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The Effects of Fat Content and Storage Temperature on the Storage Life of Smoked Sardine Fillets (*Sardina pilchardus* W.) Prepared from Frozen Sardine

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ABSTRACT

The effect of fat content and storage temperature on the biochemical and organoleptic alterations taking place during storage of smoked sardine fillets (made from sardines which were stored for three and six months at −18°C prior to being smoked and the influence of such alterations on product storage life were studied). During storage at 1°C, softening was the factor most affecting storage life in the smoked batches made from sardine frozen for three months prior to smoking or from sardine with higher fat content. In contrast, rancidity was the primary factor limiting storage life in the smoked batches made from sardine with lower lipid content and in the batches stored at −18°C. In the smoked batches made from sardines with lower fat content frozen for six months prior to smoking, the level of oxidation in the raw material was the main factor limiting the storage life of the smoked fillets; organoleptic rancidity was more perceptible in the smoked fillets stored at −18°C on account of a sharp drop in smoked flavour.

INTRODUCTION

The use of smoking as a means of preserving foods is almost as old as mankind itself. Despite this, and although there has never been a lack of traditional smoked products, consumption of smoked foods began to fall with the development of industrial cold-storage techniques. For the past ten years, however, the use of smoking has again been on the increase,

thanks to the combination of two or more preservation technologies working together to extend storage life. In the case of underutilized fish catches like sardine in Spain today, the appropriate application of smoking in combination with freezing is clearly a simple and attractive method of preservation that will also diversify the range of products available to consumers.

The chemical composition of small pelagic fish species, and sardine in particular, undergoes large fluctuations over the course of the year (Beltrán, 1988). Because of such seasonal fluctuations, the time of year, which determines the fat content of the fish, has an extremely important effect on product life and acceptability and on efforts to standardize the smoking process. In this regard, freezing the raw material intended for smoking may constitute a means of ensuring a constant supply of sardine with the same fat content for use in smoking nearly all year round. Ke and Ackman (1976) stated that high lipid contents (15–20%) were a source of difficulty during processing. However, Bhuiyan *et al.* (1986) reported that mackerel with a high lipid content was normally more suitable for smoking than mackerel with lower lipid levels.

The object of the present study was to examine the effects of fat content and storage temperature on the biochemical and organoleptic alteration taking place during the storage of smoked sardine fillets.

MATERIAL AND METHODS

Preparation and smoking of samples

The sardine (*Sardina pilchardus* W.) used in the experiment were caught in the Mediterranean Sea in March (lean sardine, e.g. a lower fat content) and in June (fatty sardine, i.e. a higher fat content). The fish were boxed in ice for transportation to the pilot plant at the Instituto del Frio, where they were frozen in a horizontal plate freezer at −40°C. Lean sardine fillets were stored for three and six months in a forced-ventilation cold store at −18°C prior to smoking; fatty sardine fillets were stored under the same conditions, but only for three months.

For smoking, the sardines were thawed at +4°C for 36 h. They were then filleted and immersed in brine (16% NaCl) for 3 min, after which they were air-dried in a forced-ventilation cold store at 0°C for 24 h. Smoking took place in an AFOS Torry-type kiln for 2 h at 30°C, followed by 45 min at 75°C. The smoked fillets were vacuum-packaged in a nylon–polyethylene film with a low permeability to oxygen. The keeping

quality of the final product was tested after 90 days at storage temperatures of +1°C ± 2°C and −18°C ± 2°C. Organoleptic and chemical analyses were performed at regular intervals during storage.

Batches of smoked sardine made from the lean sardine frozen for three months were designated L3C (chilled at +1°C) and L3F (frozen at −18°C). The batches smoked using the fatty sardine frozen for three months were designated F3C (chilled at +1°C) and F3F (frozen at −18°C). Smoked batches were also prepared from the lean sardine that had been frozen for six months; the chilled batch was designated L6C, the frozen batch L6F. Smoked batches were also made from fatty sardine that had been frozen for six months, but they were regarded as unsuitable for further processing, primarily because of problems relating to texture and appearance, and they were therefore not included in the study.

