LIPID CHANGES DURING LONG-TERM STORAGE OF CANNED TUNA

(Thunnus alalunga)

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ABSTRACT

Lipid damages were studied during a prolonged storage of canned fish. Albacore tuna was processed at two sterilization conditions (115°C, 74 min; 120°C, 40 min) and then stored up to six years. Analyses (lipid oxidation and hydrolysis, browning, fluorescent compounds formation) were achieved on the lipids extracted from the white muscle of the fish and on the packing oils. Muscle lipids were partially extracted by the dipping oil, so that an increase of the storage time produced higher levels of free fatty acids, browning and fluorescence development in the packing oils. Related to the muscle determinations, little differences were detected throughout the canned storage.

Running Title: Lipid changes during canned storage

Keywords: Canned storage, fish, lipid damages, packing oil

INTRODUCTION

Seafood lipids are known to contain a high amount of polyunsaturated fatty acids (PUFA) (1) which are supposed to play a positive role against certain diseases (2). When marine species are processed at high temperatures, PUFA damages can greatly lead to primary and secondary lipid oxidation products, which can finally produce browning (3), fluorescent compounds (4, 5), off flavour (6) and loss of essential nutrients (7). As a result, a close relationship between the lipid changes and the quality of the final product has been accorded (8, 9).

Canned fish and other marine products are of great economic significance in many countries. According to its importance, the lipid composition has been studied in the resulting canned products with a special stress in the changes occurred in the fatty acid composition and in the lipid classes (10, 11). Quality of the final product has been studied as a function of the packaging method (12), storage temperature of raw material prior to processing (13), the white muscle zone (14), the time-temperature conditions (11, 15) and the dipping medium (16, 17).

The present experience was envisaged in order to study the lipid changes in canned fish as a result of a prolonged canned storage. Lipid hydrolysis and oxidation, browning and fluorescent compounds formation were measured in the white muscle of canned albacore and in the packing oil. Two sterilization conditions (F_o = 10 and 7 min) were considered.

MATERIALS AND METHODS

Raw material and processing

The albacore tuna (Thunnus alalunga) used was caught by a tuna fishing vessel on the Atlantic Ocean (round 43°N and 27°W). The fish were kept in boxes and kept in ice for three days. After arrival at our laboratory the fish were frozen at -40 °C and then stored at -20 °C for 1 month.

The raw material was divided into three batches, that were studied separately along the whole experiment in order to carry out the statistical analysis. The fish were beheaded and eviscerated. Steam cooking was performed in our pilot plant (102-103 °C) to a final backbone temperature of 65 °C (90 min). The fish were then cooled at room temperature (14-17 °C) during five hours.

Portions of 80-90 g of cooked muscle were placed in RO-100 cans (6.52 cm diameter, 3 cm height) and olive oil (20 ml) and salt (2 g) were added. The cans were vacuum sealed and sterilized in a retort under two different time-temperature conditions: S1 (115 °C, 74 min; F_o = 10 min) and S2 (120 °C, 40 min; F_o = 7 min). Cans that contained only oil were also prepared (canned oil blanks) at both sterilization conditions. Cans were stored at room temperature in our laboratory throughout the experiment.

Sampling and lipid extraction

At several times after the sterilization step (1.5, 3, 4.5 and 6 years), cans corresponding to each batch of each sterilization condition were opened for analysis. The liquid part was drained off carefully, so that the white muscle of albacore and the packing oil were considered for analysis.

Lipids were extracted by the Bligh and Dyer (18) method from the white muscle of raw, cooked and canned albacore. Quantification was carried out according to Herbes and Allen (19). Two-gram portions of each oil (initial oil, blank oils, packing oils) were diluted with 15 ml of chloroform, and then analysed.

Lipid damage measurements

The free fatty acids (FFA) content was determined by the Lowry and Tinsley (20) method based on a complex formation with $(Ac_2O)_2Cu$-pyridine. Results are expressed as g FFA / 100 g lipids.

The conjugated dienes (CD) formation was measured at 233 nm (21). Results are calculated as: CD = B x V / w, where B is the absorbance reading, V denotes the

volume (ml) of the sample and w is the mass (mg) of the lipid material (muscle lipids or oils) measured.

The thiobarbituric acid index (TBA-i, mg malondialdehyde / Kg sample) was determined according to Vyncke (22).

