Fluorescence Formation by Interaction of Albacore (*Thunnus alalunga*) Muscle with Acetaldehyde in a Model System

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The role of acetaldehyde (AcH) in producing fluorescent compounds as a result of interaction with food constituents was studied. A model system consisting of albacore (*Thunnus alalunga*) muscle and AcH in 0.1 M phosphate buffer (pH 7.0) was used. Fish muscle was submitted to three different temperatures (5, 15, and 30 °C) and four different concentrations of AcH (0, 1, 2, and 5%) during 1 month. Resulting fluorescent compounds were analyzed in the aqueous and organic extracts at three different excitation/emission maxima wavelengths: 327/415, 393/463, and 479/516 nm. Brown color formation was also measured in organic extracts at 420 nm. A strong relationship between fluorescence formation as a function of AcH concentration, time, and temperature of reaction was evident. An augmentation in all three variables produced a significant shift of fluorescence to higher excitation/emission maxima wavelengths which was accompanied by a marked increase in browning.

INTRODUCTION

Biological material contains a wide variety of unsaturated lipids in membranes which in the presence of free-radical initiators and oxygen may experience peroxidative deterioration (Tappel, 1980; Esterbauer, 1982). It is assumed now that in vivo formation of fluorescent compounds is produced by lipid peroxidation of subcellular components as a result of a degenerative process (Miquel et al., 1978; Kikugawa and Beppu, 1987).

Fluorescence formation has also been observed in foods after processing, especially if high-temperature processes are involved. Besides primary (hydroperoxides) and secondary (carbonyl compounds) oxidation products, the determination of fluorescent compounds has been used as an analytical method for quantification of peroxidation damage to foods during processing and storage (Melton, 1983; Pikul et al., 1984, 1989).

Fluorescence determinations of treated samples have been shown on organic extracts (Bouzas et al., 1985; Maruf et al., 1990), aqueous extracts (Manwaring and Csallany, 1981; González-Garza et al., 1990), and both types of extracts (Pikul et al., 1984; Smith et al., 1990).

It has been stated that during food processing/storage fluorescent compounds are formed as a result of reaction of primary and secondary oxidation products with biological amino compounds (Gardner, 1979; Leake and Karel, 1985; Kikugawa, 1986). Among the oxidation products, aldehydes (malondialdehyde, 4-hydroxynonenal, hexanal, and others) have been found to contribute to fluorescence formation (Buttkus, 1975; Tashiro et al., 1985; Esterbauer et al., 1986; Montfoort et al., 1987) and to undergo aldolization reactions catalyzed by amino compounds of food constituents (Suyama et al., 1981; Pokorny et al., 1987).

In this paper the fluorescence formation in a fish food by the presence of a lipid damage compound such as acetaldehyde was studied. This carbonyl compound has been widely found as a result of lipid oxidation (Fujimoto and Kaneda, 1973) and has proved an extreme reactivity and volatility; it has been detected and measured as the 2,4-dinitrophenyl hydrazone derivative (McGill et al., 1977; Ammu and Devadasan, 1989).

In the present work acetaldehyde (AcH) interacted in a model system with albacore tuna (*Thunnus alalunga*) muscle. Analyses were performed in aqueous and organic extracts at several excitation/emission wavelength maxima (327/415, 393/463, and 479/516 nm). Factors such as AcH concentration, time, and temperature of reaction were studied to observe the evolution of fluorescence formation during the experiment. Analyses were complemented by the measurement of nonenzymatic browning at 420 nm.

MATERIALS AND METHODS

Raw Material and Sampling. Albacore tuna (*T. alalunga*) were caught by a tuna fishing vessel in the Atlantic Ocean (around 43° N and 27° W). The fish were kept in boxes and transported on ice for 10 days. After arrival to our laboratory, the fish were frozen at −40 °C and stored at −18 °C for 1 month prior to analysis.

The fish were eviscerated, beheaded, and randomly divided into three batches. From each batch, fish samples were homogenized and prepared for analysis.