Chemical analysis

Lipid content was determined according to AOAC (1975) procedures. The phenol content of the smoked product was estimated as per Di Cesare (1979). Lipid extraction followed the method of Bligh and Dyer (1959), and the lipids were used to measure the peroxide value (POV) (UNE, 1973).

Four replications were performed for all determinations.

Sensory analysis

Sensory analysis was carried out by a taste panel consisting of seven trained members from the Instituto del Frio's staff.

Samples were allowed to warm at room temperature for 4 h prior to evaluation by the taste panel.

Panellists rated smoked flavour on a structured scale of from 1 to 7, where 1 was extremely weak, 4 was moderate, and 7 extremely strong.

Texture was also scored according to a structured scale of from 1 to 7, with 1 extremely mushy, 4 firm, and 7 extremely hard.

Organoleptic evaluation of rancid flavour was rated by panellists on a structured scale of between 1 and 7, where 1 was very good, no trace of rancidity; 2 good, agreeable taste and only very weak rancidity; 3 moderately rancid; 4 distinctly rancid but still acceptable; 5 distinctly rancid, unacceptable; 6 very strong rancidity, distasteful; 7 completely rancid, objectionable.

Lastly, overall acceptability was assessed on a structured scale of from 1 to 7, with 1 extremely bad, 4 fair, and 7 extremely good.

Statistical treatment

Analysis of variance was carried out using BMDP PV programs (BMDP, 1981) on a CDC CYBER 180/185 computer (IBM).

RESULTS AND DISCUSSION

The lipid content of the smoked lean sardine fillets made from sardines caught in March (5·1% fat) was 7·2%, while the smoked fatty sardine fillets made from sardines caught in June (10·9% fat) has a lipid content of 12·6%.

Tables 1 and 2 set out the phenol content and the sensory analysis of smoked flavour, respectively, in the smoked sardine fillets over the storage period. According to certain workers, the phenolic fraction in the smoke is the main factor responsible for imparting the characteristic smoked flavour to smoked products (Daun, 1979). For this reason, determination of the phenol content is widely accepted as a measure expressing the degree of smoking as well as losses in smoked flavour during storage (Senesi *et al.*, 1980).

The phenol concentration (Table 1) decreased significantly during storage in all the batches except batch F3C. Many of the phenols

TABLE 1
Phenol Concentration (mg/100 g) in the Various Smoked Sardine Batches during Storage

Batch	*Days in storage*			
	0	*30*	*60*	*90*
L3C	$8{\cdot}30^{a1}$	$8{\cdot}35^{a1}$	$8{\cdot}50^{a1}$	$7{\cdot}95^{b1}$
L3F	$8{\cdot}30^{a1}$	$8{\cdot}00^{b2}$	$7{\cdot}35^{c2}$	$7{\cdot}20^{c2}$
F3C	$8{\cdot}75^{a2}$	$8{\cdot}80^{a3}$	$8{\cdot}65^{a1}$	$8{\cdot}60^{a3}$
F3F	$8{\cdot}75^{a2}$	$8{\cdot}35^{b1}$	$7{\cdot}40^{c2}$	$7{\cdot}60^{c4}$
L6C	$8{\cdot}05^{a3}$	$8{\cdot}10^{a2}$	$7{\cdot}80^{b3}$	$7{\cdot}80^{b5}$
L6F	$8{\cdot}05^{a3}$	$7{\cdot}30^{b4}$	$7{\cdot}15^{b4}$	$6{\cdot}05^{c6}$

