by fluorescence detection is used to complement other more developed measurement techniques when assessing lipid damage [13, 14]. In recent studies, the fluorescence properties of processed fatty fish samples at different excitation/emission maxima have been measured. A fluorescence shift towards higher wavelength maxima was observed as a result of increasing the time and temperature of processing. The fluorescence ratio between two of the maxima tested (393/463 nm and 327/415 nm) showed an interesting correlation with fish quality [15–17].

Lipid damage during the freezing of lean fish has been studied [18, 19]; however, most attention, especially in the case of gadoid fish species, has been given to the formation of formaldehyde (FA). During the freezing of such species, FA is produced, along with dimethylamine, by enzymatic reduction of trimethylamine oxide; it is a highly reactive molecule that leads to inter- and intramolecular linkages between protein chains. As a result, protein denaturation and the loss of quality of frozen fish have been associated with the formation of FA [20–23].

Much work has been carried out in order to clarify the possible mechanisms of FA-mediated protein denaturation [23, 24]. The interaction of FA with amino compounds has been monitored [25–27], the relative influence of FA and lipid damage products in texture changes has been evaluated [28–30] and some fluorescence development related to fish quality during freezing has been recognized [31, 32].

The present work was envisaged in order to study the influence of FA in fluorescence development during the formation of interaction compounds in processed fish. Model systems consisting of FA and a primary amine (propylamine) and fish (cod and sardine) muscle were studied. The fluorescence ratio detected during fatty fish processing was measured [15–17]. A heating temperature

S. P. Aubourg
Instituto de Investigaciones Marinas (CSIC), c/E. Cabello 6,
E-36208 Vigo, Spain

Table 1 Fluorescence ratio (δF) values[a] obtained from the aldehyde-propylamine reacting systems. (*PAM* Propylamine, *FA* formaldehyde, *Pal* propanal, *Hal* hexanal)

Reaction time (days)	Reaction mixture			
	PAM	FA+PAM	Pal+PAM	Hal+PAM
1	1.50 b (0.20)	0.77 a (0.03)	8.34 d (0.07)	2.01 c (0.07)
3	1.58 b (0.20)	0.93 a (0.05)	17.84 d (0.17)	6.09 c (0.27)
7	1.50 a (0.20)	1.04 a (0.02)	33.05 c (0.71)	15.95 b (0.16)
14	1.56 a (0.20)	1.10 a (0.04)	49.92 c (0.88)	29.93 b (0.18)
25	1.66 a (0.20)	1.12 a (0.10)	75.13 c (1.92)	49.52 b (0.53)

[a] Mean values of three independent determinations. Values in the same *row* followed by different *letters* are significanty ($P < 0.05$) different. Standard deviations are indicated in *parentheses*

of 30 °C was chosen so as to accelerate the formation of interaction compounds . The resulting fluorescence values for the FA systems were compared with those obtained by the use of common lipid oxidation products (propanal and hexanal). The presence of both aldehydes in the FA-containing mixtures was also investigated.

Materials and methods

Chemicals employed along the present work were reagent grade (Merck, Darmstadt, Germany).

Propylamine model systems. A 0.1 M solution of propylamine and a 0.5 M solution of each aldehyde (FA, propanal and hexanal) were prepared in aqueous 86% ethanol.

In the first type of reaction mixture, 10 ml of each aldehyde solution, 10 ml of propylamine solution and 10 ml of aqueous 86% ethanol solution were mixed and placed in 30-ml stoppered tubes.

In the second type of reaction mixture, 10 ml of propylamine solution, 10 ml of FA solution and 10 ml propanal or hexanal solution were mixed and placed in 30-ml stoppered tubes.

Both kinds of reaction mixtures were incubated at 30 °C in the dark without stirring and were sampled at 1, 3, 7, 14 and 25 days of storage for fluorescence analysis. Reaction mixtures were triplicated for the whole of the experiment. Blanks consisting of the starting compounds (propylamine and aldehydes) were also examined under the same reaction conditions.

Fish muscle model systems. Fresh cod (*Gadus morhua*; six individuals) was divided into three batches. In each batch, the cod white muscle was separated out. Minced cod portions (4 g) of each batch were mixed with 7 ml of aqueous 86% ethanol solution and 3 ml of each 0.5 M aldehyde (formaldehyde, propanal and hexanal) solution and placed in 30-ml stoppered tubes.

