

Recent Advances in Assessment of Marine Lipid Oxidation by Using Fluorescence

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ABSTRACT: Lipid changes during food processing are important because of their impact on the final product quality. Lipid damage detection is limited because of the ability of lipid oxidation products (i.e., hydroperoxides and carbonyl compounds) to produce interaction compounds by reacting with nucleophilic food constituents. Fluorescence quantification at a single excitation/emission maximum of these interaction compounds has been employed in a qualitative way as a complementary tool for food quality assessment. The present work reviews recent research where simultaneous detection at different excitation/emission maxima was employed to assess lipid oxidation and quality changes during fish processing. A fluorescence shift towards a higher wavelength maxima was detected as a result of lipid damage; the shift was calculated as the ratio (δF) between two of the maxima tested (393/463 nm and 327/415 nm) and was investigated along different fish processes (freezing and frozen storage, refrigerated storage, cooking, and canning) and in complementary model systems where the influence of different factors (time and temperature of processing, amine and aldehyde composition and content, formaldehyde presence, and pH value of the medium) was checked. Determination of the δF value provided better results for quality change assessment in fish products than most of the lipid quality indices, in addition to being rapid and sensitive.

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KEY WORDS: Fish processing, fluorescence, food, lipid-amine interaction compounds, lipid damages, lipid oxidation, marine species, quality assessment.

From the moment a biological species is killed, captured, or harvested until it is consumed as a food, its original properties may be modified as a result of changes of chemical constituents undergone during the specific conditions of processing. Rancidity of fats and oils has been shown to play a very important role in the degradation processes that take place in foods (1,2). During processing and storage, enzymatic and nonenzymatic lipid oxidation occur. A close relationship has been found between lipid damage and quality of the final product (3,4). Lipid degradation is especially important in foods coming from biological species where a high unsaturated lipid composition is present, such as in marine species

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that contain a high content of polyunsaturated fatty acids (PUFA) (5,6).

PUFA damages can lead to primary and secondary lipid oxidation products, which can react with amino constituents (proteins, peptides, free amino acids, and phospholipids) to produce interaction compounds (7–9). As a result, browning, flavor changes, and loss of essential nutrients occur (10–12).

Many methods have been used to measure primary (hydroperoxides) and secondary (carbonyl compounds) oxidation products to determine deterioration. There are, however, difficulties associated with common methods, especially where much damage has occurred, as in the case of biological species supporting great fat contents and PUFA proportions when processed by heat treatments. Oxidation products are unstable and tend to react with food constituents, so their detection may not be a valid tool for quality assessment in all cases (13,14). As a result, the detection of interaction compounds has recently attracted attention in assessing food quality. Fluorescence analysis has been widely explored in this regard. The present work reviews recent research employing fluorescence detection of interaction compounds as a quantitative tool for quality assessment. The formation, significance, and detection of the interaction compounds and previous use of fluorescence for food quality assessment are considered.

INTERACTION COMPOUNDS: FORMATION AND SIGNIFICANCE

The electrophilic character of most lipid oxidation compounds leads them to interact with food constituents possessing nucleophilic functions. The interactions are very important secondary reactions of oxidized lipids, particularly in protein-rich foodstuffs such as marine sources, which have a high proportion of essential and reactive amino acids such as lysine and methionine (15).

Chemical bonds of interaction compounds produced in foods may be of three types (7): physical, ionic, and covalent. The physical bonds (e.g., hydrogen bonds between polar groups and physical forces between hydrophobic groups) are usually weak, and therefore easily split by the action of solvents like methanol. However, if several such bonds are present in the molecule, their combined strength may become suf-

ficiently great to resist the polar solvents. The hydrogen bonds are formed almost immediately, even at room temperature. The formation of these bonds is manifested by a decrease in solubility of the lipid fraction in organic nonpolar solvents. The ionic bonds are also relatively weak and easily split by polar solvents, and, therefore, are less important in products of oxidized lipids with proteins.

Covalent bonds are relatively strong and resistant to the action of solvents. Their formation proceeds slowly (within several weeks at room temperature), but is accelerated to a few days by heating. The structure of covalent interaction compounds can vary, depending on the lipid and protein groups interacting (Scheme 1). Biological amines may react with lipid hydroperoxides (Reaction 1) and aldehydes (Reaction 2). In both cases, an adduct compound (Schiff base) is produced. If this reaction is repeated several times, the conjugated double bond system is enlarged and the color intensity of the molecule formed is increased (Reaction 3) (1,7,8).

As in the case of Maillard reactions between sugars and amino acids, the interactions between oxidized lipids and proteins affect the nutritional and sensory values of food. Several effects on nutritional value can be noted (7,12): (i) decrease of biological value of protein (blocking of free ϵ -amino groups of L-lysine by reacting with alkanals; oxidation of sulphide group of L-methionine; changes in other amino acids: cysteine, cystine, triptophane, histidine and tyrosine); (ii) changes in digestibility (decreased rate of lipolysis by lipases; decreased rate and extent of proteolysis by digestive enzymes); and (iii) deactivation of toxic substances produced by lipid autoxidation (lipid hydroperoxides and lower aldehydes).

Formation of interaction compounds can also influence the sensory value of food in various ways (7): flavor, color, and texture. Some flavor substances produced by lipid oxidation, e.g., lower aldehydes, may be bound to protein on storage or heating. The browning reaction is the most conspicuous change in sensory properties due to these interactions. The presence of polyenoic fatty acids and free amine groups of protein seems indispensable for the formation of brown prod-

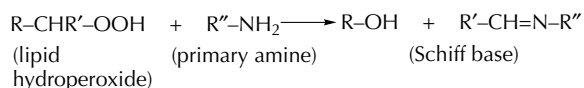
ucts. A linear correlation has been detected among browning and the extent of aldehyde-protein interaction and the formation of nonextractable lipid-protein products (7). Changes of texture are mostly due to crosslinking of peptide chains by reaction with lipid oxidation products, such as aldehydes.