L3C: Batch smoked using lean sardine frozen for three months and then stored at 1°C.
L3F: Batch smoked using lean sardine frozen for three months and then stored at −18°C.
F3C: Batch smoked using fatty sardine frozen for three months and then stored at 1°C.
F3F: Batch smoked using fatty sardine frozen for three months and then stored at −18°C.
L6C: Batch smoked using lean sardine frozen for six months and then stored at 1°C.
L6F: Batch smoked using lean sardine frozen for six months and then stored at −18°C.
Different superscripts in the same row indicate significant differences ($P < 0{\cdot}05$).
Different superscripts in the same column indicate significant differences ($P < 0{\cdot}05$).

deposited on the surface of the fish by the gaseous phase of the smoke are volatile in nature; hence losses with storage time are likely, even when products are vacuum-packaged. In addition, both the storage temperature and the length of frozen storage undergone by the raw material before smoking appeared to be important. In this respect, the phenol concentration (Table 1) and the taste panel ratings (Table 2) for the frozen batches were significantly lower than those for the chilled batches, and there were statistically significant differences between the batches prepared from the lean sardine frozen for three and for six months from the start of the storage period. To a certain extent, these differences may have been due to difficulties related to extraction of the phenols using aqueous ethanol. The reactivity between phenols and proteins has been well established (Daun, 1979). During frozen storage proteins probably present active sites for phenols, resulting in an increase in protein–phenol linkages, so that the aqueous ethanol is unable to extract as many phenols. Khammadi and Goncharov (1979) reported surprisingly low phenol concentrations in hot-smoked jack mackerel stored frozen for five months prior to smoking.

The taste panel detected constant smoked flavour levels (Table 2) in the chilled batches L3C and F3C over the 90-day storage period. This was not the case for the chilled batch prepared from the lean sardine frozen for six months (L6C), where there was a statistically significant decrease in smoked flavour after 90 days. Smoked flavour scores declined in all the frozen batches (L3F, F3F, L6F); this decrease was most pronounced in batch L6F.

Phenol concentrations (Table 1) were generally higher in the smoked batches made from the fatty sardine (caught in June) than in the batches

TABLE 2
Smoked Flavour Sensory Analysis Scores in the Various Smoked Sardine Batches during Storage

Batch	*Days in storage*			
	0	*30*	*60*	*90*
L3C	$4{\cdot}7^{a1}$	$4{\cdot}8^{a1}$	$4{\cdot}4^{a1}$	$4{\cdot}8^{a1}$
L3F	$4{\cdot}7^{a1}$	$3{\cdot}8^{b2}$	$3{\cdot}2^{b2}$	$2{\cdot}0^{c2}$
F3C	$5{\cdot}8^{a2}$	$5{\cdot}7^{a3}$	$5{\cdot}7^{a3}$	$6{\cdot}0^{a3}$
F3F	$5{\cdot}8^{a2}$	$5{\cdot}7^{a3}$	$5{\cdot}0^{b1}$	$3{\cdot}1^{c4}$
L6C	$4{\cdot}0^{a3}$	$4{\cdot}1^{a2}$	$4{\cdot}4^{a1}$	$3{\cdot}3^{b4}$
L6F	$4{\cdot}0^{a3}$	$2{\cdot}1^{b4}$	$1{\cdot}7^{c4}$	$1{\cdot}2^{d5}$

Different superscripts in the same row indicate significant differences ($P < 0{\cdot}05$).
Different superscripts in the same column indicate significant differences ($P < 0{\cdot}05$).

prepared from the lean sardine (caught in March). This was also indicated by the sensory analysis (Table 2). This may be because of the higher fat content in the fatty sardines, with the lipids probably migrating to the surface of the fish during smoking, facilitating absorption of phenols (Korhonen *et al.*, 1978). While absorption of phenols from the gaseous phase of the smoke is higher when moisture content at the surface is high (Foster & Simpson, 1961), fat also has the property of retaining liposoluble phenols from the gaseous phase. Such phenols are largely deposited towards the end of smoking, when the dispersed particles in the smoke are absorbed by the fat on the now-dry surface of the fish (Rusz & Miler, 1977).