AcH/Albacore Muscle Model System. Two-gram portions of albacore muscle were homogenized with 8 mL of 0.1 M phosphate buffer (pH 7.0). AcH was then added to obtain a final concentration of 0, 1, 2, or 5% (AcH/albacore muscle w/w).

The mixtures were incubated at 5, 15, and 30 °C and sampled at regular time intervals (3, 8, 15, and 29 days for 5 °C; 2, 7, 14, and 28 days for 15 °C; and 1, 4, 11, and 25 days for 30 °C).

The samples were centrifuged, and the supernatant was collected. The solid phase was washed with 5 mL of 0.1 M phosphate buffer (pH 7.0) and the liquid phase pooled and diluted to a total of 15 mL with 0.1 M phosphate buffer.

The remaining fish tissue was then homogenized with a mixture of 4 mL of chloroform, 4 mL of methanol, and 1 mL of water. The mixture was centrifuged and the organic layer collected. This extraction was repeated, and the organic layers were pooled, dried with anhydrous Na_2SO_4, and taken to a 5-mL volume with chloroform.

Fluorescence Measurements. The excitation and emission spectra of the aqueous and organic extracts were determined using a Perkin-Elmer LS 3B fluorescence spectrophotometer. Both kinds of extracts showed the same excitation/emission maxima for the whole experiment: 327/415, 393/463, and 479/516 nm.

Quinine sulfate solution (1 μg/mL in 0.1 N sulfuric acid) was used as a standard of fluorescence intensity (S). The fluorescence of the sample relative to the standard was calculated using the formula (Smith et al., 1990)

$$\text{fluorescence (mL/g)} = FV/Sw$$

where V is the volume of extract (15 mL for aqueous extracts and 5 mL for organic extracts) giving fluorescence F and w is the weight of the initial albacore sample (2 g).

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Table I. Fluorescence Measurements[a] of Aqueous (A) and Organic (O) Extracts from Samples Maintained at 5 °C

time of storage, days	sample[b]	327/415 nm		393/463 nm		479/516 nm	
		A	O	A	O	A	O
0	IN	1.07 e	0.20 a	0.42 ab	0.12 a	0.25 a	0.01 a
3	1	1.26 fg	0.26 ab	0.43 ab	0.17 a	0.17 a	0.03 ab
	2	1.29 g	0.29 abc	0.51 ab	0.17 a	0.23 a	0.03 ab
	3	1.11 ef	0.34 bc	0.76 bcdef	0.20 a	0.35 ab	0.04 ab
	4	0.23 b	0.36 bc	1.10 fg	0.32 abcd	1.16 d	0.09 abc
8	1	1.33 gh	0.30 abc	0.57 abc	0.21 a	0.16 a	0.05 abc
	2	1.37 ghi	0.34 bc	0.75 bcdef	0.22 abc	0.34 ab	0.05 ab
	3	0.79 d	0.33 abc	1.42 gh	0.21 ab	0.85 c	0.05 abc
	4	0.04 a	0.76 d	0.91 cdef	1.87 e	2.54 e	0.27 d
15	1	1.40 ghi	0.31 abc	0.66 abcd	0.29 abcd	0.17 a	0.06 abc
	2	1.50 i	0.30 abc	1.08 fg	0.24 abc	0.56 b	0.05 abc
	3	0.52 c	0.42 c	1.63 h	0.43 abcd	1.33 d	0.12 bc
	4	0.01 a	0.35 bc	0.68 abcde	0.73 d	3.04 g	0.32 de
29	1	1.81 j	0.41 c	0.96 cdef	0.35 abcd	0.19 a	0.08 abc
	2	1.49 hi	0.34 bc	2.08 i	0.37 abcd	1.14 d	0.10 abc
	3	0.15 ab	0.35 bc	1.78 hi	0.45 abcd	2.47 e	0.14 c
	4	0.00 a	0.25 ab	0.34 a	0.69 d	3.26 g	0.38 e

[a] Mean of three determinations. Values in the same column followed by different characters are significantly different ($p < 0.05$). [b] Samples: IN, initial; 1, 0% AcH; 2, 1% AcH; 3, 2% AcH; 4, 5% AcH.