First attempts to study the formation of interaction compounds were carried out to assess lipid oxidation in living tissues (16,17). For a long time, microscopists studied the so-called "age-pigments" or "lipofuscin" resulting from degenerative processes in cells such as aging, environmental stress, and vitamin E deficiency (18,19). They found that peroxidation of membrane phospholipids disrupts membrane structure and function so that changes may be manifested by decreased membrane fluidity, altered membrane-protein activity, and the failure of the membrane to act as a semipermeable barrier (20).

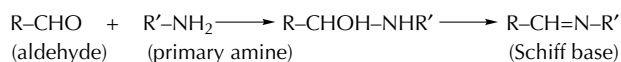
Thus, solution fluorescence spectrophotometry was used as a method to measure the extent of peroxidation in living material. Tappel *et al.* (16) studied the fluorescent chromophore $R-N=CH-CH=CH-NH-R$, which they believed to be responsible for the spectral properties observed in lipofuscin according to a malondialdehyde (MDA) interaction with $-NH_2$ groups from biological molecules, to produce the mentioned Schiff base. Later, Nair *et al.* (21,22) proved the difficulty in obtaining the proposed fluorophore starting from pure MDA in the presence of amino compounds, while Kikugawa *et al.* (17) isolated 1,4-dihydropyridines as a new type of fluorescent compound produced by the reaction of MDA and amino compounds.

As a result, evidence on the importance of other carbonyl compounds in the formation of lipofuscin-type fluorescent products surfaced. Tsuchida *et al.* (23) suggested that the water-soluble fluorescent substances in the protein fraction of mouse and human sera might not be conjugated Schiff bases formed from protein and MDA but due to some other stable compounds. It was suggested (24,25) that fluorescent chromolipids formed by lipid peroxidation are derived from 4-hydroxynonenal or similar reactive aldehydes, since the formation of high molecular weight proteins more or less parallels the formation of chromolipids, but not the formation of MDA.

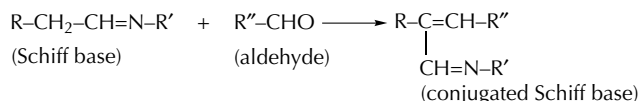
Reaction 1:



Reaction 2:



Reaction 3:



SCHEME 1

DETECTION OF INTERACTION COMPOUNDS

Different analytical procedures have been employed in order to follow the interaction between oxidized lipids and proteins. Remaining $-SH$ and $-NH_2$ groups in different kinds of protein extracts have been assessed by means of specific reactions; techniques such as sodium dodecyl sulfate-electrophoresis, permeation chromatography, high-performance liquid chromatography (HPLC), ultraviolet-absorption, and brown color development have been employed (26–28). Capillary zone electrophoresis (29) proved useful for separating small amounts of typical complex mixtures of Maillard reaction products arising from mixtures including free amino acids and aldehydic sugars; separation and partial characterization were carried out. The advantages of capillary elec-

trophoresis in separating melanoidin compounds were assessed by comparison with reversed HPLC (30).

Once fluorescent compounds were successfully used as an analytical method for quantifying peroxidation damage to biological tissues, this technique was widely employed to assess lipid oxidation in foods. Recently, this kind of detection has become the most employed method for analyzing interaction compounds during food processing. In the next section, some examples of this application will be reviewed.

Fluorescence is a rapid and sensitive method for characterizing molecular environments and events. Its theory and methodology have been extensively employed for studies of molecular structure and function in the disciplines of both chemistry and biochemistry (20). Fluorescence spectrophotometry is characterized by two distinct spectra: excitation and emission. Emission occurs at a longer wavelength than the incident light (excitation) because the energy of the emitted radiation differs from that absorbed by an amount equal to the vibrational energy lost to the surroundings (31).

LIPID OXIDATION ASSESSMENT IN FOODS BY FLUORESCENCE DETECTION

Fluorescence research has been conducted on different kinds of foods and processes during the last three decades. Frequently, the fluorescence spectrum is studied and then a single excitation/emission maximum is chosen to be quantified and employed for lipid oxidation assessment; however, in most applications, fluorescence is used as a qualitative tool complementary with other more developed techniques. Quantitative determinations and correlations with accepted traditional quality indices have been very scarce in fluorescence research. The next two subsections are divided into marine and non-marine sources because of differences in the PUFA content and the impact on fluorescence development.

Some controversy exists in the literature regarding the kind of extract or material that should be analyzed by fluorometry. Fluorescence determinations of treated samples have been demonstrated in organic extracts (32,33), aqueous extracts (19,34), both types of extracts (35,36), and solid sample spectrofluorometry (37).

Organic extracts have been widely employed for studying fluorescence development during damage of living tissues. Membrane lipids (i.e., phospholipids) are involved and a water-insoluble fluorescent complex was reported to be formed, since phospholipids acted as amino donors (16,38). However, if protein amino compounds are involved as amino donors, fluorescent substances formed would remain attached to the amino constituent (39,40); in this case, fluorescent compounds could not be extracted with organic solvents, but rather with aqueous ones. Depending on the nature of the amino donor, the resulting Schiff base complex may have either polar or nonpolar properties.

Nonmarine foods. Detection of fluorescent compounds extracted with an organic solvent mixture provided reproducible results reflecting the progress of oxidation in milk (32) and

frying oils (41). Comparison of absorption and intrinsic fluorescence of aromatic residues of proteins of soft wheat flour and gluten gave information on the interactions of lipids and proteins in these complex systems (42).

