Production and Quality Assessment of a Smoked Tuna (Euthynnus affinis) Product

A. Zotos, D. Petridis, I. Siskos, and C. Gougoulias

ABSTRACT: The sensory, instrumental, and chemical profile of a smoked tuna product comparable and competitive to smoked turkey and pork was studied, based on four experimental factors. Despite their different brining times, all brined, sliced portions of tuna were assessed by panelists as quite acceptable products in terms of firmness, juiciness, color, and saltiness. Protein denaturation seemed to be affected by the brining time. Lipid oxidation seemed quite extensive; the ratio of C22:6n-3/C16:0 was decreased at 15% and 20%. Histamine content was between 3.7 mg/100 g and 7.5 mg/100 g. After 3 mo in refrigeration, the aerobic bacteria was 19.10^5 to 250.10^6 in contrast to the unprocessed samples at 28.10^2.

Keywords: tuna, frozen and smoked, new products, sensory, physicochemical

Introduction

Hard curing by salting and smoking permits lengthy preservation of fish and widens its acceptability by conferring traditional flavors that are relished as a condiment by many people, particularly in Greece, home country of this study’s authors. The main cured products of commercial interest are salted-dried sardines, anchovies, hard salted-cold smoked mackerel, and herring.

Greece’s fish-smoking industry is very traditionally based and due to shortages of fish (mainly mackerel and tuna), fish are imported in frozen condition for later smoking (mackerel), or canning in oil (tuna).

Since transport has improved with industrialization, preferences have changed to milder cured products, which do not keep very long and therefore require careful handling.

It is known from previous studies that the frozen storage history of fish does not strongly affect the sensory quality of the smoked products, as judged by 10 expert panelists. However, after 22-w frozen storage of the fish, lipid oxidation was found to be quite extensive (PV=108 meq.Kg^-1) (Zotos 1991; Zotos and others 1995), although the quality of protein is quite similar to that of fresh fish (Opstevdt 1988).

Histamine content was found to be less than 9.4 mg/100 g in smoked mackerel products (Zotos and others 1995), but over 10 mg/100 g in more than 56% of canned samples (Yeannes and Casales 1995). Thus the aim of this investigation was to produce a relatively new milder smoked product from frozen tuna which could be introduced into the market in large portions, as well as to estimate the effect of salt, tripolyphosphate and lactose addition. Also evaluated was the effect of the smoking process, the technological effect of smoking on protein and on lipid quality, the sensory properties of such a product, and the relationship between sensory, instrumental, and chemical methods.

Materials and Methods

Tuna samples in frozen condition (500 to 700 g) were supplied by Diatrophiaki S.A. (Thessaloniki, Greece) to the Fish Technology Laboratory of Technological Educational Institution. The samples were frozen by the Greek company using an air blast freezer, and kept at -20 °C for 2 mo.

Smoking process

Half of the tuna samples were thawed overnight in a 1% sodium tripolyphosphate (STPP) solution, in order to improve their water-holding capacity and texture quality (BK Giulini Chemie and Co. 1998), while the other half were thawed in water. The following day they were brined in 15% solution for 0, 2, 4, 6, and 10 h, (in order to identify the effect of salt on the products and the panelists’ preference), then allowed to dry at 10 ± 2 °C overnight. Half of the samples were coated with lactose to accelerate the browning reaction. Subsequently, all samples were placed in a kiln and smoked, using oak wood. The smoking processes were as follows: a) 30 min at 30 °C; 30 min at 50 °C and 15 min at 75 °C; b) 60 min at 30 °C, 30 min at 50 °C, and 15 min at 75 °C. (The different time at 30 °C was chosen in order to find out the effect on the quality and appearance of the final products). The above temperatures were monitored by a thermocouple placed in the center of the largest sample. The overall smoking process was approximately 5 to 6 h. After smoking, the tuna filets were cooled and individually vacuum-packed in plastic bags and stored at 4 °C for up to 3 days, then were subjected to sensory analysis by a trained panel of 12 assessors.

Moisture content

Moisture content was determined by the CEC (Commission of European Communities) recommended method ISOR 1442 (EEC 1979).