The brown colour formation (BCF) was determined at 420 nm according to Labuza and Massaro (23). Results were calculated using the following formula: BCF = B x V / w, where B is the absorbance reading obtained, V is the volume (ml) of the sample, and w is the mass (g) of the lipid material (muscle lipids or oils) employed.

Fluorescence formation was studied at 393/463 nm and 327/415 nm according to previous experiences (15, 24). The relative fluorescences (RF) were calculated as follows: $RF = F / F_{st}$, where F is the sample fluorescence at each excitation/emission maximum, and F_{st} is the corresponding fluorescence intensity of a quinine sulphate solution (1 μg / ml in 0.05 M H_2SO_4). The fluorescence shift (δF) was calculated as the ratio between both RF values: $\delta F = RF_{393/463nm} / RF_{327/415nm}$, and was studied in the lipid extracts from albacore muscle and in the oils.

Chemicals employed along the present work were reagent grade (E. Merck, Darmstadt, Germany). All spectrophotometric determinations were carried out in a Beckman DV-64 spectrophotometer, all fluorescence determinations in a Perkin-Elmer LS 3B fluorescence spectrophotometer.

Statistical analysis

Data resulting from lipid damages measurements were subjected to the ANOVA one-way method, according to Sokal and Rohlf (25). Significance was declared at $p < 0.05$.

RESULTS AND DISCUSSION

Lipid hydrolysis

Both sterilization processes (S1 and S2) followed by the shortest storage time tested in this experiment (1.5 years) produced a hydrolytic effect on the muscle lipids (Tables 1 and 2) according to previous results related to short storage times (14, 16). Then, a slight increase in the FFA proportion was detected as a result of the storage. However, little differences were obtained.

No significant differences between the initial oil and any of the blank oils were detected (Tables 3 and 4). However, packing oils showed higher values of FFA than the blank ones, and also a progressive increase was obtained as a result of increasing the time of canned storage.

Results can be explained on the basis of the lipid classes extraction carried out by the packing oil (11, 17). Vegetable oils employed for fish canning are basically composed of triglycerides (26). Thus, this lipid class is faster extracted from the muscle lipids, so that the remaining lipid classes increase their proportions in the muscle lipids (14). As a result of the storage time, a slight increase in the FFA proportion of the muscle was obtained (Tables 1 and 2). At the same time, FFA from the fish muscle accumulated in the packing oil leading to a continuous increase in the proportion of this lipid class (Tables 3 and 4).

Lipid oxidation

The muscle lipids showed little significant variations of the CD content (Tables 1 and 2); a clear tendency could not be concluded as a result of the canned storage. The most interesting results were a sharp increase after 4.5 years of storage, followed by a

big decrease after six years. Variations in the CD value could be attributed to the extraction ability of the oil.

The packing oils showed higher values than the initial and blank ones (Tables 3 and 4), caused by the extraction of such molecules from the canned muscle. However, no significant differences were obtained between the coating oils throughout the storage.

Samples resulting from both sterilization processes (S1 and S2) produced the same behaviour for the TBA-i along the canned storage (Tables 1 and 2). A big increase after cooking was followed by a sharp decrease in the canned samples, and no significant differences were obtained as a result of the canned storage time.

Lipid oxidation detection (primary, CD; and secondary, TBA-i) has not shown to be able to assess differences and a clear tendency for the canned samples during the present experience. No oxidation development has been observed to occur in the muscle nor in the packing oil during the canned storage.

Interaction compounds formation

The browning measurement in the muscle lipids showed an increase after the sterilization step (in both conditions) and the shortest canned storage tested (1.5 years; Tables 1 and 2), according to previous results related to short storage times (15). Then, a decrease was observed after 4.5 years and then again after 6 years.

The browning formation in the different kinds of oils indicated the following increasing sequence (Tables 3 and 4): initial < blanks < packing. No differences were obtained between the blank oils as a result of the canned storage. In the case of the packing oils, a slight increase was observed throughout the storage, although differences were not significant in all cases.

It is concluded that browning compounds are widely formed in the muscle as a result of the interaction between oxidated lipids and nucleophilic constituents (aminated, mainly) (3, 27) during the thermal treatments (cooking and sterilization). Then, such adduct compounds are progressively extracted by the oil throughout the canned storage. Browning forming, measured as the absorbance at 420 nm, was not detected during the canned storage, as was indicated by the constant value of the blank oils and the decreasing value of the muscle.

