QUALITY DIFFERENCES ASSESSMENT IN CANNED SARDINE (*Sardina pilchardus*) BY FLUORESCENCE DETECTION

S. Aubourg* and I. Medina

Instituto de Investigaciones Marinas (CSIC)

c/ E. Cabello, 6

36208-VIGO (Spain)

Phone: +34-86-231930

Fax: +34-86-292762

E-mail: saubourg@iim.csic.es

ABSTRACT

Chilled (0, 2, 6, 10, 13 and 15 days) and frozen (0, 0.5, 2, 4, 8 and 12 months) sardines were used to determine the influence of such storage times of fish over the quality of the final canned product. Traditional determinations of lipid quality (free fatty acids content, thiobarbituric acid index and polyene index) were studied and compared with the formation of fluorescent compounds expressed as the ratio between fluorescence readings taken at two excitation/emission maxima (393/463 nm and 327/415 nm). No clear correlations were found between the common measurements of lipid deterioration and the time of storage prior to canning. Satisfactory correlations were found between the fluorescence ratio obtained from the filling medium of cans and the time of storage of the starting material ($r = 0.90$ and $r = 0.91$ in brine and oil canned samples, respectively). According to the present results, fluorescence detection of interaction compounds can provide a rapid and sensible method to assess quality differences in the final product as it relates to the quality of the raw material used.

Running title: Quality determination of canned sardine by fluorescence

Keywords: Canning, fluorescence, lipid deterioration, quality, sardine.

INTRODUCTION

Canned fish and other processed marine species are products of great economic importance in several countries. The majority of quality problems found in canned products can be related to the quality of raw material, which continuously changes during storage which can include freezing and chilling (Cheftel and Cheftel, 1976; Pigott and Tucker, 1987). Marine lipid composition is highly unsaturated (Ackman, 1989) and oxidation during storage and processing is likely to occur, leading to quality loss (Pearson et al., 1977; Ackman, 1988).

Many methods have been used to measure primary (peroxides) and secondary (carbonyl compounds) oxidation products as a degree determination of quality deterioration (Melton, 1983; Kim and Labella, 1987). However, most of these methods are difficult to use for quality assessment during heating since oxidation products are unstable and tend to react with compounds with free amine groups (proteins, peptides, free amino acids and some phospholipids) (Gardner, 1979; Leake and Karel, 1985). As a result of this kind of reactions, browning, flavor changes (Pearson et al., 1977; Pokorný, 1981) and loss of essential nutrients (Nielsen et al., 1985; Hidalgo et al., 1992) have been observed during processing.

The analysis of the compounds resulting from these interactions has been carried out by measuring the fluorescence formation at a single excitation/emission wavelength maximum (Bouzas et al., 1985; Lubis and Buckle, 1990; Maruf et al., 1990).

Previous work carried out on fish muscle after thermal treatment has found a bathochromic shift of fluorescence related to an increase of lipid oxidation depending on time and temperature of processing (Aubourg et al., 1992a, 1992b and 1995b). The

fluorescence ratio between two of the maxima investigated (393/463 nm and 327/415 nm) has shown to be correlated with quality during chilling (Aubourg et al., 1997).

In the present work the influence of the storage time of fish previous to canning was determined. Raw sardines were stored at two of the most common storage conditions (chilling: 0°C, on ice; and freezing: -18°C) and then canned according to the typical process employed in canneries. Qualities of the resulting canned products were compared by means of the traditional lipid quality determinations (free fatty acids content, thiobarbituric acid index and polyene index) and fluorescence formation. The usefulness of the fluorescence detection was analyzed for two types of filling media (brine and oil) widely employed for canning and also on the organic and aqueous extracts resulting from the lipid extraction of sardine muscle.

EXPERIMENTAL PROCEDURES

Raw material, chilled and frozen storages

Fresh sardines (*Sardina pilchardus*) were obtained in a local market (twelve hours after capture). Upon arrival at our laboratory, individual fish were stored in isothermal rooms at 0°C (on ice) and -18°C. Inside each temperature, sardines were randomly distributed into three groups that were studied independently. Chilled sardines from each batch were taken for canning after: 0, 2, 6, 10, 13 and 15 days of storage. Frozen sardines were taken for canning after: 0, 0.5, 2, 4, 8 and 12 months of storage.

Canning process

Chilled and frozen sardines were steam cooked in our pilot plant (102-103°C) to a final backbone temperature of 65°C (Pérez-Martín, et al., 1989); the fish were then cooled at room temperature (14°C) for about 3 hours, headed and eviscerated.