Fluorescence levels measured in the organic and aqueous layers arising from Folch lipid extraction were effective in assessing damage during chicken breast and leg meat processing (35). The initial levels of either MDA or fluorescent products in meat are of primary importance in determining the final MDA and fluorescence levels after processing. The extent of warmed-over flavor (WOF) formation in cooked beef was studied (43). Correlation of WOF with sensory evaluations, in which a trained sensory panel evaluated WOF smell and WOF taste, showed that thiobarbituric acid reactive substances (TBARS) and fluorescence analysis of the organic extract of the muscle are suitable for monitoring the development of WOF in reheated cooked beef during storage. Spectrofluorometry technique was directly applied on solid food samples during freeze-drying of pork and egg yolk (44); fluorescence intensity showed a high positive correlation with the absorbed oxygen level; however, thiobarbituric acid index (TBA-i) and peroxide value (PV) were not suitably correlated with the absorbed oxygen in all cases.

Fluorescence also has been used for food authenticity identification. A multispectral fluorescence imaging system was tested to distinguish several vegetable food products (maize, pea, soybean, and wheat). Application of stepwise discriminant analysis showed that identification of the four products was possible with fluorescence (45). Future work should include the study of mixtures of food products and the identification of contaminants in grain and seed samples. Fluorescence spectra of apple juice were evaluated to relate picking date of the apple with traditional harvest indices (46). Apples with different picking dates could be correctly classified by their fluorescence spectra; however, the authors concluded that a model based on a broader set of parameters should be tested to overcome the problem of large interapple variation.

New possibilities for analytical characterization of virgin olive oil, refined olive oil, and hask oil were obtained by studying their fluorescence spectra (47). Four types of edible oils were characterized by their three-dimensional (excitation and emission wavelength and emission intensity fluorescence spectra) fluorescence topograms; all oils displayed characteristic topograms, whose curve patterns were distinctly different (48). In both experiments (47,48), complementary research should be carried out with more oil types and mixtures to create methods to use against fraud.

Marine foods. Changes were observed in the muscle fluorescence of many species of fish during storage in the frozen state; it was suggested that careful visual examination of fish muscle fluorescence, before and after thawing, could be useful in the assessment of cold storage history (49). Two fluorescent emissions developed in fish muscle during frozen storage were investigated and related to fish quality, although quantitative results were not obtained (50); carbonyl compounds arising from lipid oxidation compounds and for-

maldehyde produced by degradation of trimethylamine oxide were thought to be involved in both fluorescences.

Determination of interaction compounds such as polymers, fluorescent material in the lipid extract, and browning matter was found to be reliable in evaluating oxidative deterioration during sardine freeze-drying (51). Rancidity scores and several chemical assessments of lipid degradation (PV, conjugated dienes content, browning, fluorescence and amino acid composition changes) were made on dried, salted sardines stored at three temperatures (5, 20, and 30°C) for 24 wk; the presence of fluorescent products measured in the lipid fraction showed a significant correlation with sensory scores (52). Sun-dried or salted fatty fish showed highly positive correlations between the odor of the stored products and the fluorescent pigment production measured in the lipid fraction (33,36). Determination of extractable fluorescence (aqueous and organic phases resulting from lipid extraction) and soluble brown color were found to be relevant indicators of the lipid oxidation in salted sun-dried fish (36).

Fish meats were measured directly before and after lipid extraction by solid sample spectrofluorometry (37) for the quantitative assessment of oxidative deterioration in freeze-dried fish. For it, two fluorescences (370/460 nm and 450/500 nm) were tested. PV, TBA-i, and fluorescence intensities of organic solvent extracts did not correlate well with the extent of lipid oxidation determined by oxygen absorption. However, fluorescence measured at 450/500 nm in defatted fish muscle provided a high correlation with oxygen absorption. It was concluded that such solid sample fluorescence detection could be valuable to quantify the oxidative deterioration; thus, more experiments concerning a wider range of fish material and processing conditions are necessary.

QUALITY ASSESSMENT BY FLUORESCENCE SHIFT DETECTION DURING FISH PROCESSING

During the last ten years, experiments carried out in our research group have focused on the formation of interaction compounds. With this purpose, simultaneous fluorescence detection at several excitation/emission maxima during fish processing was employed. The research was aimed in employing fluorescence detection as a quantitative tool for quality assessment during food processing.

In our first attempts (53,54), it was observed that fluorescent compounds formed during the first stages of the reaction or processing gave rise to other fluorescent compounds showing higher wavelength maxima as a result of increased food damage. This observed bathochromic shift agrees with the general theory of progressive formation of Schiff bases and other interaction compounds with increasing molecular weights and unsaturated bond number (7–9) (Scheme 1). From these initial experiments, it was observed that this fluorescence shift could be assessed by calculating the ratio (δF) between two of the excitation/emission maxima tested (393/463 nm and 327/415 nm).

The following research steps were focused on correlating

this δF value with common fish quality indices along different fish processes. Since no previous information was available concerning the kinds of compounds that were measured at fluorescence maxima, both the aqueous and organic (lipid extract) phases resulting from the lipid extraction (δF_{aq} and δF_{or} , respectively) were analyzed for all experiments.

Freezing and frozen storage. Sardine (*Sardina pilchardus*) fish was kept frozen (–18 and –10°C) up to two years (55). Fluorescence results (δF_{aq} and δF_{or}) were compared to common quality indices [total volatile base-nitrogen (TVB-N), conjugated dienes (CD), TBA-i, and free fatty acids (FFA)].

At –18°C, the δF_{aq} value showed significant ($P < 0.01$) correlations with storage time ($r = 0.80$), TBA-i ($r = 0.92$), and FFA content ($r = 0.83$). The two classical indices TBA-i and FFA have been recognized as useful in this kind of storage, since enzymatic lipid damage has been shown to be a decisive degradation pathway (56,57). Indeed, lipid hydrolysis has been reported to facilitate further oxidation (58,59). Principal component analysis (Fig. 1) showed that the δF_{aq} was grouped together with the storage time, FFA, and TBA-i at high loading in the Factor 1 axis, whereas their loading values for Factor 2 were low. The δF_{or} detection, however, did not afford satisfactory quality assessment.