Table II. Fluorescence Measurements[a] of Aqueous (A) and Organic (O) Extracts from Samples Maintained at 15 °C

time of storage, days	sample[b]	327/415 nm		393/463 nm		479/516 nm	
		A	O	A	O	A	O
0	IN	1.07 c	0.20 ab	0.42 a	0.12 a	0.25 a	0.01 a
2	1	1.24 c	0.37 de	0.54 a	0.27 ab	0.20 a	0.05 a
	2	1.35 c	0.39 de	0.61 ab	0.30 ab	0.27 a	0.07 a
	3	1.13 c	0.38 de	1.05 cd	0.29 ab	0.48 a	0.07 a
	4	0.16 a	0.34 cde	1.20 de	0.51 bc	1.60 b	0.17 ab
7	1	1.82 d	0.34 de	1.31 de	0.34 ab	0.25 a	0.09 a
	2	1.79 d	0.37 de	1.56 e	0.42 b	0.74 a	0.10 a
	3	0.60 b	0.31 bcd	2.17 f	0.39 ab	1.72 b	0.11 ab
	4	0.02 a	0.29 bcd	0.76 abc	0.76 c	3.31 cd	0.35 bc
14	1	2.76 e	0.38 de	1.22 de	0.35 ab	0.19 a	0.08 a
	2	1.90 d	0.35 de	3.17 g	0.78 c	1.38 b	0.19 ab
	3	0.17 a	0.31 bcd	2.40 f	0.78 c	3.29 cd	0.24 ab
	4	0.00 a	0.23 abc	0.39 a	1.09 d	3.90 d	0.58 c
28	1	3.16 f	0.45 e	1.00 bcd	0.37 ab	0.18 a	0.08 a
	2	1.27 c	0.21 ab	4.75 h	1.60 e	2.99 c	0.57 c
	3	0.07 a	0.20 ab	2.23 f	1.43 e	4.72 e	0.57 c
	4	0.00 a	0.12 a	0.52 a	1.47 e	5.29 e	0.90 d

[a] Mean of three determinations. Values in the same column followed by different characters are signficantly different ($p < 0.05$). [b] Sample names as expressed in Table I.

Spectrophotometric Measurement of Brown Color Formation. Measurement of brown color formation in organic extracts was achieved at 420 nm (Kato et al., 1986; Labuza and Massaro, 1990) in a Beckman DU-64 spectrophotometer. Results shown (Table IV) were calculated using the formula (Smith et al., 1990)

$$\text{absorbance} = BV/w$$

where B is the absorbance lecture obtained, V is the volume of the organic extract (5 mL), and w is the weight of the initial albacore sample (2 g).

Statistical Analysis. Data resulting from the fluorescence and nonenzymatic browning measures were subjected to the ANOVA one-way method, according to the method of Sokal and Rohlf (1981).

RESULTS AND DISCUSSION

Tables I–III show the results of fluorescence determinations of the aqueous and organic extracts at three different temperatures. For each time of storage, samples are referred to as 1, 2, 3, and 4 corresponding to 0, 1, 2, and 5% AcH/albacore muscle (w/w), respectively. The brown color formation in the same samples is shown in Table IV.

Blank samples without albacore muscle but with similar mixtures of phosphate buffer and AcH were also analyzed and showed negligible fluorescence formation and brown color development.

Aqueous Extracts. Tables I–III show the fluorescence measurements of aqueous extracts. The initial samples showed low levels at 393/463 and 479/516 nm but considerable fluorescence at 327/415 nm.

Results obtained at 5 °C are shown in Table I. At 327/415 nm an increase is observed in samples 1 and 2, while samples having higher AcH content (3 and 4) show an appreciable decrease. At 393/463 nm samples containing 0, 1, and 2% AcH reached higher values for all of the experiments, while the 5% sample decreased. At 479/516 nm all samples showed an increase with time.

Table II gives the results obtained at 15 °C. At 327/415 nm sample 1 showed an increase, while sample 4 showed a decrease with time. At 393/463 nm samples 2 and 3 reached higher values. At 479/516 nm all samples showed an increase with time.