Fluorescence detection of interaction compounds during food processing has been largely carried out by measuring the fluorescence formation at a single excitation/emission maximum (4, 5). Previous work carried out on thermally treated fish muscle indicated a bathochromic shift of fluorescence related to an increase of lipid oxidation and depending on time and temperature of processing (15, 24). The fluorescence shift (δF) calculated as the ratio between two of the maxima investigated (393/463 nm and 327/415 nm) showed to be interesting to be subsequently correlated with quality.

In the present experiment, the δF value was studied. The following increasing sequence was obtained for the muscle lipid extracts (Tables 1 and 2): raw < cooked < canned. However, δF value showed to be relatively constant along the whole storage.

The fluorescence detection (δF) in the oils (Tables 3 and 4) indicated little differences as a result of the canning process and the shortest storage time (1.5 years). However, the prolonged canned storage produced an increase in the δF value for both the packing and blank oils. A comparison between the corresponding blank and coating oils, indicated that significantly higher values were obtained for the packing oils after 6 years of storage.

In a previous work (15), a short canned storage (four months) was tested in three common sterilization conditions. According to the present results, no differences were obtained between the packing oils and the corresponding blank oils. Indeed, no differences were detected between the initial oil and the blank oils, that could be explained on the basis of the short storage time employed.

According to the present results, it is concluded that fluorescent compounds are formed in the oils during the canned storage by condensation reaction of lipids and other compounds present in the starting oil. At the same time, fluorescent compounds are also progressively formed as a result of the interaction between the packing oil and some constituents of the fish muscle. The content of fluorescent compounds (δF) formed in the muscle during the thermal treatment (Schiff bases and aldolic condensation compounds (28-31) remains almost constant along the canned storage.

CONCLUSIONS

Canned samples resulting from both sterilization treatments (S1 and S2) have led to very similar results and conclusions. Differences in the time-temperature conditions did not provide a different evolution of lipid changes during a prolonged canned storage.

Lipid-lipid interactions have been observed between both kinds of lipid material. The packing oil has progressively extracted the lipid components of the muscle along the canned storage, including metabolites that could be employed as quality indexes. As a result, the analysis of some lipid parameters (FFA content, browning development and fluorescence formation) in the packing oils was found sensible in order to assess lipid changes during a prolonged canned storage.

Results obtained from this study reinforce the role of fluorescence measurements as a complementary test of quality control in processed foods. Fluorescence detection as the δF value in the packing oil has proved to accord with the time of storage and traditional quality indexes (FFA, browning development). Detection of interaction compounds with fluorescent properties has recently attracted a great attention since nutritional and toxicological consequences are involved (7, 32, 33).

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TABLE 1: Lipid damages* measured in tuna muscle after cooking, S1 sterilization (115 °C, 74 min) and canned storage **.

Sample	FFA	CD	TBA- i	BCF	δF
Raw	2.47 a (0.26)	1.15 ab (0.01)	4.35 b (0.55)	0.89 a (0.25)	1.28 a (0.25)
Cooked	3.32 a (0.38)	1.38 b (0.21)	8.13 c (2.31)	2.04 a (0.19)	2.72 b (0.55)
S1(1.5)	8.93 b (1.20)	1.23 ab (0.05)	0.15 a (0.02)	6.82 c (1.61)	4.09 de (0.39)
S1(3)	11.33 bc (1.53)	1.26 ab (0.25)	0.13 a (0.03)	5.70 c (0.73)	3.56 cd (0.24)
S1(4.5)	12.36 c (1.38)	1.98 c (0.26)	0.14 a (0.05)	3.91 b (0.26)	3.29 bc (0.07)
S1(6)	13.80 c (3.96)	1.03 a (0.22)	0.14 a (0.02)	1.40 a (0.22)	4.55 e (0.65)

* 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 brackets.

** FFA (free fatty acids; g / 100 g lipids); TBA-i (thiobarbituric acid index; mg malondialdehyde / Kg muscle sample); CD (conjugated dienes), BCF (brown colour formation), δF (fluorescence shift) were calculated as expressed in the Materials and methods section. Canned samples names: S1(1.5), S1(3), S1(4.5) and S1(6), where the canned storage time (years) is indicated in brackets.

TABLE 2: Lipid damages* measured in tuna muscle after cooking, S2 sterilization (120 °C, 40 min) and canned storage **.