Table 3 presents the sensory evaluation of texture for the smoked sardine fillets. Taste panel ratings for the frozen batches were constant over the storage period studied. In contrast, the chilled batches exhibited progressive softening over the 90 days of storage. In the earlier paper Beltrán *et al.* (1989) discussed microbial growth in chilled smoke sardine, and consequently the softening detected may have been the result of proteolysis caused by microbial enzymes. Those same investigators also reported higher viable bacterial counts in smoked sardine prepared from fatty sardine, implying more intense proteolysis than in batches made from lean sardine, irrespective of the higher levels of endogenous protease activity recorded in sardine caught in summer as compared to sardine caught in winter. These two factors accounted for the generally lower scores (i.e. greater softening) over the storage period, awarded by the taste panel to the batches made from the fatty sardine, than to those made from the lean sardine.

Table 4 gives the sensory analysis scores for rancid flavour in the smoked sardine fillets during storage. In the batches smoked using

TABLE 3
Texture Sensory Analysis Scores in the Various Smoked Sardine Batches during Storage

Batch	*Days in storage*			
	0	*30*	*60*	*90*
L3C	$4{\cdot}4^{a1}$	$4{\cdot}1^{a1}$	$4{\cdot}0^{a1}$	$2{\cdot}7^{b1}$
L3F	$4{\cdot}4^{a1}$	$4{\cdot}5^{a2}$	$4{\cdot}1^{a1}$	$4{\cdot}5^{a2}$
F3C	$3{\cdot}7^{a2}$	$3{\cdot}8^{a1}$	$2{\cdot}3^{b2}$	$1{\cdot}8^{c4}$
F3F	$3{\cdot}7^{a2}$	$4{\cdot}0^{a1}$	$4{\cdot}0^{a1}$	$3{\cdot}9^{a3}$
L6C	$4{\cdot}2^{a1}$	$4{\cdot}0^{a1}$	$3{\cdot}3^{b3}$	$2{\cdot}9^{c1}$
L6F	$4{\cdot}2^{a1}$	$4{\cdot}0^{a1}$	$3{\cdot}9^{a1}$	$4{\cdot}0^{a3}$

Different superscripts in the same row indicate significant differences ($P < 0{\cdot}05$).
Diffffferent superscripts in the same column indicate significant differences ($P < 0{\cdot}05$).

TABLE 4
Rancid Flavour Sensory Analysis Scores in the Various Smoked Sardine Batches during Storage

Batch	*Days in storage*			
	0	*30*	*60*	*90*
L3C	$2{\cdot}1^{a1}$	$2{\cdot}6^{b1}$	$3{\cdot}2^{c1}$	$4{\cdot}3^{d1}$
L3F	$2{\cdot}1^{a1}$	$3{\cdot}0^{b2}$	$3{\cdot}7^{c2}$	$4{\cdot}8^{d2}$
F3C	$1{\cdot}6^{a2}$	$1{\cdot}8^{a3}$	$2{\cdot}4^{b3}$	$3{\cdot}7^{c3}$
F3F	$1{\cdot}6^{a2}$	$2{\cdot}2^{b4}$	$2{\cdot}9^{c4}$	$4{\cdot}2^{d1}$
L6C	$3{\cdot}4^{a3}$	$4{\cdot}0^{b5}$	$5{\cdot}7^{c5}$	$5{\cdot}8^{c4}$
L6F	$3{\cdot}4^{a3}$	$5{\cdot}9^{b6}$	$6{\cdot}4^{c6}$	$6{\cdot}3^{c5}$

Different superscripts in the same row indicate significant differences ($P < 0{\cdot}05$).
Different superscripts in the same column indicate significant differences ($P < 0{\cdot}05$).