Fresh sardine (*Sardina pilchardus*; three dozen) was divided into three batches. In each batch, the sardine white muscle was separated out. Minced sardine portions (4 g) of each batch were mixed with 7 ml of aqueous 86% ethanol solution and 3 ml of 0.5 M FA solution and placed in 30-ml stoppered tubes.

For both kinds of fish muscle systems, the reaction mixtures were held at 30 °C in the dark without stirring and, at definite time intervals (1, 7 and 25 days) were subjected to fluorescence analysis. Blanks consisting of the starting reagents (minced cod and sardine muscles, FA, propanal and hexanal) were also examined using the same reaction conditions throughout.

Fluorescence analysis. Reaction mixtures containing fish muscle were centrifuged (3000 *g* for 10 min) and filtered. The liquid part was exposed to UV light (350 nm) for 30 s to destroy any retinol present in the extract and then analysed by fluorimetry. In the case of model systems containing propylamine, the reaction mixtures were analysed directly.

Fluorescence measurements (Perkin-Elmer LS 3B) were made at 327/415 nm and 393/463 nm excitation/emission maxima. Relative fluorescence values (RF) were calculated as $RF = F/F_{st}$, where F is the sample fluorescence at each excitation/emission maximum, and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 μg/ml in 0.05 M H_2SO_4) at the corresponding wavelength. The fluorescence ratio (δF) was calculated as: $\delta F = RF_{393/463\ nm}/RF_{327/415}$ nm [16, 17].

Statistical analysis. Data corresponding to δF were subjected to the ANOVA one-way method. Mean results were compared by the LSD test using the Statistica package Statsoft, Tulsa, USA. Significance was declared at $P < 0.05$.

Results

As a first step, the role of FA in the formation of fluorescent compounds in a model system with a primary amine (propylamine) was studied. The δF value of the FA-containing medium increased a little after 25 days of reaction (Table 1). A comparison with two lipid oxidation compounds was carried out; propanal and hexanal where chosen because of their widespread formation during ω3-PUFA and ω6-PUFA (respectively) oxidation [33, 34]. Both aldehyde systems led to a progressive increase in δF as the reaction proceeded, reaching values at the end of storage that were higher than those achieved in the FA medium. None of the aldehyde (FA, propanal and hexanal) blank solutions produced any significant changes throughout the course of the experiment. The same result was observed in the case of propylamine (Table 1).

Propanal gave rise to a higher δF value than hexanal, which can be explained on the basis of it being a smaller molecule with greater electrophilic reactivity. The positive inductive effect of the pentyl radical is known to be greater [35] than that of the ethyl radical, resulting in a more reactive carbonilic carbon (a stronger electrophile) in the case of propanal.

A different conclusion about the effect of aldehyde chain length was obtained by Montfoort et al. [36], who carried out a comparative study of fluorescence formation by exposing liposomes containing phosphatidyl ethanolamine

Table 2 Fluorescence ratio (δF) values[a] obtained by reaction of FA with PAM in the presence of other aldehydes

Reaction time (days)	Reaction mixture		
	FA+PAM	FA+PAM+Pal	FA+PAM+Hal
1	0.84 c (0.01)	0.77 b (0.04)	0.58 a (0.03)
3	0.90 b (0.04)	0.96 b (0.01)	0.78 a (0.03)
7	1.07 a (0.06)	1.23 b (0.04)	1.28 b (0.06)
14	1.13 a (0.01)	1.57 b (0.05)	2.28 c (0.07)
25	1.17 a (0.02)	1.58 b (0.04)	3.11 c (0.05)

[a] Mean values of three independent determinations. Values in the same *row* followed by different *letters* are significantly ($P < 0.05$) different. Standard deviations are indicated in *parentheses*

(PE) to a variety of aldehydes. The fluorescence formation increased with the chain length of saturated aldehydes; however, fluorescence was investigated at a single excitation/emission maximum, so that a bathochromic shift could not be evaluated. Another difference with the present experiment is that the amine used (i.e. PE) was more lipophilic, so that an aldehyde of longer chain length could facilitate the interaction with such an amine.

