Microbiological and nutritional quality of extended shelf life refrigerated pasteurized fish

M.C. García-Linares¹, E. Gonzalez-Fandos*², M.C. García-Fernández¹ and M.T. García-Arias¹

SUMMARY
Extended shelf life refrigerated pasteurized foods are foods that have received a mild heat treatment in hermetically sealed packages, or heated and packaged without recontamination and that require refrigeration. These products have an enhance but limited shelf-life. The balance between extension of shelf life, microbiological safety, fresh-like appearance and high nutritional value must be assesed. The aim of this work was to determine the effect of fat content of extended shelf life refrigerated pasteurized fish on heat survival of some microbial groups (mesophilic, psychrotrophic, Enterobacteriaceae, Micrococaceae and anaerobes) and their control during chilled storage for 3, 20 and 45 days.

An increase of fat content is associated to a significant decrease of moisture content. Higher fat levels in all products resulted in fewer microbial reductions. Bacterial cells suspended in fat are typically more difficult to destroy than in an aqueous medium due to reduction in water activity.

Keywords
refrigerated pasteurized foods, fish, microbiological quality.

1. Food Science and Food Technology Institute – University of León – León.
* Correspondance : elena.gonzalez@daa.unirioja.es

Qualité microbiologique et nutritionnelle du poisson conservé sous vide et réfrigéré

Mots clés
conservation sous vide et réfrigérée, poisson, qualité microbiologique.
1 – INTRODUCTION

Spain, Norway and Portugal are by far the highest consumers of fish in Europe (in Spain 71 g per person per day-VARELA et al., 1991). Over the last few years an increase in consumption has even been observed. This change in food habits has been stimulated by the diffusion of information on the beneficial effects of fish consumption on health. It has a high protein quality and content (similar to meat) and a high content in hydrosoluble and liposoluble vitamins, minerals and polyunsaturated fatty acids of the n-3 family (PUFA n-3) (SANCHEZ-MUNIZ et. al., 1991). In fact, nutrition recommendations in many countries suggest an increased consumption of fish and seafood. However, fish and fish products are vulnerable to various biochemical, physical and microbial forms of deterioration when going through the production chain (catch to retail sale) chain. In order to increase the average amount of fish eaten at home, good quality seafood that is well prepared and conveniently packed should be available (SCHELLEKENS, 1996).

The market of refrigerated processed food of extended durability has increased over the last ten years. In Spain, sales in 1997 of these products were 145.000 tons with an annual increase of 5%. Moreover, there is a higher degree of convenience as these products do not need thawing, and they are easier to prepare; taste and texture are much closer to the fresh product.

Extended shelf life refrigerated pasteurized foods are foods that have received a mild heat treatment in hermetically sealed packages, or heated and packaged without recontamination and that require refrigeration (MARTH, 1998; MOSSEL and STRUIJK, 1991). The three main factors which determine the microbiological safety of extended shelf life refrigerated pasteurized foods are: (i) the intensity of heat treatment (ii) the rapidity of cooling and the temperature reached and (iii) the control of chilled storage (temperature and time).

Over the last ten years, various authors (JUNEJA, 1998; RYBKA et al., 1999) have studied the microbiological quality of extended shelf life refrigerated pasteurized foods. However, there is little information available on the nutritional and microbiological aspects of extended shelf life refrigerated pasteurized fish. (MIYAZAWA et al., 1994; THORSELL and VINSMO, 1991).

The UK Advisory Committee on the Microbiological Safety of Food recommends for cooked-chill products with an extended shelf life of more than 10 days a heat treatment of 90°C for 10 minutes or equivalent lethality and strict chill conditions to control Clostridium botulinum (ACMSF, 1992). An adequate heat treatment must achieve at least a six log reduction cycle in the psychrotrophic strains of Clostridium botulinum type E spores.

There are few data published on the nutritional aspects of extended shelf life refrigerated pasteurized fish and obviously more accurate data on the acceptability of minimally processed foods by consumers are needed (GHIZALA et al., 1996; WATIER, 1988).

The nature of food (fat content, pH, aw, presence of osmoprotectans, essential amino acids and lytic enzymes) is an important determinant of the lethality of a heat treatment and also of the possibility of pathogen growth (SCHELLEKENS, 1996). It would thus be important to study the influence of each factor on the growth and microorganisms inactivation in order to establish additional hurdles when the first barrier (temperature adequate refrigeration) is not fulfilled.