The highest temperature (–10°C) studied in this experiment (55) produced the most lipid damage. The best fluorescence results were obtained, again, in the case of the δF_{aq} value, showing a significant ($P < 0.01$) correlation with the TBA-i ($r = 0.81$) and also significant ($P < 0.05$) correlation with storage time ($r = 0.72$), FFA ($r = 0.59$), and TVB-N ($r = 0.60$). A principal component analysis (Fig. 2) showed that δF_{aq} was grouped with the same indices as for –18°C (storage time, FFA, and TBA-i) and also with TVB-N index, probably due to some microbiological activity development (60,61); all these parameters showed a high loading in the Factor 1 axis.

Refrigerated storage. Sardine fish was kept refrigerated (0°C on ice) up to 16 d (62). Fish muscle alterations were checked by measuring the fluorescence shift (δF_{aq} and δF_{or}) value and compared again with common quality indices (TVB-N, FFA, CD, and TBA-i). The TVB-N detection was emphasized because it is an important index for microbiological activity during refrigerated storage (60,61). In our results (62), a high and significant ($P < 0.05$) correlation was found (Table 1) for the δF_{aq} value with the TVB-N ($r = 0.93$) and the refrigerated storage time ($r = 0.94$). Among the lipid indices, the δF_{aq} value showed the best correlation with refrigerated storage time, even better than the TVB-N. Regarding frozen storage, the δF_{or} value did not provide satisfactory results for assessing quality changes and for correlating with other quality indices.

A complementary study was carried out by holding sardines at 15°C for 8 d (62). Common indices and fluorescence analysis (δF_{aq} and δF_{or} values) showed more damage than during refrigerated storage. Correlation results between parameters can be seen in Table 1. The δF_{or} is not included, since no satisfactory results were obtained. The storage time

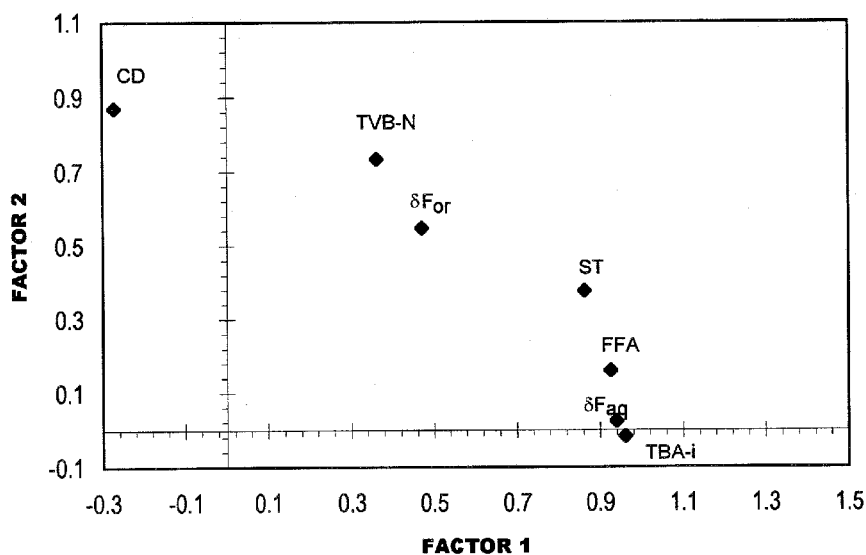


FIG. 1. Principal component analysis for different parameters measured during sardine frozen storage at -18°C . Abbreviations: ST, storage time; TVB-N, total volatile base-nitrogen; CD, conjugate dienes; TBA-i, thiobarbituric acid index; FFA, free fatty acids; δF_{aq} and δF_{or} , fluorescence ratios measured in the aqueous and organic phases, respectively. Adapted from Reference 55.

showed significant ($P < 0.05$) correlation values with all the indices, especially with the TVB-N, FFA, and δF_{aq} value. At the same time, the δF_{aq} value also provided significant ($P < 0.05$) correlations with all the parameters studied.

Changes during refrigerated (0°C in ice) storage of a lean fish species (*Micromesistius poutassou*) were studied (63). Again, different kinds of lipid damage indices [PV, CD, TBA-i, FFA, polyene index (PI), δF_{aq} , and δF_{or}] were investigated and compared to TVB-N content. Similarly to previous results regarding a fatty fish species (sardine), fluorescence detection of interaction compounds formed in the aqueous

medium (δF_{aq} value) showed the best correlation with the time of storage (nonlinear relationship; Fig. 3) and the TVB-N evolution (linear relationship; Fig. 4), and provided the highest independent contribution to time prediction during refrigerated storage.

Cooking. Two tuna species (big eye, *Thunnus obesus*; and bluefin, *Thunnus thynnus*) were steam cooked ($102\text{--}103^{\circ}\text{C}$, to a final backbone temperature of 65°C) (64). The lipid damage was investigated using conventional (FFA, CD, carbonyls, and browning) and fluorescence (δF_{aq} and δF_{or} values) detection methods, and the results were compared. As a