The next experiment was to study the formation of fluorescence in mixtures containing both FA and the same primary amine in the presence of lipid oxidation compounds. Again, propanal and hexanal were employed. Results are indicated in Table 2. It can be concluded that such mixtures produced a greater δF compared with model systems that contained FA and amine alone (Table 1). However, hexanal and propanal systems but no FA produced higher δF values. This result could be explained on the basis of the higher reactivity of FA (smaller molecule having a more electrophilic carbonilic carbon) that is able to interact more quickly with the amine, which is five times less concentrated than any of the aldehydes in the reaction medium.

The following step was to study a more realistic system consisting of fish muscle. The effect of addition of FA, propanal and hexanal to the white muscle of cod (Table 3) was studied. As a control, the δF value of the blank muscle medium did not significantly increase during storage. At the same time, addition of FA to cod muscle produced a significant increase in δF after 25 days of reaction. Accordingly, it was deduced that FA exerts a positive effect on fluorescence formation. However, the value of δF obtained was clearly lower than those obtained from reaction mixtures containing both lipid oxidation aldehydes. Again, fluorescence development was greater in the propanal medium than in the hexanal medium.

Finally, the role of FA in the formation of interaction compounds with fluorescence properties was studied using a reaction medium consisting of sardine muscle. The results are indicated in Table 3.

The addition of FA to a fatty fish muscle led to a greater increase in δF than in the case of cod. A significant increase was obtained after 7 days of reaction. This greater fluorescence development can be explained by the fact that fatty fish undergoes greater lipid oxidation than lean fish [1, 9]. At the same time, the δF of the blank sardine muscle was significantly increased at the end of the storage, which can be explained by the interaction between amino compounds from the muscle and oxidation molecules formed from muscle lipids under the particular reaction conditions used [15–17]. This increase is clearly smaller than that obtained

Table 3 Fluorescence ratio (δF) values[a] obtained from the aldehyde-fish muscle reaction systems. (*MCM* Minced cod muscle, *MSM* minced sardine muscle). Aldehydes as expressed in Table 1

Reaction mixture	Reaction time (days)		
	1	7	25
MCM	0.88 ab (0.10)	0.97 a (0.16)	1.18 a (0.31)
FA+MCM	1.18 b (0.01)	1.18 a (0.04)	1.53 a (0.10)
Pal+MCM	6.03 d (0.79)	22.63 d (2.61)	37.08 d (1.57)
Hal+MCM	3.23 c (0.23)	18.17 c (1.48)	26.81 c (2.18)
MSM	0.53 a (0.07)	0.65 a (0.11)	1.21 a (0.09)
FA+MSM	1.48 b (0.11)	3.46 b (0.09)	6.73 b (0.30)

[a] Mean values of three independent determinations. Values in the same *column* followed by different *letters* are significantly ($P < 0.05$) different. Standard deviations are indicated in *parentheses*

in the presence of FA, indicating that FA plays a significant role in fluorescence formation, as in the case of the cod system.

Discussion

In previous studies of fatty fish processing [15–17], lipid deterioration compounds have caused the formation of interaction compounds, whose fluorescence properties have provided a valuable method for quality assessment. The present experiment was envisaged in order to study the formation of such fluorescent compounds in fish samples containing FA and lipid oxidation compounds as a result of processing damage.

Different kinds of reaction model systems were tested. All kinds of FA experiments showed a bathochromic shift (δF increase), although of varying size, according to the general theory of progressive formation of addition compounds where amino and aldehyde compounds are involved [5, 6, 8].

In spite of its greater reactivity, FA has less ability than the lipid oxidation compounds to lead to fluorescence development. However, the results obtained in the studies of both kinds of fish model systems indicated that FA had a positive effect on δF value, so that an interaction could be observed by fluorescence spectroscopy. On the basis of these results it is postulated that fluorescence detection of interaction compounds could be employed as a complementary tool to assess the quality of fish species during processing, during which FA formation (freezing of gadoid species) and lipid oxidation (enzymatic and non-enzymatic) take place. In this sense, further research is needed in order to compare such fluorescence detection with commonly used damage indices during the freezing of gadoid species.

The implication of FA in toxicological and nutritional issues has attracted much attention too [23]. The participation of FA in the formation of fluorescence compounds could bridge gaps in our knowledge of the relationship observed between formation of interaction compounds during the storage/processing of foods and the pigmented and fluorescent granules found in human and animal tissues (lipofuscin) [7, 37, 38].

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