Protein analysis

The method used for salt-soluble protein content was that of Cowie and Mackie (1968). In the procedure adopted, soluble protein, nonprotein, and total nitrogen were determined.

Salt content

Salt content was determined by the volumetric method of the AOAC (Official Method 1995).

Lipid extraction and analysis

The lipid content was determined by the Bligh and Dyer
Production and Quality Assessment of a Smoked Tuna . . .

Table 1—This table shows arrangement of treatments based on the complete combination of the four experimental factors.

<table>
<thead>
<tr>
<th>Polyphosphates</th>
<th>Smoking Process</th>
<th>0 h Brining</th>
<th>Lactose</th>
<th>2 h Brining</th>
<th>Lactose</th>
<th>4 h Brining</th>
<th>Lactose</th>
<th>6 h Brining</th>
<th>Lactose</th>
<th>10 h Brining</th>
<th>Lactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>60 min</td>
<td>A1</td>
<td>yes</td>
<td>A2</td>
<td>yes</td>
<td>A3</td>
<td>yes</td>
<td>A4</td>
<td>yes</td>
<td>A5</td>
<td>yes</td>
</tr>
<tr>
<td>Yes</td>
<td>30 min</td>
<td>B1</td>
<td>yes</td>
<td>B2</td>
<td>yes</td>
<td>B3</td>
<td>yes</td>
<td>B4</td>
<td>yes</td>
<td>B5</td>
<td>yes</td>
</tr>
<tr>
<td>No</td>
<td>60 min</td>
<td>C1</td>
<td>yes</td>
<td>C2</td>
<td>yes</td>
<td>C3</td>
<td>yes</td>
<td>C4</td>
<td>yes</td>
<td>C5</td>
<td>yes</td>
</tr>
<tr>
<td>No</td>
<td>30 min</td>
<td>D1</td>
<td>yes</td>
<td>D2</td>
<td>yes</td>
<td>D3</td>
<td>yes</td>
<td>D4</td>
<td>yes</td>
<td>D5</td>
<td>yes</td>
</tr>
</tbody>
</table>

All A, C, and D treatments were hot-smoked for 60 min at 30 °C, 30 min at 50 °C and 15 min at 75 °C. All B and D treatments were hot-smoked for 30 min at 30 °C, 30 min at 50 °C and 15 min at 75 °C. All A and B treatments were thawed in 1% sodium tripolyphosphate (STPP) solution. All C and D treatments were thawed in water. All A, B, C, and D treatments followed with an odd number were coated with lactose. All A, B, C, and D treatments followed with an even number were not coated with lactose.

(1959) method as modified by Hanson and Olley (1963).

Fatty acid analysis

The fatty acid profile was performed according to a simple and quick method of Humberside Polytechnic (1989), as described in Zotos and others (1995).

Histamine analysis

Histamine was measured by a thin-layer chromatography method (TLC) based on that developed at the Torry Research Station, Aberdeen, U.K. (1984) as described in Zotos and others (1995).

Instrumental analysis

The instrumental analysis was performed using an Instron UTM Analyser, Model 1140 (Instron Ltd., U.K.) with a flat probe of 3.6 cm dia. The 40 different tuna samples were prepared as cylinders, measuring 2.5 cm in height and 3 cm in dia. The firmness was measured by compressing the samples with a weight of 25 kg at a speed of 1 cm/min, and a pressure distance of 10 mm, thus measuring the peak height of the first significant break of the sample. The water-holding capacity (WHC) was measured by placing the samples on a dried and pre-weighed filter paper. A pressure of 25 kg was then applied for 1 min. After accurately re-weighing the filter paper, the loss of water from the samples was calculated.