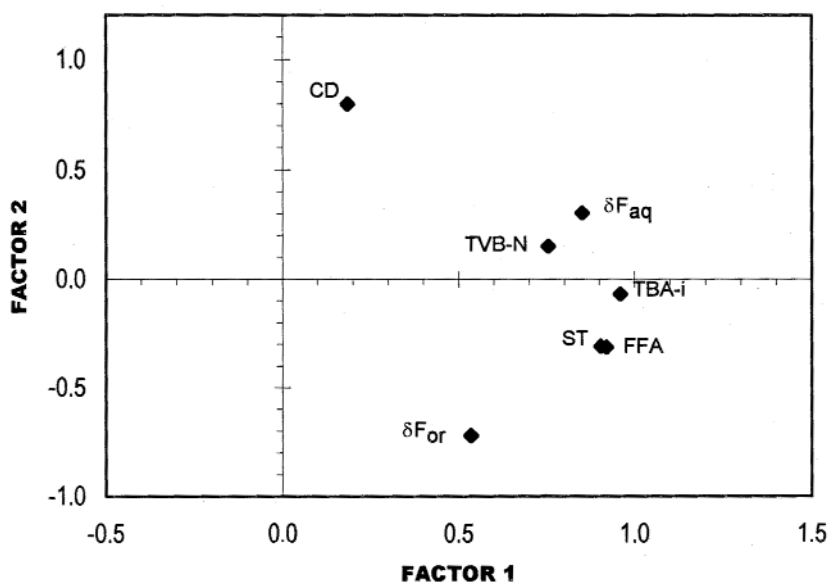


FIG. 2. Principal component analysis for different parameters measured during sardine frozen storage at -10°C storage. See Figure 1 for abbreviations. Adapted from Reference 55.

TABLE 1
Correlation Coefficient Matrix for Different Parameters^a Measured During Refrigerated Storage of Sardines^b

	TVB-N	CD	TBA-i	FFA	δF_{aq}
Storage at 0°C (on ice)					
ST	0.86 ^c	0.04	0.50	0.50	0.94 ^c
TVB-N		-0.11	0.06	0.31	0.93 ^c
CD			0.48	0.57 ^c	-0.03
TBA-i				0.78 ^c	0.28
FFA					0.42
Storage at 15°C					
	TVB-N	CD	TBA-i	FFA	δF_{aq}
ST	0.99 ^c	0.85 ^c	0.58 ^c	0.93 ^c	0.93 ^c
TVB-N		0.84 ^c	0.51	0.93 ^c	0.92 ^c
CD			0.61 ^c	0.84 ^c	0.90 ^c
TBA-i				0.61 ^c	0.59 ^c
FFA					0.92 ^c

^aAbbreviations: ST, storage time; TVB-N, total volatile base-nitrogen; CD, conjugated dienes; TBA-i, thiobarbituric acid index; FFA, free fatty acids; and δF_{aq} , fluorescence shift ratio measured in the aqueous phase resulting after the lipid extraction.

^bAdapted from Reference 62.

^cSignificance: $P < 0.05$.

consequence of the thermal treatment, δF_{or} value increased (cooked/raw ratios > 1 , Table 2), according to changes undergone by other conventional indices of lipid damage (i.e., carbonyls, browning, and FFA). Different individual fish were studied in both species (BE1–BE5 and BF1–BF4, Table 2); as a result, the δF_{or} value showed a lower variation coefficient than did the remaining conventional methods, showing that the fluorescence detection was less dependent on the individ-

TABLE 2
Lipid Damage Indices Expressed as the Cooked/Raw Measurement Ratio in the Different Samples Studied^a

Sample	FFA	CD	Carbonyls	Browning	δF_{or}
BE1	2.10	0.63	3.17	1.60	1.56
BE2	1.76	0.67	2.96	2.85	1.55
BE3	1.27	0.39	2.08	1.13	1.32
BE4	1.77	0.47	3.94	1.81	1.27
BE5	2.24	0.57	3.56	1.83	1.63
BF1	1.36	0.89	3.65	2.88	1.59
BF2	2.36	2.36	4.03	4.17	1.68
BF3	3.44	1.03	3.73	4.45	1.60
BF4	2.26	2.42	2.27	2.34	1.26
Mean	2.06	1.05	3.27	2.56	1.50
SD	0.61	0.74	0.67	1.08	0.15
VC	29.47	70.48	20.49	42.19	10.00

^aBE1–BE5, five individual fish samples of big eye tuna (*Thunnus obesus*); BF1–BF4, four individual fish samples of bluefin tuna (*Thunnus thynnus*). Adapted from Reference 64. Abbreviations: δF_{or} , fluorescence shift ratio measured in the lipid extract; SD, standard deviation; and VC, variation coefficient. See Table 1 for other abbreviation.

ual fish sample studied. Similar conclusions were obtained by analyzing the δF_{aq} value.

Canning. Thermal treatment during sterilization has been reported to produce many changes in fish muscle; however, many of the problems encountered with poor quality canned fish can be related to the quality of the raw material, which continuously changes during storage (2). Refrigerated and frozen storages are the most utilized methods in canneries for preserving fish prior to canning. Accordingly, refrigerated and frozen sardines were employed by our group to determine the influence of the storage time of fish (raw material quality)