sardine frozen for three months (L3C, L3F, F3C and F3F), organoleptic rancidity was more perceptible in the frozen batches, even though oxidation levels indicated by the POVs were higher in the chilled batches than in the frozen ones (Table 5). This may have been due to the decreased smoked flavour in the frozen batches (Table 2), which made the rancid flavour more readily detectable by the taste panel. The batches smoked using the lean sardine frozen for three months, displayed significantly higher scores than did the batches smoked using the frozen fatty sardine, which was in consonance with the results of the biochemical indices (Table 5). This was related to the characteristics of the raw material, e.g. such factors as tocopherol content, lipid:pigment ratio, degree of unsaturation of the fatty acids, etc., all of which varied in accordance with the seasonal fluctuations in chemical composition of the sardine (Beltrán,

TABLE 5
Peroxide Values (meq./kg fat) in the Various Smoked Sardine Batches during Storage

Batch	*Days in storage*			
	0	*30*	*60*	*90*
L3C	$9{\cdot}26^{a1}$	$9{\cdot}56^{a1}$	$10{\cdot}83^{b1}$	$4{\cdot}49^{c1}$
L3F	$9{\cdot}26^{a1}$	$8{\cdot}21^{a2}$	$9{\cdot}12^{a2}$	$6{\cdot}40^{b2}$
F3C	$4{\cdot}92^{a2}$	$9{\cdot}89^{b1}$	$7{\cdot}40^{c3}$	$5{\cdot}03^{a1}$
F3F	$4{\cdot}92^{a2}$	$7{\cdot}40^{b3}$	$8{\cdot}03^{c3}$	$8{\cdot}82^{c3}$
L6C	$9{\cdot}61^{a1}$	$10{\cdot}14^{a1}$	$12{\cdot}92^{b4}$	$6{\cdot}28^{c2}$
L6F	$9{\cdot}61^{a1}$	$11{\cdot}44^{b4}$	$10{\cdot}42^{a1}$	$7{\cdot}89^{c4}$

Different superscripts in the same row indicate significant differences ($P < 0{\cdot}05$).
Different superscripts in the same column indicate significant differences ($P < 0{\cdot}05$).

1988). The level of rancidity attained in L3F, L3C, F3F and F3C, was apparent in the gradually higher rancidity ratings awarded by the taste panel as storage advanced. This progressive increase in organoleptic rancidity led to scores that exceeded the acceptability threshold (greater than 4) by the end of the storage period (90 days) in batches L3F, L3C and F3F.

In the smoked batches made from the lean sardine frozen for six months (L6C and L6F), rancid flavour scores (Table 4) in the first sensory analysis were close to 4.0 (the acceptability limit). The limit was exceeded after 30 days in batch L6F and after 60 days in batch L6C. Organoleptic rancidity was manifested later in the chilled batch (L6C) than in the frozen batch (L6F). This was again because the smoked flavour was able to mask somewhat the strong rancid flavour in batch L6C, whereas in batch L6F smoke flavour was already rather low after 30 days of storage (Table 2).

Chandrasekhar *et al.* (1979) stated that POV levels below 20 meq/kg could be considered acceptable in smoked sardine. The peroxide values (POVs) in the smoked fillets used in this study (Table 5) did not reach those levels, although it should be pointed out that in the case of batches L6C and L6F, the raw material used in smoking (lean sardine frozen for 180 days) had already passed the maximum POV levels (Fig. 1).