Sensory analysis

Sensory evaluation was performed using 12 experienced members of the academic staff, instead of the 82 required, for 41 different treatments. Therefore, the 12 panelists were used approximately 7 times, each panelist tasting 5 treatments every time. The panelists were male (n = 7) and female (n = 5), and ranged in age from 40 to 52. Their experience on evaluating smoked fish and meat products were studied based on the following 4 experimental factors: (a) use or not of tripolyphosphates (soaking) (2 levels); (b) use or not of lactose (2 levels); (c) 5 different salt levels (0, 2, 4, 6 and 10 h in brine), and (d) 2 different initial smoking processes (60 and 30 min at 30 °C) (2 levels). Thus a combination of 2 × 2 × 5 × 2 = 40 treatments (samples) was achieved, and the design (Table 1) was increased with a sample of smoked turkey (as a product similar in appearance, obtained from the local market), in order to conform to the particular Plan 13.17a found in Cochran and Cox (1957). This balanced incomplete design included 10 replicates per treatment with only 1 pair of similar treatments allowed: t = 41 treatments, b = 82 panelists, k = 5 treatments per panelist, r = 10 replicates per tuna treatment, and λ = 1 similar pair of treatments for the same panelist. Treatments were presented to the panelists through a particular precedence, carried out by the use of Minitab Statistical Software Package (Ver. 12.0). Adjusted sensory mean scores were deduced for the 41 treatments and at that point, the smoked turkey treatment was excluded from further investigation.

All tuna products, as handled at the various levels of the 4 factors under investigation, with all variables considered, were statistically analyzed using 4 different approaches: (a) A multivariate analysis of variance (MANOVA) for the factors and their interaction terms was attempted on the whole set of sensory and physicochemical variables. An analysis of variance (ANOVA) was then employed, using only the statistically significant factors found and their interaction terms. Statistically significant differences between brining time levels were tested using the SNK (Student-Newman-Keuls) test for comparison of level mean values (Zar 1984). (b) The procedure continued with a discriminant analysis, one for the sensory set and another for the physicochemical set, to find out which variables are most important in discriminating between the levels of factors. (c) The discriminating procedure was further enhanced by using Classification Trees Analysis, as developed by Breimen (1993). This technique splits each variable at specific points over which some levels of the factors under study are discriminated. The classifica-
Results and Discussion

Analysis of variance

As applied on all the variables for the 4 experimental factors considered, MANOVA procedure revealed that the brining time, the smoking process, and the addition of tripolyphosphates were statistically significant, but the addition of lactose did not have any effect (Table 2). MANOVA further revealed that the interaction term of brining time x smoking process was also significant.

Significant main effects of the experimental factors were tested through one-way ANOVA on the sensory and physicochemical variables. The brining factor had significant effect on firmness ($p = 0.005$), juiciness ($p < 0.001$) and saltiness ($p < 0.001$) (Figure 1). According to the SNK test of mean level comparisons, the mean value of firmness (9.42) of the samples brined for 10 h was significantly higher than all other means that were statistically equal. Additionally, the mean value of juiciness (5.2) of the samples which were not brined was significantly lower than all other mean values which were also statistically equal.

SNK test procedure revealed significant mean differences of saltiness between the different times of brining as follows: $0 \text{ h} < 2 \text{ h} = 4 \text{ h} = 6 \text{ h} < 10 \text{ h} (1.5 < 5.5 = 6.5 = 7.5 < 10.3)$. Thus, an increasing trend of saltiness with the increase of brining time occurred (Figure 1).

As expected, the different brining time of the samples significantly influenced the concentration of salt ($p < 0.001$), as well as the concentration of the flesh molecules, such as protein ($p = 0.007$) and lipid ($p = 0.002$), but they did not show any effect on the instrumental analysis (Figure 1).

The salt content of the samples and the time of brining showed an increasing trend that was most accentuated in the tuna samples of 0 h and 10 h (Figure 1). The SNK test revealed exactly the same mean pattern as that of saltiness ($0.00\% < 0.76\% = 0.92\% = 1.04\% < 2.39\%$). Thus, the salt content and sensory saltiness were found to show a strong positive relationship.

The concentration of crude protein and lipid (both on dry weight basis) had the highest mean values in the samples which were not brined (Figure 1). This concentration of protein and lipid of the above samples was significantly different from all the samples with various brining time levels, which did not significantly differentiate each other. The significant difference between samples untreated with brine and all others was probably a result of some possible minor losses of nitrogenous and lipid compounds during brining (into the brine).