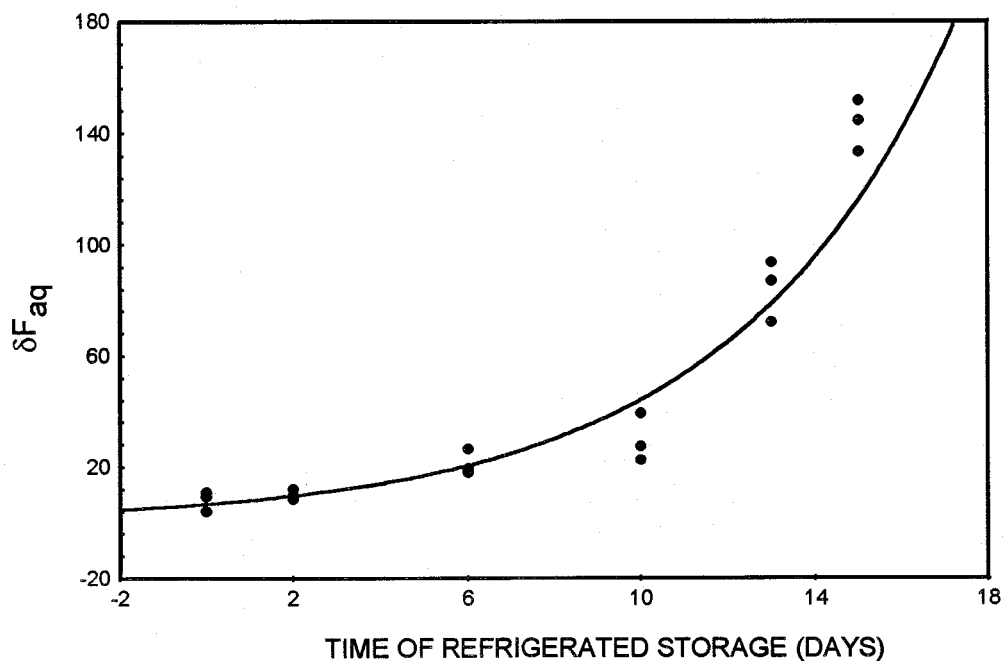


FIG. 3. Nonlinear relationship between δF_{aq} and time of refrigerated storage. See Figure 1 for abbreviation. Adapted from Reference 63.

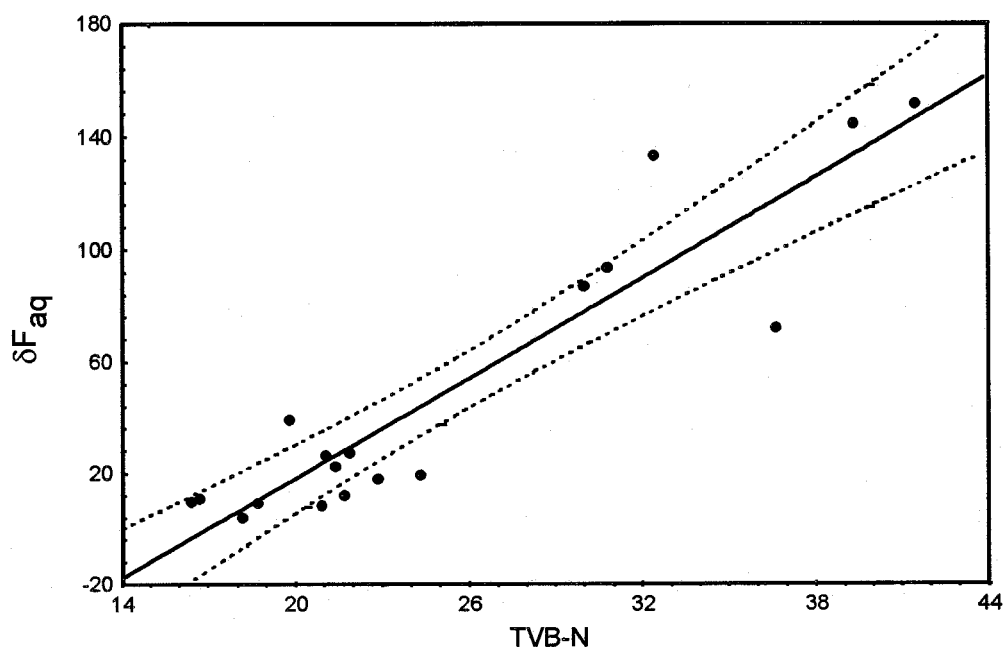


FIG. 4. Linear relationship between δF_{aq} and TVB-N. See Figure 1 for abbreviations. Adapted from Reference 63.

over the quality of the final canned product measured by the δF value and traditional indices (65). Refrigerated (0°C , on ice) sardines were canned in brine (Table 3), while frozen (-18°C) sardines were canned in oil (Table 4); sterilization was carried out in a retort (115°C , 45 min).

The common indices of lipid degradation (FFA, CD, TBA-i, and PI) measured in canned sardines did not reflect quality changes according to the previous time of storage of the raw material (Tables 3 and 4). It could be argued that degradation products measured in such indices can either be distributed into different phases of the can (packing medium, exudate, fish muscle), be partly destroyed during the heat process, or interact with other constituents so that their assessment was not an accurate measure of quality.

Fluorescence analysis of fish muscle extracts (δF_{aq} and δF_{or} ; Tables 3 and 4) afforded poor results for quality changes

assessment. These results might be explained by the presence in the can of a packing liquid (brine or oil), which could act as an extraction medium of the solid phase. The analysis of the fluorescence ratio value, however, in both packing media (brine and oil; δF_{pm}) showed a satisfactory quadratic correlation ($r = 0.90$ in brine; $r = 0.91$ in oil) with the time of storage of the raw material employed before canning. Therefore, quality differences in the final canned product could be assessed according to quality of raw material employed.

FACTORS AFFECTING THE δF VALUE

Complementary research was carried out in our group to study the influence of different factors in the δF value. Fluorescence detection was checked in accurate model systems and results are shown in the following subsections.

TABLE 3
Lipid Damage Measurements in Brine-Canned Sardine (BCS)^a

Sample	FFA	TBA-i	PI	δF_{aq}	δF_{or}	δF_{pm}
IN-CH	0.20 ^a	0.98 ^a	1.66 ^a	0.78 ^a	1.50 ^a	—
BCS-0	4.11 ^b	2.10 ^c	1.72 ^a	2.22 ^{b,c}	2.71 ^{a,b}	1.75 ^a
BCS-2	3.98 ^b	1.89 ^{b,c}	1.65 ^a	2.36 ^{b,c}	2.87 ^{a,b}	1.73 ^a
BCS-6	4.35 ^{b,c}	1.73 ^{b,c}	1.62 ^a	2.52 ^{b,c}	6.99 ^{a,b,c}	2.22 ^{a,b}
BCS-10	4.68 ^{b,c}	1.81 ^{b,c}	1.65 ^a	2.22 ^{b,c}	9.00 ^{b,c}	3.35 ^b
BCS-13	5.84 ^{c,d}	2.02 ^c	1.58 ^a	2.04 ^b	12.22 ^c	5.08 ^c
BCS-15	6.93 ^d	1.49 ^{a,b}	1.59 ^a	2.78 ^c	10.93 ^c	6.92 ^d