The aim of this work was to study the microbiological and nutritional quality and possible correlation between fat content and microbial growth in extended shelf life refrigerated pasteurized fish.
2 – MATERIAL AND METHODS

Sixteen slices from four different salmons were salted and divided into two batches: raw salmon (RS), processed salmon (PS) which was vacuum packed using a vacuum sealing machine (TECNOTRIP, Barcelona, Spain), pasteurized in a steam oven (SURDRY A-142, Bilbao, Spain) (90°C for 12 min), cooled in a blast cooler (MATACHANA, Barcelona, Spain) and stored at 4°C for 3 (PS3), 20 (PS20) and 45 days (PS45).

The same study was carried out on other fish with a lower fat content: trout. Eight trouts were cut into two filets, salted and divided into two batches: raw trout (RT) and processed trout (PT) which was vacuum packed, pasteurized (90°C for 12 min) and refrigerated at 4°C for 3 (PT3), 20 (PT20) and 45 (PT45) days.

The following determinations were made in each batch: pH, water activity, microbiological quality and proximate composition.

2.1 Physicochemical analyses

For the pH determination, 10 g of fish were blended with 10 ml of distilled water. The pH of the homogenized sample was measured with a Crison Basic 20 pHmeter (Crison Instruments, Barcelona, Spain) (AOAC, 1995). The water activity was determined using an AqualabTM 2000 water activity instrument (Decagon, Inc., USA) (AOAC, 1998). The determinations were performed in duplicate.

2.2 Microbiological analyses

Using a sterile scalpel, twenty-five grams of sample were removed and placed in a stomacher bag containing 225 ml of 0.1% (w/v) sterile peptone water (Unipath Ltd., Basingstoke, UK). Samples were homogenized using a 400 Stomacher model (A.J. Seward, London) for 2 minutes. Then, decimal dilutions were carried out using the same diluent. Aerobic mesophilic microorganisms were counted on Plate Count Agar (PCA, Unipath Ltd.) for duplicate plates incubated 72h at 30°C (ICMSF, 1978). Psychrotrophic microorganisms were counted on Plate Count Agar (PCA, Unipath Ltd.) incubated 7°C for 10 days (GILLILAND et al., 1984). For Enterobacteriaceae enumeration, duplicate 1ml pour plates of Violet Red Bile Glucose Agar (VRBGA, Unipath Ltd.) with overlay were prepared using appropriate dilutions. The plates were incubated at 30°C for 24h and then counted (ICMSF, 1978). The enumeration of Micrococccae and Staphylococcus aureus was carried out using the surface plating procedure on Baird-Parker (Unipath, Ltd.) medium with incubation at 35°C for 48 h (ICMSF, 1978). Anaerobes counts were carried out by deep seeding in PCA medium (Unipath Ltd.) and then incubated in anaerobic jar at 35°C for 48 h (ICMSF, 1978). E. coli and Salmonella determination was carried out in Violet Red Bile Agar (VRBA, Unipath, Ltd.) (ICMSF, 1978) and by the Iso-Grid Hydrophobic Grid membrane Filter (Iso Grid HGMF™; QA Lab, Toronto, Ontario) procedure.

2.3 Proximate composition

For water content determination five homogeneous samples (3 g) were dried at 100°C up to constant weight (method 240.03; AOAC, 1980).

Protein content was calculated in six homogeneous freeze-dried samples from Kjeldahl nitrogen using a 6.25 conversion factor (AOAC, 1984).

For total fat content determination six homogeneous freeze-dried samples (0.5 g) were extracted with petroleum ether (BP 40-60), in an extracting unit (Soxtec System 1040 Tecator, Sweden). Extracted fat was gravimetrically evaluated.

Ash content was determined by heating at 500°C to constant weight using a muffle furnace (method 310.12; AOAC, 1975).
For Fatty Acid Composition fish fat was extracted following the Bligh & Dyer method (1959) and was saponified with 0.5M sodium hydroxide in methanol, and then methylated following the Metcalfe et al method (1966). The fatty acid methyl esters of fish fat were analyzed by gas chromatography. A Hewlett-Packard 6890A with mass detector HP5973A was used.

2.4 Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA). The Newman Keuls test was used to compare means when a significant variation was highlighted by ANOVA. Significance was established at p<0.05 level.

3 – RESULTS AND DISCUSSION

Water and nutrient contents of raw trout (T) and processed trout stored for 3 (PT3), 20 (PT20) and 45 days (PT45) are shown in table 1. They are similar to those reported by other authors (MOREIRAS et al., 1992; SOUCI et al., 1998). After processing, the trout lost 5% of its water (relative value), figure which reached to 7% after a storage of 45 days. These data are in good agreement with those reported by MOREIRAS et al. (1992) on other species of fish and for other processes.