The applied smoking process of 60 min at 30 °C showed significantly higher mean values than that of 30 min at 30 °C, both on firmness ($p = 0.001$) and on color ($p < 0.001$), indicating that the longer exposure time at 30 °C may result in either tougher products and/or more brown color (Figure 2). This longer smoking exposure also significantly lowered the mean juiciness of the samples ($p = 0.008$).

The physicochemical analysis showed that the smoking process of 60 min at 30 °C gave significantly higher mean values on instrumental firmness ($p = 0.001$) and significantly lower values on salt concentration ($p = 0.006$) (Figure 2).

The addition of tripolyphosphates increased the salt content of the products ($p = 0.008$) and the instrumentally measured WHC ($p = 0.018$), but decreased the crude protein content of the products ($p = 0.007$), as well as the concentration of the flesh molecules, such as protein ($p = 0.007$) and lipid ($p = 0.002$), but they did not show any effect on the instrumental analysis (Figure 1).

Table 2—Statistically significant results of the general multiple analysis of variance for four factors and interaction terms

<table>
<thead>
<tr>
<th>Factor</th>
<th>Pillai’s statistic</th>
<th>F-value</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brining time</td>
<td>3.043</td>
<td>3.50</td>
<td>32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking process</td>
<td>0.937</td>
<td>11.85</td>
<td>8</td>
<td>0.001</td>
</tr>
<tr>
<td>Tripolyphosphates</td>
<td>0.922</td>
<td>9.41</td>
<td>8</td>
<td>0.002</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.541</td>
<td>0.94</td>
<td>8</td>
<td>0.544*</td>
</tr>
<tr>
<td>Brining × Smoking</td>
<td>2.905</td>
<td>2.92</td>
<td>44</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*non-significant at 0.05 probability level
The brining time and smoking process interacted significantly for saltiness (\(p = 0.001\)), salt content (\(p = 0.020\)), and lipid content (\(p = 0.016\)). The mean changes of saltiness and salt content at 4-h brining time differentiated between the 2 smoking processes (Figure 4), but the most striking effect is focused on the lipid content at the longer smoking exposure, in which inverse mean changes between lipid content and both salt content and saltiness seemed to occur (Figure 4).

### Discriminant analysis

Unlike analysis of variance where the experimental factors are considered as an independent set, in discriminant analysis the variables are the predictors (sensory and physicochemical), and the 4 factors are the dependent set. The discriminant procedure began with backward elimination of the least important variables backed up by the partial \(\lambda\) values, standardized coefficients for canonical variables, and means of canonical variables per canonical root. A selected variable with probability value less than 0.05, combined with low value of the partial \(\lambda\) and high value of the standardized canonical coefficient, indicates significant discriminatory power. Moreover, the larger the distance between the means of the canonical variables per root, the higher the efficiency of discrimination.

Regarding the sensory variables in this investigation, the addition of salt (brining time) was best discriminated by saltiness (\(p < 0.001, \lambda = 0.214\)) and, to a lesser degree, by firmness (\(p = 0.008, \lambda = 0.676\)). The juiciness did not have a significant effect on discrimination, although such an effect was observed at the samples of 0 h in brine when ANOVA was performed. The greatest distance values between the canonical means were observed at salt levels of 0 h (root1:3.862) and 10 h (root1:1.736) in brine, and all the rest of the values were very close. The discriminatory power summed up 70% for all salt levels, with 100% correctness for the levels 0 h and 10 h, and 75%, 25%, and 50% for the levels 2 h, 4 h, and 6 h, respectively.

The smoking process was best discriminated by color with an 85% discrimination effect for each smoking process (\(p = 0.001, \lambda = 0.910\)).

From the physicochemical variables, the brining time was best discriminated by salt content (\(p < 0.001, \lambda = 0.152\)), by instrumental firmness (\(p = 0.014, \lambda = 0.673\)) and by lipid content (\(p = 0.002, \lambda = 0.599\)). The classification power was 75% and a 100% correctness was observed for the levels 0 h and 10 h in brine, while for the levels 2 h, 4 h, and 6 h, it was 62.5%, 50% and 62.5%, respectively. The mean canonical values were very distinct for the 0 h (root1:3.672) and 10 h (root1:4.286) levels. Thus, a similar classification pattern was observed between the sensory variables (saltiness and firmness) and the physicochemical variables (salt content, instrumental firmness, and lipid content) in reference to brining time.