Two individual sardines were placed in each small flat rectangular can (105 x 60 x 25 mm; 150 ml). Two filling media were tested. For it, brine (20 ml of 2 % NaCl in water) was employed as filling liquid in cans containing sardines that had been kept chilled; olive oil (20 ml) and salt (2 g) were employed in cans containing sardine that had been kept frozen. Cans containing only oil were also prepared (canned oil blanks).

The cans were vacuum sealed and sterilized in a retort (115°C, 45 minutes; F_o = 7 min). After 4 months of storage at room temperature, the cans were opened and the liquid (brine or oil) was carefully drained off. The white muscle of sardine and the filling medium were used for analysis.

Basic analyses

Water content was determined by weight difference of the homogenized muscle (1-2 g) before and after 24 hours at 105°C; results are expressed as g water/100 g muscle. Lipids were extracted by the Bligh and Dyer (1959) method and quantification was carried out according to Herbes and Allen (1983). Results are expressed as g lipids /100 g muscle.

Lipid damage measurements

Free fatty acids (FFA) content was determined by the Lowry and Tinsley (1976) method based on a complex formation with $(Ac_2O)_2Cu$-pyridine. Results are expressed as g FFA/100 g lipids.

The thiobarbituric acid index (TBA-i) (mg malondialdehyde/Kg muscle) was determined according to Vyncke (1970).

Lipid extracts corresponding to brine canned samples were converted into fatty acid methyl esters and analyzed by Gas-Chromatography according to Lepage and Roy

(1986). The polyene index (PI) was calculated as the following fatty acids ratio: 20:5 + 22:6 / 16:0 (Lubis and Buckle, 1990).

Fluorescence analysis

A Perkin-Elmer LS 3B fluorescence spectrophotometer was used for fluorescence determination. The fluorescence shift (δF) was calculated as follows:

$$\delta F = \frac{F3/F3_{st}}{F1/F1_{st}}$$

where F3 and F1 are the fluorescence intensities of the sample at 393/463 nm and 327/415 nm, respectively. $F3_{st}$ and $F1_{st}$ are the fluorescence intensities of a quinine sulfate solution (1 μg/ml in 0.05 M H_2SO_4) at the corresponding wavelength.

The fluorescence shift was studied on the aqueous (δF_{aq}) and organic (δF_{or}) phases resulting from lipid extraction (Bligh and Dyer, 1959) of white muscle and also on the liquid medium of the can (filling or packing medium) (δF_{pm}).

For the fluorescence analysis of the packing media, the liquid part of the can was carefully drained off. In the case of the canned with oil samples, 1g of each filling oil was made up to 15 ml with chloroform. For the brine canned samples, the whole packing brine was made up to 100 ml with aq. 2% NaCl. Both kinds of solutions were then directly employed for fluorescence analysis.

Statistical analyses

Data resulting from basic analyses, lipid damage measurements and fluorescence shifts were subjected to the ANOVA one-way method, according to Sokal and Rohlf (1981). Regression models were assayed with all variables studied; linear and non linear correlations were carried out using the Statistica package (Statsoft, 1994). Significance was declared at $p < 0.05$.

RESULTS

A) Chilled storage and brine canning

Basic and lipid damage determinations

No differences in water content in canned sardines were obtained as a result of differences in chilling time (Table 1). Lipid contents in the canned material did not show clear effects resulting from the previous storage and few significant differences were observed (Table 1).

The common quality indexes related to lipids showed different tendencies relative to the time of storage. An increase in the FFA content after canning can be inferred by comparing the RAW_{CH} and BCS_0 samples (0.20 ± 0.05 and 4.11 ± 0.23, respectively) (Table 2 and Figure 1.A.), according to the thermal physical breakdown of lipids in canned products (Medina et al., 1994). Regarding the time of previous chilled storage, a progressive increase in the FFA proportion of canned products was observed (Figure 1.A.). Data obtained showed a significant linear correlation with the time of storage, and subsequently with the quality of the starting material ($r = 0.51$).

The determination of the TBA reactive substances (TBARS) in the canned samples did not provide a clear tendency by comparison with the previous time of storage (Figure 1.B.). No significant linear and non linear correlations could be obtained

between both parameters. A large difference was obtained in TBA values between RAW_{CH} and BCS_0 samples (Table 2 and Figure 1.B.).

The PI value showed a decreasing tendency as long as the storage time increased (Figure 1.C.). However, no significant correlations could be obtained. By comparing the different canned values, no significant differences were obtained. Some previous research on cooking (Hearn et al., 1987; Gallardo et al., 1989) and canning (Hale and Brown, 1983; Aubourg et al., 1995a) has shown that the polyunsaturated fatty acids (PUFA) proportion in total lipids did not diminish as a result of such thermal processes.