Table III shows the evolution of fluorescence at 30 °C. At 327/415 nm an increase in sample 1 was observed, while

Table III. Fluorescence Measurements[a] of Aqueous (A) and Organic (O) Extracts from Samples Maintained at 30 °C

time of storage, days	sample[b]	327/415 nm		393/463 nm		479/516 nm	
		A	O	A	O	A	O
0	IN	1.07 bc	0.20 bc	0.42 ab	0.12 a	0.25 abcd	0.01 a
1	1	1.59 ef	0.34 defg	1.40 cde	0.24 a	0.20 abc	0.04 a
	2	1.72 f	0.38 fg	1.12 bcd	0.30 ab	0.45 abcd	0.06 a
	3	0.77 b	0.36 efg	1.46 de	0.35 abc	0.99 de	0.09 abc
	4	0.20 a	0.23 bcd	0.62 ab	0.67 de	2.35 fg	0.29 d
4	1	3.18 g	0.35 defg	1.58 de	0.26 a	0.13 a	0.05 a
	2	1.37 cde	0.33 def	2.70 f	0.38 abc	1.58 ef	0.09 abc
	3	0.16 a	0.25 cde	1.86 e	0.53 bcde	2.92 gh	0.19 abcd
	4	0.00 a	0.12 ab	0.37 a	0.71 e	4.11 ij	0.50 e
11	1	3.71 h	0.44 fg	2.81 f	0.60 cde	0.17 ab	0.14 abcd
	2	1.21 cd	0.46 g	4.17 g	1.40 h	2.53 gh	0.47 e
	3	0.04 a	0.23 bcd	1.88 de	1.28 gh	4.39 jk	0.57 ef
	4	0.00 a	0.14 abc	0.44 ab	1.09 fg	5.17 k	0.81 g
25	1	3.98 h	0.37 defg	3.26 f	0.40 abcd	0.20 ab	0.09 ab
	2	1.51 def	0.36 defg	5.77 h	1.75 i	3.39 hi	0.74 fg
	3	0.06 a	0.25 cde	1.51 de	1.26 gh	4.82 jk	0.59 ef
	4	0.00 a	0.07 a	0.70 abc	0.98 f	6.49 l	0.79 g

[a] Mean of three determinations. Values in the same column followed by different characters are signifciantly different ($p < 0.05$). [b] Sample names as expressed in Table I.

Table IV. Spectrophotometric Absorbance (×1000, 420 nm)[a] of Organic Extracts from Samples Maintained at 5, 15, and 30 °C

sampling[b]	sample[c]	5 °C	15 °C	30 °C
initial	IN	25.8 a	25.8 a	25.8 a
1st storage	1	38.3 ab	62.5 a	59.3 ab
	2	41.8 ab	75.8 ab	75.8 ab
	3	62.5 abc	80.0 abc	86.8 ab
	4	60.8 abc	119.3 abc	177.5 bc
2nd storage	1	65.0 abc	101.8 abc	88.3 ab
	2	53.3 abc	100.0 abc	112.5 ab
	3	73.8 abcd	105.8 abc	181.8 bc
	4	197.5 fg	190.8 cd	315.8 de
3rd storage	1	82.5 bcde	111.8 abc	174.3 bc
	2	81.8 bcd	185.0 bcd	293.3 cd
	3	103.3 cdef	182.5 bcd	438.3 ef
	4	152.5 fg	270.8 d	522.5 fg
4th storage	1	129.3 def	116.8 abc	121.3 ab
	2	134.3 ef	443.3 e	532.5 fg
	3	151.8 fg	426.8 e	570.8 g
	4	217.5 g	521.8 e	857.5 h

[a] Mean of three determinations. Values in the same column followed by different characters are significantly different ($p < 0.05$). [b] Besides the initial sampling, four times of storage were considered at each temperature (3, 8, 15, and 29 days at 5 °C; 2, 7, 14, and 28 days at 15 °C; 1, 4, 11, and 25 days at 30 °C). [c] Sample names as expressed in Table I.

samples 3 and 4 decreased with time. At 393/463 nm sample 1 reached higher values. At 479/516 nm a general increase in all samples with time was observed.