The smoking process of 30 min at 30 °C was uniquely discriminated by instrumental firmness with a total correctness of 80%. The correctness of the first smoking process (30 min at 30 °C) was 90%, while that of the second process (60 min at 30 °C) was 70% (\(p = 0.001, \lambda = 0.754\)).

The addition of tripolyphosphates was uniquely discriminated by the instrumental measurement of WHC with 70% correctness (\(p = 0.012, \lambda = 0.840\)), despite the fact that this was not detected by the panelists.

### Classification trees

CART split selection has proved a superb analysis in discriminating sensory and physicochemical data. The efficiency of this method is mainly based on global validation costs, tree sequence, and misclassification costs (Breiman 1993). Low values of the above costs indicate a valid data classification.

From the sensory variables, saltiness discriminated the levels 1 h in brine at a split value of < 3.23, whereas the levels 2 h, 4 h, and 6 h in brine were clustered at a split value of < 8.86. Values on saltiness higher than 8.86 were indicative of samples brined up to 10 h (Figure 5). Saltiness was the most discriminant variable (ranking score 100), followed by firm-
ness (ranking score 55), juiciness (ranking score 34), and color (ranking score 22).

Color was the most important variable to classify the smoking process (ranking score 100), followed by firmness (ranking score 60). Color discriminated the two smoking processes at a split value of 7.34 (lower values indicated the smoking process for 30 min at 30°C). The addition of tripolyphosphates and lactose was not effectively classified by the sensory variables (data not shown).

From the physicochemical variables, salt content was the most important variable (ranking score 100) to classify the brining time, followed to a lesser extent by crude protein (ranking score 63), and lipid content (ranking score 46). The brining process of 10 h was determined at a split salt content value of >1.60% and the samples with 0-h brining time were identified at a split value of <0.15% (Figure 6). The samples with brining times of 2 h, 4 h, and 6 h varied between 0.15% and 1.60% salt content.

The smoking process was best discriminated by the instrumental firmness (ranking score 100). Instrumental values on firmness, <5.34 cm (peak height), indicated the smoking process of 30 min at 30°C, while higher values indicated the smoking process of 60 min at 30°C.

Salt content was the most important variable for classification of tripolyphosphates (ranking score 100), followed by the instrumental WHC (ranking score 64), and crude protein (ranking score 57). A concentration of salt <0.75% classified the absence of tripolyphosphates while higher values indicated their presence, although not too efficiently.

**Multiple regression**

All physicochemical variables were backward eliminated against each of the sensory variables. Only one strong relation was established, that between saltiness and salt, and lipid content:

\[
\text{Saltiness} = 11.5 + 2.93 \times (\% \text{Salt}) - 0.718 \times (\% \text{Lipid content}), \ R^2 = 0.82, \ n = 40.
\]

It easily follows that saltiness increases with salt content increase and lipid content decrease, thereby suggesting an antagonistic effect between lipid and salt content. This effect was also adequately depicted in Figure 4 and was ascribed to the impact of the longer smoking exposure (60 min at 30°C).

**Correlation matrix**

Sensory and physicochemical variables were poorly to fairly correlated (Table 3), with the exception of saltiness and salt content (r = 0.87), indicating that the saltiness of the smoked tuna samples was the most influential sensory variable for the panelists.

**Protein denaturation**

In contrast to the small protein denaturation change due

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![Figure 4](image-url)  
**Figure 4**—Effect of brining time and smoking process interaction on saltiness, salt content, and lipid content, after data standardization.
to frozen storage (salt-soluble protein of the frozen tuna samples at 95%), the salt-soluble protein of the smoked samples was 38, 28, 25, 22 and 12% for the samples of 0-, 2-, 4-, 6-, and 10-h brining, respectively. These results indicate that the extent of brining may, with smoking, synergistically contribute to the extent of protein denaturation. According to Obstvedt (1988), 90% of the fish protein is denatured at about 60 to 65 °C; however, in those smoking processes, the tuna samples were exposed at 75 °C and the protein denaturation was 60, 70, 74, 77 and 87% for the samples of 0-, 2-, 4-, 6-, and 10-h brining prior to smoking, respectively.