Fluorescence analysis

The fluorescence shift measured in the aqueous phase (δF_{aq}) did not show a good relationship with the previous storage time (Figure 2.A.). Different storage times of the starting chilled material did not provide significant differences between the corresponding canned products. The best results were obtained by using an exponential model but the correlation calculated was poor ($r = 0.24$). An increase was observed resulting from the canning process (comparison of RAW_{CH} and BCS_0 samples; Table 2 and Figure 2.A.) according with data previously described (Aubourg et al., 1992b).

The analysis of the organic phase (lipid extract) showed a significant linear dependence of the δF_{or} value of the canned samples with the previous storage time (Figure 2.B., $r = 0.47$). By comparing with the RAW_{CH} sample (Table 2), a significant increase in canned samples was only detected after 10 days of previous storage (BCS_{10}, Figure 2.B.).

The most satisfactory results were obtained in the analysis of the fluorescence shift from the filling liquid (δF_{pm}) (Figure 2.C.). A stronger dependence with the

previous storage time than the other measurements was obtained. The best results were obtained using a non linear fitting (r = 0.90).

B) Frozen storage and oil canning

Basic and lipid damage determinations

By comparing the RAW_{FR} and OCS_0 samples, a loss in the water content is concluded as a result of the canning process (Table 1). However, the different frozen storage times did not provide significant differences in the water content in the canned samples.

Regarding the lipid content, little significant differences were observed (Table 1). Different storage times of the frozen material did not provide differences in the canned samples. An important loss of lipids from muscle was observed by comparing the raw material (RAW_{FR}) and most of the oil canned samples. This effect is probably due to the extraction carried out by the filling oil (Aubourg et al., 1990).

The FFA content of the canned samples did not show a clear tendency in comparison with the previous frozen storage time (Figure 3.A.). No significant correlations between both parameters could be obtained. Canned samples without previous frozen storage (OCS_0) showed a very low FFA level compared with the remaining canned ones.

Results obtained for the brine canned samples (Figure 1.A.) showed that the thermal treatment itself causes a great increase in the FFA content. However, in the case of oil canning, no increase was observed (comparison of RAW_{FR} and OCS_0 samples; Table 2 and Figure 3.A.), that could be explained as a result of the presence of the oil medium in the muscle and a partial extraction of lipid classes from the muscle into the oil (Aubourg et al., 1990).

Results obtained for the TBA-i are shown in Figure 3.B. An increasing storage time of the starting material did not produce a constant increase throughout the experiment. Significant correlations obtained using exponential models were poor (r = 0.23).

Fluorescence analysis

A significant correlation was found by comparing the fluorescence shift measured in the aqueous phase (δF_{aq}) of the canned samples with the time of frozen storage using exponential regression models (r = 0.49; Figure 4.A.).

Best results were obtained in the case of measuring the fluorescence shift in the organic phase (δF_{or}) of the canned muscle (Figure 4.B.). A significant linear correlation with the previous storage time was obtained (r = 0.62). A high increase was observed as a result of canning (comparison of RAW_{FR} and OCS_0 samples; Table 2 and Figure 4.B.) according to previous results (Aubourg et al., 1992b). Among canned sardines, an important increase of δF_{or} value was obtained after 4 months of frozen storage (Figure 4.B.).

Regarding the fluorescence shift measured in the packing oil (δF_{pm}) a strong dependence with the time of previous storage was obtained (Figure 4.C.). Good regressions, linear and quadratic were found (r = 0.85 and r = 0.91, respectively). A high increase in the fluorescence value of canned samples was obtained after 4 months of frozen storage (Figure 4.C.). Results using a multiple regression model with the three measurements of fluorescence (δF_{aq}, δF_{or} and δF_{pm}) did not improve the correlation obtained using only the δF_{pm}.

According to previous results (Aubourg et al., 1992b), no differences between the fluorescence measured in the initial oil (δF = 0.92) and the oil that had been

separately canned (blank, $\delta F = 0.94$) were observed. Both measurements did not differ from the packing oil corresponding to OCS_0 sample (Figure 4.C.), but were significantly different compared to the filling oil from samples that had been stored 0.5 months ($OCS_{0.5}$, Figure 4.C.).

DISCUSSION

Many previous reports have proved that fish quality progressively decreases throughout the chilling (El Marrakchi et al., 1990; Bennour et al., 1991) and frozen (Shewfelt, 1981; Verma et al., 1995) storages. In both treatments, lipid changes have shown to play an important role as quality indexes during the quality loss (Hwang and Regenstein, 1993; Han and Liston, 1988).