^aMeasurement abbreviations: PI, polyene index and δF_{pm} , fluorescence shift measured in the packing medium. See Tables 1 and 2 for other abbreviations. Sample names: IN-CH, initial raw sardine; BCS-0, BCS-2, BCS-6, BCS-10, BCS-13, and BCS-15, canned sardine that was previously kept refrigerated at 0°C on ice during 0, 2, 6, 10, 13, and 15 d, respectively. Mean values of three determinations. Values in the same column followed by different superscript letters are significantly different ($P < 0.05$). Adapted from Reference 65.

TABLE 4
Lipid Damage Measurements in Oil-Canned Sardine (OCS)^a

Sample	FFA	TBA-i	δF_{aq}	δF_{or}	δF_{pm}
IN-FR	1.11 ^a	0.48 ^a	0.37 ^a	0.43 ^a	—
OCS-0	0.62 ^a	1.53 ^c	1.74 ^{a,b}	3.53 ^b	0.93 ^a
OCS-0.5	3.17 ^b	1.77 ^{c,d}	3.75 ^c	4.65 ^{b,c}	1.20 ^a
OCS-2	4.75 ^c	1.13 ^b	5.98 ^d	4.13 ^{b,c}	1.21 ^a
OCS-4	3.16 ^b	1.11 ^b	2.62 ^{b,c}	4.99 ^c	1.88 ^b
OCS-8	2.83 ^b	1.96 ^d	3.48 ^c	8.08 ^d	2.25 ^b
OCS-12	4.81 ^c	1.39 ^{b,c}	7.51 ^d	6.85 ^d	4.56 ^c

^aSee Table 3 for measurement abbreviations. Sample names: IN-FR, initial raw sardine; OCS-0, OCS-0.5, OCS-2, OCS-4, OCS-8, and OCS-12, canned sardine that was previously kept frozen at -18°C during 0, 0.5, 2, 4, 8, and 12 mon, respectively). Mean values of three determinations. Values in the same column followed by different superscript letters are significantly different ($P < 0.05$). Adapted from Reference 65. See Tables 1–3 for other abbreviations.

Time and temperature of processing. In a first attempt (53), a model system mixture consisting of a lipid oxidation compound (acetaldehyde) and albacore (*Thunnus alalunga*) white muscle was studied. Several fluorescence maxima (327/415, 393/463, and 479/516 nm) were detected and studied accordingly in the aqueous reaction medium and in the lipid extract of the mixture. A strong relationship between fluorescence formation in both media and time and temperature of reaction was evident. An augmentation in these parameters produced a significant shift of fluorescence formed to higher excitation/emission maxima wavelengths, leading to higher δF values in both phases.

In the previously mentioned experiments (55,62,63), different times and temperatures during frozen and refrigerated storages were tested. A higher δF value in the aqueous phase (δF_{aq}) occurred when increasing the time and temperature of storage.

Four different sterilization conditions (110°C, 120 min; 130°C, 120 min; 115°C, 60 min; 130°C, 27 min) were tested for albacore canning (54). Different fluorescence maxima (327/415, 356/416, 393/463, and 446/490 nm) were evaluated. A fluorescence shift to higher wavelength maxima measured in both the aqueous (δF_{aq}) and organic (δF_{or}) phases was observed as a result of processing. The strongest sterilization treatment (130°C, 120 min) had the greatest lipid damage and δF value in both phases.

Amine composition and content. Different kinds of amines (different substitution degree and steric hindrance) were tested in model systems to quantify the δF value of the reaction medium (66). Primary (butylamine, iso-butylamine, *sec*-butylamine, and *tert*-butylamine), secondary (dibutylamine) and tertiary (tributylamine) amines produced changes in the fluorescent properties of the systems during reaction at 30°C for 25 d with cod liver oil and glutaraldehyde. Greater δF values were associated with a lower degree of substitution (primary amines) and steric hindrance (butylamine and iso-butylamine) of the $-NH_2$ group, caused by a higher nucleophilic ability for reacting with lipid oxidation compounds.

Influence of the chainlength also was studied (66) in different primary amines (methylamine, ethylamine, propylamine, butylamine, and octylamine) by reacting with cod liver oil at 30°C (Table 5). Greater δF values were obtained at the end of the reaction time as a result of increased chainlength. Longer chainlengths may provide the amine molecule with a more lipid-type structure, so that interaction with lipid oxidation compounds would be facilitated (25).

Different amine (butylamine, dibutylamine, and tributylamine) concentrations (0.2, 0.8, 4.0, and 20.0 mM) were tested by reacting with cod liver oil for 25 days at 30°C (66). In all cases, a higher δF value in the reaction medium was observed as the amine content increased, showing that the formation of fluorescent compounds depended not only on the presence of lipid oxidation compounds, but also on the amine content present in the biological matter.