Lipid oxidation

The oxidation of lipids was detected with changes on fatty acid profiles. A significant decrease of polyunsaturated fatty acids (decrease from 55% to 49% due to thawing and smoking process, and from 55% to 43% due to thawing, 10-h brining, and smoking process) was followed by a slight increase of the saturated fatty acids, and a significant increase of the monounsaturated ones (increase from 18% to 23% due to thawing and smoking process, and from 18% to 28% due to thawing, 10-h brining, and smoking process). These results indicate an extensive lipid oxidation during the different experimental processes of the tuna samples. This is also confirmed by the ratio of C22:6n–3/C16:0 which was used as an oxidative indicator. Thus, according to Castrillon and others (1996), a ratio of less than 8 indicated lipid oxidation in sardines; in our investigation, the ratio for the oven was 15% due to thawing and smoking, and 20% due to thawing, 10 hours of brining, and smoking.

Histamine

According to European Union (EU) Regulations, the acceptable histamine levels are < 20 mg/100 g in fresh fish and < 40 mg/100 g in brined fish. It was found by Casales (1995) that 56% of canned mackerel samples contained histamine at a level of more than 10 mg/100 g and in some samples, more than 30 mg/100 g. It was also observed by the author that the later samples produced an unpleasant flavor.

The histamine in these smoked tuna samples varied between 3.7 mg/100 g to 7.55 mg/100 g of tuna flesh, indicating that the histamine in the smoked products remained at levels which would not be expected to cause symptoms of scombrototoxic poisoning.

Aerobic plate count

It was observed by Hansen and others (1998) that the preservation of smoked salmon at 5 °C under vacuum conditions was dependent upon the size of the samples; the larger the products, the longer their preservation time. It was also found by Leroi and others (1998) that on the 6th day of cold-smoked salmon preserved at 8 °C, the total *Mesophilus* bacteria was 3 × 10⁶ cfu/g. Similar results were also observed by Himelbloom and others (1996). They found that the total *Mesophilus* bacteria increased from 1.4 × 10⁴ to 250.10⁶ cfu/g in 3 d at ambient temperature.

The aerobic bacteria in this study were 28.10⁵/g in the unprocessed samples but 19.10⁵/g in the smoked samples after 3-m storage at 5 °C. According to Liston and Matches (1976), total aerobic count in smoked products equal to or above 10⁶/g indicates spoilage.

Conclusions

Strong linear trends between sensory and physicochemical variable sets were not confirmed in this investigation as demonstrated by the correlation matrix. However, a good concordance was evidently observed as a result of the various flexible statistical techniques applied.

Lipid oxidation, as detected with changes in fatty acid profiles, was quite extensive. This extensive oxidation may explain the ineffective role of lactose on color formation, since the oxidation of lipid produced the necessary carbonyls for the carbonyl-amino reactions (browning), and therefore, the formation of the attractive color of the smoked products.

An acceptable product can be produced if it is brined for up to 10 h, treated with polyphosphates to improve its water-holding capacity, making it more adaptable to slicing, and smoked for about 5 to 6 h as follows:

- 60 min at 30 °C, 30 min at 50 °C and 15 to 20 min at 75 °C in the center of the product.

The above was confirmed by a comparison of the adjusted mean sensory values for the above combined level (treat-
ments A9 and A10 in Table 1), which were as follows (95% confidence intervals are shown in parentheses): firmness 9.9 (8.6 to 11.2), juiciness 7.9 (6.6 to 9.2), saltiness 10.1 (8.9 to 11.3), and surface color 7.4 (6.0 to 8.8). The respective values for the smoked turkey used in this investigation, were: 9.2 (7.2 to 11.1), 9.3 (7.3 to 11.2), 6.3 (4.7 to 7.9), and 4.5 (2.7 to 6.3).

In conclusion, a hot-smoked tuna product can be produced and introduced into the market and sold in slices, quite similar to and competitive with smoked turkey.

References


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