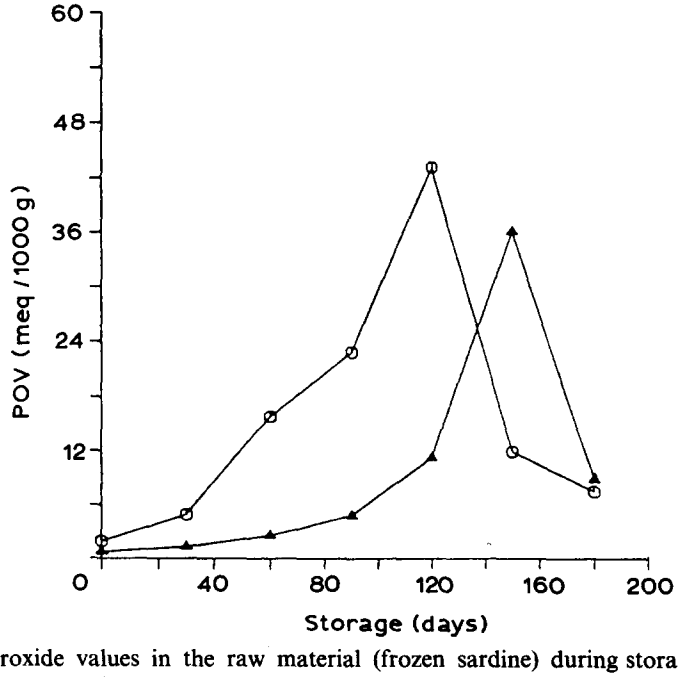

Fig. 1. Peroxide values in the raw material (frozen sardine) during storage at −18°C prior to smoking [○: sardine caught in March (lipid content 5.1%); ▲: sardine caught in June (lipid content 10.9%)]

Consequently, even though the levels recorded in this study were not very high, this is not a basis for stating that these batches displayed acceptable rancidity levels, as the sensory analysis showed. It is therefore important to know the history of the product since, when determining POV levels, it is the primary oxidation products rather than the end products (low molecular weight compounds responsible for rancid odours and flavours) that serve as a measure of incipient rancidity (Cole & Keay, 1976).

The overall acceptability ratings for the smoked batches made from sardine frozen for three months (L3C, L3F, F3C and F3F) (Table 6) were dependent upon the levels of both organoleptic rancidity and softening detected. Thus, the score for batch F3C fell below the acceptability limit after 60 days in storage, chiefly due to the excessively soft texture of this batch. The acceptability limit was reached in batches L3F, L3C and F3F after 90 days, mainly because of rancid flavours, accompanied by weak smoked flavour in batches L3F and F3F and by softening in batch L3C. Basically, therefore, softening was the main limiting factor in the chilled batches and the batches prepared from the fatty sardine, while rancidity was the primary limiting factor in the frozen batches and the batches made from the lean sardine.

In the batches smoked using the sardine frozen for six months (L6C and L6F), rancidity was the main factor limiting overall acceptability (Table 6) and was more pronounced in the frozen batch. Despite the higher POV level in batch L6C compared with batch L6F, in the chilled batch rancid flavours were masked to some extent by the smoked flavour, and as a result the rating for batch L6C was still within the limits of acceptability after 30 days in storage. The batch stored at −18°C (L6F) was evaluated as unacceptable by the taste panel after 30 days.

TABLE 6

Overall Acceptability Scores of Smoked Sardine Fillets over the Storage Period as Assessed by a Taste Panel

Batch	*Days in storage*			
	0	*30*	*60*	*90*
L3C	$4{\cdot}8^{a1}$	$4{\cdot}4^{b1}$	$4{\cdot}0^{c1}$	$2{\cdot}9^{d1}$
L3F	$4{\cdot}8^{a1}$	$4{\cdot}3^{b1}$	$4{\cdot}1^{b1}$	$3{\cdot}1^{c1}$
F3C	$4{\cdot}7^{a1}$	$4{\cdot}2^{b1}$	$3{\cdot}0^{c2}$	$2{\cdot}1^{d2}$
F3F	$4{\cdot}7^{a1}$	$4{\cdot}8^{a2}$	$4{\cdot}0^{b1}$	$3{\cdot}2^{c1}$
L6C	$4{\cdot}2^{a2}$	$4{\cdot}1^{b1}$	$2{\cdot}4^{c3}$	$1{\cdot}5^{d3}$
L6F	$4{\cdot}2^{a2}$	$2{\cdot}0^{b3}$	$1{\cdot}0^{c4}$	$1{\cdot}1^{c4}$

Different superscripts in the same row indicate significant differences ($P < 0{\cdot}05$).
Different superscripts in the same column indicate significant differences ($P < 0{\cdot}05$).

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