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# Deliverable D6.4:

## Prototype of an innovative cold- smoked salmon product with high nutritional value and desired sensorial characteristics


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# Table of Contents

1

## Executive summary

The FutureEUAqua project developed protocols for optimising an innovative cryo-smoking technology to obtain ready-to-eat slightly smoked salmon, with reduced interruption of the cold chain of fillets to produce high quality smoked salmon. The effect of the quality of the raw material, using fish bred with traditional and innovative diets was also assessed. The results were used for shelf-life studies of fish products and enabled the development of a prototype of cryo-smoked salmon at 5 °C, which is characterised by high nutritional value and longer shelf-life compared to the analogous conventional cold smoked salmon (processed at about 20 °C). In the first part of this deliverable, the selection of the appropriate processing parameters, based on the effect on the product stability is described while, in the second part, the developed innovative product is presented with its physicochemical, nutritional, and microbiological properties compared to the conventional one.

# Introduction

Historically, the main positive effects of fish consumption on human health have been attributed to the high content of n-3 long chain polyunsaturated fatty acids (LC PUFA). N-3 LC PUFA have been shown to significantly reduce the risk for sudden death caused by cardiac arrhythmias and all-cause mortality in patients with known coronary heart disease. Fatty fish, such as salmon and tuna, and fish oil are rich sources of the n-3 LC PUFA eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) [1]. Salmon has been well recognized as the major source of commercial fish oil [2], and indeed, it represents an important source of dietary n-3 LC PUFA. It is known since long ago that the fatty acid composition of salmon muscle is dependent on the diet, among other factors [3].

For increasing shelf-life of perishable fish products, smoking is one of the oldest preservation techniques that humans have used. Smoking technologies are increasingly used to impart special organoleptic properties to fish products and to preserve and extend the shelf-life of these products. Twenty-eight % of salmon products on the EU market are smoked. Smoked salmon is a product of great economic importance in the world fish market and, in recent years, has gained increasing popularity among consumers.

The smoking process includes several steps, and, for each, the choice of control parameters such as salting method, concentration of brine, and/or smoking temperature determines specific finished product characteristics. Smoke contains several phenolic compounds that impart flavour to the product and the absorption of these compounds depends on the temperature and smoking time. Traditional cold-smoking is generally performed within a temperature range of 20–30 °C [4].

The quality of smoked products can be influenced by several parameters relating to raw material composition [5], processing conditions [6, 7, 8] and “post processing” parameters (shelf-life and storage temperature of the product). Today, the smoking process is still based on traditional principles and methods. The biggest change has been the replacement of wild salmon by farmed salmon as the raw material in the cold smoked production. However, several problems have arisen related to quality loss, such as increased liquid loss, discolouration, gap formation and too soft texture [9]. Such quality defects lead to the downgrading of the product, resulting in great economic losses for producers. From a nutritional point of view, one of the main challenges of this innovation is to keep constant or even increase, the concentration of n-3 LC PUFA in the final product.

In the FutureEUAqua project, an innovative cryo-smoking technology, patented by the partner CS, has been used to obtain ready-to-eat lightly smoked salmon, with the aim of developing an innovative fish product characterized by increased shelf-life, high nutritional value and desired sensorial characteristics.

In the first part of this deliverable, the optimization of processing parameters for cryo-smoking was carried out, and the selected ones were used for the development of an innovative product prototype, that was characterized and compared to a traditional one in the second part.

# Cryo-smoking process optimization

## Prototype of smoker

The innovative prototype developed by the partner CS (**Figure 1**) used for the cryo-smoking process consists of two parts: the combustion cell and the treatment chamber. In the combustion cell, the pyrolysis of the wood, and thus the production of smoke, takes place. Connected to the combustion cell there is an external tank containing liquid nitrogen ($N_2$), which enables the low-boiling components of the smoke to be selected and conveyed into the treatment chamber by means of a fan and Venturi effect. The high-boiling components are instead removed by venting from the same chamber.

Internally, the treatment chamber consists of a fan for air circulation and grid supports on which the fillets are placed. Outside the treatment chamber is a cavity that allows the circulation of liquid nitrogen, which is used as the cooling fluid for the cryo-smoking process.

Process parameters, such as temperature and air circulation speed, are adjustable from the control panel located on the opening door of the treatment chamber (**Figure 2**). The temperature range within which the machine operates goes from -10°C to +60°C and it is also possible to carry out a drying phase before the smoking phase.