In this work the common indexes of lipid degradation measured in canned sardines did not show good correlations with the previous time of storage of the raw material, so that quality differences in the final product were difficult to assess. As an explanation, degradation products that are susceptible to be measured in such indexes can either be distributed into different phases of the can (packing medium, exudate, fish muscle), be partially destroyed during the heat process, or interact with other constituents, so that the determination of such degradation compounds can not always afford an accurate method for the quality assessment.

Fluorescence detection of interaction compounds formed between damaged lipids and other biological constituents (aminated, mainly) of the muscle has provided some interesting results. Fluorescence analysis of muscle lipids extracts (δF_{aq} and δF_{or}) afforded poor results that could be explained by the presence in the can of a filling liquid (brine or oil). In a previous paper (Aubourg et al., 1997), good correlations were

obtained between the chilled storage time and the δF_{aq} and δF_{or} values in chilled sardine.

However, the analysis of both packing media in the present work showed a satisfactory correlation with the time of storage of the raw material employed for canning. Values obtained for the linear and non linear regressions were always higher than in the case of classical indexes related to lipid damages. This kind of measurement showed to remain valid in spite of the fact that a strong thermal treatment (cooking and sterilization) is included.

Results obtained in this work reinforce the role of fluorescence detection of interaction compounds as a tool for quality assessment. Further experiments (comparison of different storage conditions, comparison of different filling media, and so on) focused in the fluorescence detection in the filling medium during canning are still necessary in order to consolidate such a rapid and sensible measurement as an accurate method to be correlated with the quality of the starting material.

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TABLE 1

Basic analyses* in brine canned sardine (BCS)** and oil canned sardine (OCS)***

Brine Canned Sardine			Oil Canned Sardine		
	Water Content	**Lipid Content**		**Water Content**	**Lipid Content**
RAW_{CH}	69.02 [a]	3.69 [a]	**RAW_{FR}**	68.20 [b]	7.11 [c]
BCS_0	71.04 [a]	3.74 [a]	**OCS_0**	59.87 [a]	3.99 [a]
BCS_2	67.95 [a]	4.07 [ab]	**$OCS_{0.5}$**	60.26 [a]	4.23 [ab]
BCS_6	71.00 [a]	5.38 [bcd]	**OCS_2**	57.98 [a]	4.42 [ab]
BCS_{10}	68.84 [a]	5.99 [d]	**OCS_4**	58.09 [a]	3.63 [a]
BCS_{13}	70.67 [a]	5.56 [cd]	**OCS_8**	60.08 [a]	5.94 [bc]
BCS_{15}	70.56 [a]	4.49 [abc]	**OCS_{12}**	59.02 [a]	3.60 [a]

* Mean values of three determinations. For each set of canned sardine, values in the same column followed by different letters are significantly different ($p<0.05$).

** Sample names: RAW_{CH} (raw sardine); BCS_0, BCS_2, BCS_6, BCS_{10}, BCS_{13} and BCS_{15} (canned sardine that was kept chilled during 0, 2, 6, 10, 13 and 15 days, respectively).

*** Sample names: RAW_{FR} (raw sardine); OCS_0, $OCS_{0.5}$, OCS_2, OCS_4, OCS_8 and OCS_{12} (canned sardine that was kept frozen during 0, 0.5, 2, 4, 8 and 12 months, respectively).

TABLE 2

Measurements* of lipid degradation for raw samples employed in both experiments**.

	Brine Canned Sardine (RAW_{CH})	Oil Canned Sardine (RAW_{FR})
FFA	0.20±0.50	1.11±0.09
TBA-i	0.98±0.34	0.47±0.24
PI	1.66±0.04	
δF_{aq}	0.78±0.13	0.37±0.04
δF_{or}	1.50±0.44	0.43±0.06

*FFA: free fatty acids, TBA-i: thiobarbituric acid index, PI: polyene index, δF_{aq} and δF_{or} fluorescence shifts of the aqueous and organic phases, respectively.

** Mean values of three determinations ± standard deviations.

FIGURES

Figure 1:

Lipid damage measurements in brine canned sardine after chilled storage. **A.** FFA, **B.** TBA-i. and **C.** PI. Abbreviations as specified in Table 2.

Figure 2:

Fluorescence shifts in brine canned sardine after chilled storage. **A.** δF_{aq}, **B.** δF_{or}. and **C.** δF_{pm}. Abbreviations as specified in Table 2; δF_{pm}: fluorescence shift of the packing medium.

Figure 3:

Lipid damage measurements in oil canned sardine after frozen storage. **A.** FFA and **B.** TBA-i. Abbreviations as specified in Table 2.

Figure 4:

Fluorescence shifts in oil canned sardine after frozen storage. **A.** δF_{aq}, **B.** δF_{or}. and **C.** δF_{pm}. Abbreviations as specified in Table 2 and Figure 2.