Table 1

<table>
<thead>
<tr>
<th>Moisture</th>
<th>Protein</th>
<th>Fat</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>75.27 ± 0.46</td>
<td>16.04 ± 0.52</td>
<td>7.03 ± 0.48</td>
</tr>
<tr>
<td>PT3</td>
<td>69.85 ± 0.09*</td>
<td>20.15 ± 0.07*</td>
<td>7.45 ± 0.22*</td>
</tr>
<tr>
<td>PT20</td>
<td>69.56 ± 0.38*</td>
<td>21.90 ± 0.07*</td>
<td>8.26 ± 0.18*</td>
</tr>
<tr>
<td>PT45</td>
<td>68.78 ± 0.39*</td>
<td>21.99 ± 0.26*</td>
<td>7.73 ± 0.17*</td>
</tr>
<tr>
<td>S</td>
<td>66.66 ± 0.70*</td>
<td>18.13 ± 0.37*</td>
<td>13.71 ± 0.14*</td>
</tr>
<tr>
<td>PS3</td>
<td>61.44 ± 0.46**</td>
<td>15.49 ± 0.50**</td>
<td>19.66 ± 0.42**</td>
</tr>
<tr>
<td>PS20</td>
<td>63.58 ± 0.60**</td>
<td>20.43 ± 0.22**</td>
<td>15.33 ± 0.15**</td>
</tr>
<tr>
<td>PS45</td>
<td>62.78 ± 0.57**</td>
<td>20.17 ± 0.10**</td>
<td>15.88 ± 0.21**</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations of six homogeneous samples.

T or S: raw trout or salmon; PT3 or PS3, PT20 or PS20, PT45 or PS45: processed trout or salmon after a storage of 3, 20 and 45 days, respectively.

*: Significantly different (p < 0.05) (*) vs the same raw fish species; (●) vs trout submitted to the same process and the same storage time.

This decrease in water content resulted in a significant increase (p < 0.05) in protein content after processing and subsequent storage for 20 and 45 days.

There was an increase in fat levels after processing (PT3) and after 20 days in storage (PT20). Nevertheless, when it was stored for another 25 days, there was a significant decrease in fat content. The ash content increased significantly in processed trout.

The moisture content in processed salmon (PS) was significantly (p < 0.05) lower than in the raw sample (S) (table 1). However, as fat and ash content increased, protein content dropped. The exchange of water and fat in processed salmon was inversely correlated; this phenomenon has been explained by various authors for other seafood (BEAMONTE and CASTRILLO, 1989; MAI et al., 1978). Nevertheless, it should be taken into account that this processing was carried out without adding oil.
The fatty acid compositions of both fish were significantly different. The trout had a higher content in saturated and total polyunsaturated fatty acids, and lower monounsaturated total content (table 2). The total content of saturated and monounsaturated fatty acids did not vary throughout the 45 days of storage in both fish. However, the total PUFA content of trout fell significantly throughout storage. This drop caused a decrease in the P/S ratio.

Table 2
Fat content and saturated, monounsaturated and polyunsaturated fatty acids content of raw salmon and trout (g fatty acid/100 g total fatty acid).

<table>
<thead>
<tr>
<th></th>
<th>Salmon</th>
<th>Trout</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat Content</td>
<td>13.71 ± 0.14</td>
<td>7.03 ± 0.48</td>
</tr>
<tr>
<td>SFA total</td>
<td>19.97</td>
<td>24.04</td>
</tr>
<tr>
<td>MUFA total</td>
<td>37.93</td>
<td>31.77</td>
</tr>
<tr>
<td>PUFA total</td>
<td>31.48</td>
<td>35.62</td>
</tr>
<tr>
<td>P/S ratio</td>
<td>1.58</td>
<td>1.48</td>
</tr>
</tbody>
</table>

SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.

As regards sanitary quality, it must be pointed out that in all samples of both raw fishes (T and S) and samples processed and stored for 3 (PT3, PS3), 20 (PT20, PS20) and 45 (PT45, PS45) days, Salmonella spp. enterotoxigenic Staphylococcus and Escherichia coli were absent.

The evolution of the microbial contamination in both fish can be seen in Figures 1-5. Raw trout showed a high contamination by mesophilic bacteria above 4 log cfu/g and raw salmon has a high contamination by mesophilic and psychrotrophic microorganisms (counts above 5 log cfu/g for both microbial groups).

As regards the behaviour of the surviving flora, in both processed fishes stored for 20 days (PT20 and PS20), it was observed that salmon maintained its stability while trout showed a lower mesophilic, psychrotrophic, Enterobacteriaceae and Micrococaceae counts compared to the same fish stored for three days. However, in the last stage of storage (20-45 days) there was a dramatic change in the behaviour of the flora in both fish. A significant increase in the counts of mesophilic and psychrotrophic was observed in trout samples, while in salmon only a slight increase in the viable mesophilic flora and the Enterobacteriaceae was observed. Anaerobic flora was not detected during refrigerated storage in the first 20 days because it was below 10^1 cfu/g (detection limit of the technique used) and only a small increase was observed in the final stage of storage (20-45 days) in salmon samples (figure 5).