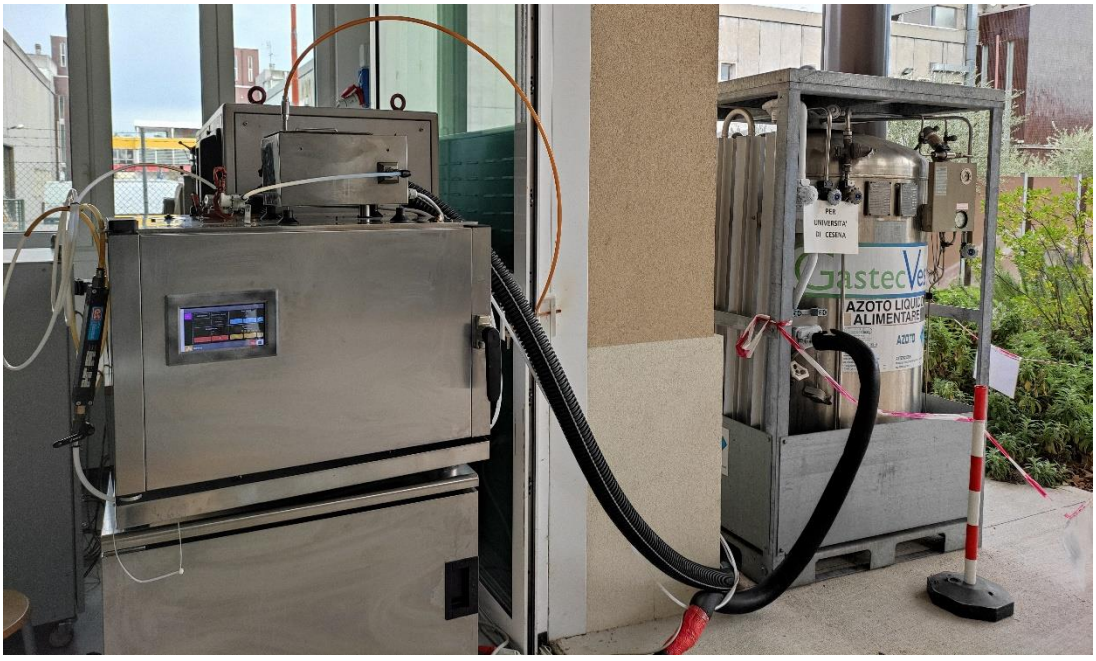


***Figure 1.*** *Prototype of salmon cryo-smoker and liquid nitrogen tank connected to the machine.*

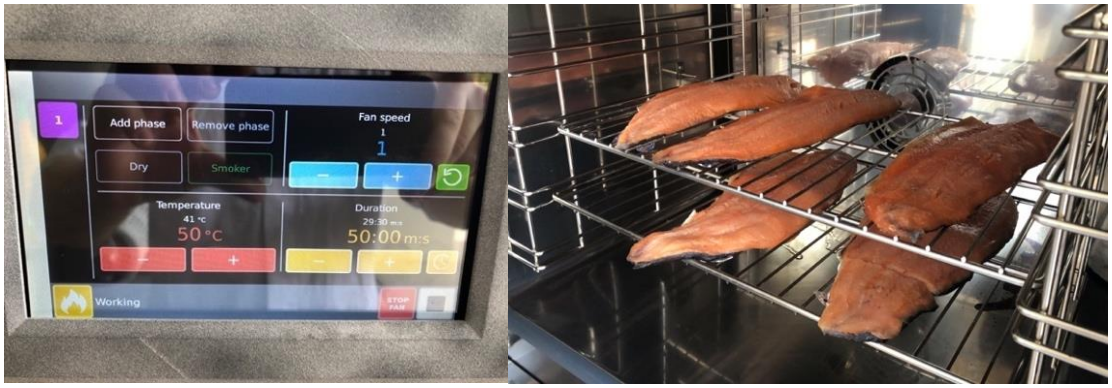


***Figure 2.*** *Cryo-smoker parameters and smoking chamber of the pilot system.*

## Selection of smoking time

The salmons (*Salmo salar*) were obtained from the Salmar farm (Kverva, Norway); after killing and evisceration, they were placed in polystyrene containers, evenly distributed between layers of ice, stored in superchilling conditions and sent by plane to Italy. After 48 hours, they were received in Cesenatico at EMAR (Economia del Mare, Cesenatico, Italy) where they were filleted, frozen at -45°C, vacuum-packed and kept at this temperature until processing (2 weeks). Before the salting and smoking procedures, salmons were thawed overnight at 4 °C.