Organic Extracts. Fluorescence measurements of organic extracts are shown in Tables I–III. Values did not undergo so many significant differences as in the case of aqueous extracts. The initial sample showed low values at 327/415 and 393/463 nm and negligible values at 479/516 nm.

At 5 °C (Table I) the only significant behavior was found in sample 4, reaching its top value after 9 days (327/415 and 393/463 nm) and at the end of the experiment (479/516 nm).

Table II shows the evolution of fluorescence at 15 °C. At 327/415 nm there is a general tendency to decrease, especially in samples with high AcH content. At 393/463 nm and 479/516 nm all samples reached higher values; indeed, great differences were found in the case of samples with a high AcH content.

Table III (30 °C) shows no significant differences at 327/415 nm for any of the samples. At 393/463 nm there is a general increasing tendency, especially in samples 2 and 3. At 479/516 nm all of the samples followed a general behavior reaching higher values.

Measurement of brown color formation (420 nm) is shown in Table IV. Besides the initial measure, four successive determinations were carried out on samples of each temperature at the same time as fluorescence measurements (3, 8, 15, and 29 days for 5 °C; 2, 7, 14, and 28 days for 15 °C; and 1, 4, 11, and 25 days for 30 °C) were performed.

A gradual increase in absorbance was evident with increasing AcH concentration, time, and temperature. Values become higher as long as the three factors increase together. Samples with acetaldehyde (2, 3, and 4) showed a gradual increase with time.

Discussion. A fluorescence shift to higher wavelengths with increasing AcH concentration, time, and temperature of reaction was evident when acetaldehyde was incubated with tuna muscle. Accelerated by these three factors, fluorescent compounds formed in the first stages of interaction lead to the formation of other fluorescent compounds showing excitation/emission maxima at higher wavelengths than their precursors.

At the same time and according to this fluorescence shift, a gradual increase in brown color formation was obtained. Suyama and Adachi (1979) had already obtained a linear increase in browning as a result of a phosphatidylethanolamine–acetaldehyde interaction in a model system at 25 °C.

Samples containing fish muscle and phosphate buffer have shown some fluorescent development at 327/415 and 393/463 nm and browning increase as a result of thermal treatment. However, the addition of a reactive lipid oxidation compound (acetaldehyde) has led to a fluorescence shift to higher wavelength maxima (479/516 nm) and to a largely greater increase in browning. Furthermore, blank samples (mixtures of phosphate buffer and AcH) showed no fluorescence or brown color development.

The results agree with the general theory of progressive formation of Schiff bases and other interaction products with increasing molecular weights and unsaturated bonds

(Pokorny, 1977; Gardner, 1979; Kikugawa and Beppu, 1987). AcH has proved to play an important role in these transformations because of its high reactivity.

When the aqueous and organic extracts are compared, both show the same excitation/emission maxima and general behavior related to AcH concentration, time, and temperature. However, the values obtained for aqueous extracts were quantitatively higher and the differences were significant in more cases. Previous experiences (Shimasaki et al., 1984; Iio and Yoden, 1988) showed that fluorescent substances formed from oxidized membrane lipids with amino compounds would remain attached to the amino constituent; in the case of amino substrates such as proteins, fluorescent compounds could not be extracted with organic solvents but rather with aqueous solvents. For this reason the extent of lipid oxidation might appear lower than the actual amount if only organic extracts are considered (Pokorny, 1977).

Another interesting result obtained is the sensitive role played by AcH in the formation of fluorescent compounds. For a long time, this role was mainly supposed to be played by malondialdehyde (Gardner, 1979; Tappel, 1980), but many recent works have proved that other carbonyl compounds (unsaturated and with oxygenated functions mainly) may participate in the fluorescence formation with a higher contribution than malonaldehyde (Leake and Karel, 1985; Esterbauer et al., 1986; Montfoort et al., 1987).

Results obtained from this study reinforce the role of fluorescence measurements as a complementary test of quality control in processed foods and underline the interest of studying fluorescence formation at different wavelengths to follow the evolution of fluorescent compounds formed in the first stages of in vitro experiments and food processing.

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