The microbial reduction of mesophilic, psychrotrophic, Enterobacteriaceae, Micrococaceae and anaerobic counts, and the evolution of pH and a_w in processed trout and salmon can be seen in table 3. No variations in pH and a_w were found, which does not support a different behaviour of the microorganism throughout the storage period.

As regards the hygienic quality indices studied in salmon and trout, it can be said that the treatment studied significantly reduced (p < 0.05) microbial contamination, which was to be expected given that this type of process involve an established heat treatment that destroys non-heat resistant contaminating flora.

This increase in mesophilic flora throughout storage in processed fish with a lower fat content coincide with the results obtained by Rosnes et al. (1999) in other studies also carried out on two cooked fish (salmon and cod).

Scientific norms for pasteurization values (temperature/time), cooling rates, storage temperatures and shelf life must be established. One relevant factor to be taken into account and scarcely investigated is the composition of food (Gibbs, 1999; Schellekens, 1996).
Studies on heat resistance carried out on fish with different fat contents and in broth have concluded that a greater fat content in fish (i.e., 20% opposed to 0.5%) acts as a protecting agent in the heat resistance of pathogenic microorganisms such as *Listeria monocytogenes* and *Escherichia coli* (BEM EMBAREK and HUSS, 1993; MACKEY and BRATCHELL, 1989).

However, (MACKEY et al., 1990; BUNCIC et al., 1992) in two other studies carried out on ground beef with the same percentage of fat (20%) showed that no significant protective effect of fat can be demonstrated.

The reason proposed by these authors is that fat was added to ground meat and this may not have the same protective effect as fatty meat tissue. However, it must also be considered that the type of food studied is different (fish and meat).

### Table 3

<table>
<thead>
<tr>
<th>Microbial reductions</th>
<th>pH</th>
<th>Fat</th>
<th>Mesophilic</th>
<th>Psychrotrrophic</th>
<th>Enterobacteriaceae</th>
<th>Micrococaceae</th>
<th>Anaerobes</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>6.56</td>
<td>0.997</td>
<td>7.03</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>S</td>
<td>6.33</td>
<td>0.995</td>
<td>13.71</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Processing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT3</td>
<td>6.56</td>
<td>0.992</td>
<td>7.45</td>
<td>1.7</td>
<td>0.8</td>
<td>1.6</td>
<td>0.4</td>
</tr>
<tr>
<td>PS3</td>
<td>6.47</td>
<td>1.000</td>
<td>19.66</td>
<td>2.9*</td>
<td>3.0*</td>
<td>1.0</td>
<td>0.8*</td>
</tr>
<tr>
<td>Storage 3-20 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT20</td>
<td>6.61</td>
<td>1.000</td>
<td>8.26</td>
<td>0.4</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>PS20</td>
<td>6.43</td>
<td>0.995</td>
<td>15.33</td>
<td>0.1*</td>
<td>0.1*</td>
<td>0.1*</td>
<td>0.0*</td>
</tr>
<tr>
<td>Storage 20-45 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT45</td>
<td>6.51</td>
<td>0.998</td>
<td>7.73</td>
<td>–1.7</td>
<td>–1.2</td>
<td>–0.8</td>
<td>0.0</td>
</tr>
<tr>
<td>PS45</td>
<td>6.52</td>
<td>0.995</td>
<td>15.88</td>
<td>–0.9*</td>
<td>–0.1*</td>
<td>–0.2*</td>
<td>0.1*</td>
</tr>
</tbody>
</table>

T or S: raw trout or salmon; PT3 or PS3, PT20 or PS20, PT45 or PS45: processed trout or salmon after a storage of 3, 20 and 45 days, respectively.

(*) indicate significantly differences (p<0.05) vs trout submitted to the same process and the same storage time.

Figures 1

Evolution of mesophilic counts in processed salmon and trout. Salmon (●), trout (▲).
Figure 2
Evolution of psychrotrophic counts in processed salmon and trout. Salmon (●), trout (▲).

Figure 3
Evolution of Enterobacteriaceae counts in processed salmon and trout. Salmon (●), trout (▲).

Figure 4
Evolution of Microccocaceae counts in processed salmon and trout. Salmon (●), trout (▲).

Figure 5
Evolution of anaerobes counts in processed salmon and trout. Salmon (●), trout (▲).
4 – CONCLUSIONS

The proximate composition of fish, and particularly its total fat content has a significant (p < 0.05) influence on the microbial survival and development of the group indicators of hygienic quality during the refrigeration storage (mesophilic, psychrotrophic and Enterobacteriaceae).

REFERENCES