Sample	FFA	CD	TBA- i	BCF	δF
Raw	2.47 a (0.26)	1.15 b (0.01)	4.35 b (0.55)	0.89 a (0.25)	1.28 a (0.25)
Cooked	3.32 a (0.38)	1.38 c (0.21)	8.13 c (2.31)	2.04 ab (0.19)	2.72 b (0.55)
S2(1.5)	6.38 ab (0.89)	1.14 b (0.02)	0.15 a (0.03)	4.42 cd (0.95)	3.95 cd (0.43)
S2(3)	10.37 bc (2.63)	1.22 bc (0.11)	0.12 a (0.02)	6.25 d (2.73)	3.70 cd (0.11)
S2(4.5)	12.70 cd (3.59)	1.85 d (0.08)	0.12 a (0.01)	4.00 bc (0.32)	3.30 bc (0.20)
S2(6)	14.71 d (3.60)	0.89 a (0.15)	0.11 a (0.01)	1.39 a (0.36)	4.32 d (0.66)

* 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 brackets.

** Names and units of damage measurements as expressed in Table 1. Canned samples names: S2(1.5), S2(3), S2(4.5) and S2(6), where the canned storage time (years) is indicated in brackets.

TABLE 3: Lipid damages* measured in oil samples after the S1 sterilization (115 °C, 74 min) and canned storage **.

Sample	FFA	CD	BCF	δF
INO	0.06 a (0.00)	0.21 a (0.01)	0.081 a (0.003)	1.02 a (0.03)
BO-S1(1.5)	0.06 a (0.00)	0.23 a (0.01)	0.182 b (0.005)	1.14 a (0.03)
BO-S1(3)	0.12 a (0.00)	0.24 a (0.01)	0.202 b (0.005)	1.41 ab (0.04)
BO-S1(4.5)	0.11 a (0.00)	0.26 a (0.01)	0.204 b (0.004)	1.80 bc (0.04)
BO-S1(6)	0.13 a (0.00)	0.22 a (0.01)	0.205 b (0.006)	2.27 d (0.10)
PO-S1(1.5)	0.46 b (0.05)	0.32 b (0.06)	0.382 c (0.014)	1.60 b (0.31)
PO-S1(3)	0.82 c (0.18)	0.36 b (0.04)	0.466 d (0.034)	1.64 b (0.03)
PO-S1(4.5)	1.22 d (0.12)	0.32 b (0.01)	0.501 e (0.012)	2.19 cd (0.59)
PO-S1(6)	1.71 e (0.35)	0.31 b (0.04)	0.533 e (0.041)	3.13 e (0.21)

* Names and units of damage measurements as specified in Table 1. 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 brackets.

** INO (initial oil). Blank oils: BO-S1(1.5), BO-S1(3), BO-S1(4.5) and BO-S1(6); packing oils: PO-S1(1.5), PO-S1(3), PO-S1(4.5) and PO-S1(6); in both kinds of oils the canned storage time (years) is indicated in brackets.

TABLE 4: Lipid damages* measured in oil samples after the S2 sterilization (120 °C, 40 min) and canned storage **.

Sample	FFA	CD	BCF	δF
INO	0.06 a (0.00)	0.21 a (0.01)	0.081 a (0.003)	1.02 a (0.03)
BO-S2(1.5)	0.05 a (0.00)	0.22 a (0.01)	0.205 b (0.005)	1.09 ab (0.03)
BO-S2(3)	0.12 a (0.00)	0.23 a (0.01)	0.219 b (0.005)	1.50 bc (0.04)
BO-S2(4.5)	0.12 a (0.00)	0.28 b (0.01)	0.213 b (0.005)	1.86 cd (0.05)
BO-S2(6)	0.14 a (0.00)	0.21 a (0.01)	0.200 b (0.006)	2.25 d (0.10)
PO-S2(1.5)	0.73 b (0.12)	0.32 c (0.01)	0.420 c (0.054)	1.33 ab (0.29)
PO-S2(3)	0.92 b (0.23)	0.35 c (0.05)	0.437 cd (0.033)	1.64 c (0.23)
PO-S2(4.5)	1.33 c (0.08)	0.35 c (0.01)	0.490 d (0.011)	2.22 cd (0.66)
PO-S2(6)	1.87 d (0.28)	0.32 c (0.04)	0.494 d (0.089)	3.37 e (0.39)

* Names and units of damage measurements as specified in Table 1. 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 brackets.

** INO (initial oil). Blank oils: BO-S2(1.5), BO-S2(3), BO-S2(4.5) and BO-S2(6); packing oils: PO-S2(1.5), PO-S2(3), PO-S2(4.5) and PO-S2(6); in both kinds of oils the canned storage time (years) is indicated in brackets.