Aldehyde composition and content. During the model system interaction between acetaldehyde and tuna muscle just

TABLE 5
Fluorescence Ratio (δF) Values Obtained by Reaction at 30°C of Cod Liver Oil and Primary Amines with Different Chainlengths^a

Reaction mixture	Reaction time		
	1 d	7 d	25 d
CLO	2.30 ^a	2.26 ^a	2.37 ^a
CLO + MA	6.16 ^c	17.6 ^{c,d}	29.5 ^b
CLO + EA	5.09 ^b	14.5 ^b	31.5 ^b
CLO + PA	5.35 ^{b,c}	16.2 ^{b,c}	35.1 ^c
CLO + BA	5.85 ^c	17.2 ^{c,d}	37.6 ^c
CLO + OA	5.70 ^c	19.7 ^d	44.9 ^d

^aValues in the same column followed by different superscript letters are significantly different ($P < 0.05$). Abbreviations: CLO, cod liver oil; MA, methylamine; EA, ethylamine; PA, propylamine; BA, butylamine; OA, octylamine. Adapted from Reference 66.

mentioned (53), different aldehyde contents (0, 1, 2, and 5%, acetaldehyde/albacore muscle, w/w) were tested. A bigger fluorescence shift was produced according to the acetaldehyde content that was present in the reaction medium. This bathochromic shift suggests a progressive formation of Schiff bases with increased molecular weights and unsaturated bond numbers (7–9) (Scheme 1).

The relative ability of different aldehydes (formaldehyde, acetaldehyde, propanal, hexanal, 2-hexenal, and benzaldehyde) in producing fluorescent compounds related to the δF value was studied (67). Aldehydes were compared in model systems by reacting for 25 d at 30°C with *n*-propylamine and minced sardine white muscle as nucleophilic compound sources. A fluorescence development in the aqueous reaction medium was detected for all the aldehydes during the reaction time. Great differences, however, were observed among aldehydes according to their chemical structure and reactivity. A sharp shift to higher wavelength maxima was detected during storage for 2-hexenal and acetaldehyde because of a greater reactivity detected in reactions involving unsaturated and short carbonylic compounds (24,25). Few changes in fluorescence formation were observed for formaldehyde and benzaldehyde.

Formaldehyde presence and pH value. During the frozen storage of gadoid fish species, formaldehyde (FA) is produced, along with dimethylamine, by enzymatic reduction of trimethylamine oxide. FA has been recognized as a highly reactive molecule leading to inter- and intramolecular linkages between protein chains. As a result, protein denaturation and quality loss in frozen fish have been associated with FA formation (68,69).

In a recent work, the influence of FA on the δF value measured in the aqueous reaction medium was studied (70). A model system of fluorescence formation was set up, in which FA reacted at 30°C for 25 d with *n*-propylamine and minced fish muscle. The FA was less able to produce fluorescent compounds than common fish oxidation compounds, which were also tested (i.e., propanal and hexanal). Model systems consisting of FA and fatty fish (sardine) muscle, however, produced a higher δF value than did FA and lean fish (cod), because of greater lipid oxidation compound formation of the

TABLE 6
 δF Values Obtained from the Aqueous Medium of the Aldehyde-Fish Muscle Reaction Systems at 30°C^a

Reaction mixture	Reaction time		
	1 d	7 d	25 d
MCM	0.88 ^{a,b}	0.97 ^a	1.18 ^a
FA + MCM	1.18 ^b	1.18 ^a	1.53 ^a
Pal + MCM	6.03 ^d	22.6 ^d	37.1 ^d
Hal + MCM	3.23 ^c	18.2 ^c	26.8 ^c
MSM	0.53 ^a	0.65 ^a	1.21 ^a
FA + MSM	1.48 ^b	3.46 ^b	6.73 ^b

^aValues in the same column followed by different superscript letters are significantly different ($P < 0.05$). Abbreviations: MCM, minced cod muscle; MSM, minced sardine muscle; FA, formaldehyde; Pal, propanal; and Hal, hexanal. Adapted from Reference 70. See Table 5 for other abbreviation.

fatty fish system under the reaction conditions. Results obtained are summarized in Table 6.

The effect of the pH value of the medium on the fluorescence development was evaluated (71). For this purpose, a model system consisting of minced hake (*Merluccius merluccius*) muscle in 0.1 M phosphate buffer of different pH values (5.0, 6.0, 7.0, and 8.0) stored at 30°C for up to 30 d was studied. The presence/absence of FA in the reaction medium also was evaluated. In the absence of FA, no significant ($P < 0.05$) differences were obtained for the δF value measured in the aqueous reaction medium as pH changed. The presence of FA in the same reaction mixture, however, had a positive effect on the fluorescence shift development (Table 7), so that the following increasing δF values were obtained: pH 5, pH 6 < pH 7 < pH 8. This result can be explained by analyzing the pK_a values of the biological amine compounds involved (72). As long as the pH increases, the nucleophilic ability of N-terminal groups of proteins and R-terminal groups of basic amino acids (lysine, tyrosine, and arginine) is increased, so that the formation of interaction compounds is favored.

The interesting results obtained for the δF value detection suggest several areas of further research: (i) a better knowledge of the formation mechanism and identification of fluorescent compounds involved; (ii) application of this detection method to a wide range of foods, including nonmarine bio-

TABLE 7
 δF Values Obtained from the Aqueous Medium of the Reaction Systems with Formaldehyde and Minced Hake at Different pH at 30°C^a

Reaction time (d)	pH value			
	5.0	6.0	7.0	8.0
1	0.80 ^a	0.96 ^a	1.88 ^c	1.59 ^b
2	1.00 ^a	1.30 ^a	2.68 ^b	2.43 ^b
4	1.36 ^a	1.51 ^a	7.38 ^c	5.36 ^b
7	1.78 ^a	2.46 ^a	9.08 ^b	9.79 ^b
15	2.94 ^a	3.51 ^a	16.41 ^b	17.94 ^b
30	4.98 ^a	6.57 ^a	42.49 ^b	63.79 ^c

^aMean values of three independent determinations. Values in the same row followed by different superscript letters are significantly different ($P < 0.05$). Adapted from Reference 71. See Table 5 for abbreviation.

logical species; (iii) detection of the δF value in a wide range of food processes, including new and traditional ones, where lipid oxidation plays an important role (under-vacuum cooking, lipid-derived coatings, modified atmospheres, addition of natural and synthetic antioxidants, drying, and salting).

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