After thawing, the salmon fillets were hand-cut into 600 g pieces, removing the end and the lower part of the fillet. The fillets were then subjected to a preliminary osmotic dehydration process (salting) and vacuum-packed with a mixture of sodium chloride and sucrose. 2:1 (w:w) for 24h at 4°C (**Figure 3**).

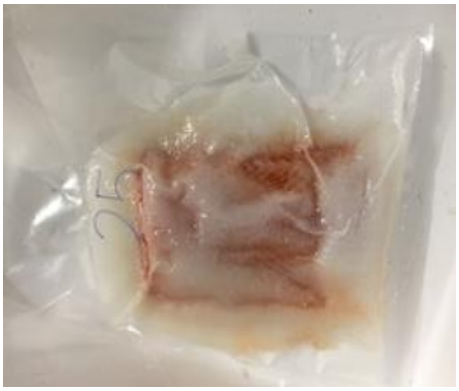

***Figure 3.*** *osmotic dehydration process*

At the end of the osmotic dehydration process, the fillets surface was rapidly washed to remove excess mixture and subjected to the cold smoking process optimised in Task 6.1. The determination of the process parameters was based on the results of the preliminary sensory and physicochemical analyses, testing 2 different temperatures and 3 different smoking times (see **Table 1**).

**Table 1.** Parameters for the optimisation of cryo-smoking

| Temperature | Time |
|---|---|
| 5 °C | 1h |
| 5 °C | 2h |
| **5 °C** | **3h** |
| 20°C | 1h |
| 20°C | 2h |
| **20 °C** | **3h** |

Subsequently, the two smoking temperatures of 5 (cryo-) and 20°C (cold-smoking) and the smoking time of 3 hours were selected for the sample preparation for shelf-life tests. This process was carried out in industrial environment, at the partner TP facilities, where two tween cryo-smokers are present (**Figure 4**).

***Figure 4.*** *Cryo-smoking of salmon fillets using the innovative smoking plant*

At the end of the smoking treatments, all fillets were weighed, vacuum packed, and stored in cold storage at 4 ± 2°C for 3 days, to allow fillet internal equilibration, with a consequent redistribution of smoke compounds and salt within the tissue.

The choice of the optimal treatment was based on the results of the sensorial analysis, that was conducted by a panel of 20 male and female tasters, aged 20-65 years. The test included visual, gustatory, olfactory, texture and overall liking evaluation of the smoked samples. The evaluation of the different smoked samples was conducted by the panel taking using the evaluation form shown in **Table 2**. The attributes were rated based on a hedonic scale of 1 to 9, where 1 corresponded to "extremely unpleasant" and 9 to "extremely pleasant" and 5 was the threshold for acceptability. The panellists were

chosen among usual consumers of fish products and trained in internal training sessions by expert panellists. During sensorial analysis, samples were coded using randomized alphanumerical codes.

**Table 2:** Sensory evaluation scheme used for smoked samples evaluation

| ATTRIBUTE | SCORE* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Color homogeneity | | | | | | | | | |
| Intensity of red color | | | | | | | | | |
| Smoke smell intensity | | | | | | | | | |
| Smoke flavour intensity | | | | | | | | | |
| Rancid taste | | | | | | | | | |
| Salty taste intensity | | | | | | | | | |
| Fatty texture | | | | | | | | | |
| Firmness | | | | | | | | | |
| Overall liking | | | | | | | | | |

* 1= extremely unpleasant; 9= extremely pleasant

The results of the descriptive sensory analysis, reported in **Figure 5**, showed that the overall acceptability scores of salmon smoked for 3 hours were higher at both temperatures compared to the results obtained after smoking for 1 and 2 hours. This result might be correlated to the smoke flavour intensity that was perceived higher in samples smoked for longer times. For the other parameters, samples smoked at 5°C by the innovative method were similar for all smoking times to those smoked at 20°C, a temperature comparable to that used in the food industry for conventional smoking.

Based on these preliminary experiments, the following smoking procedures were carried out for 3 h.

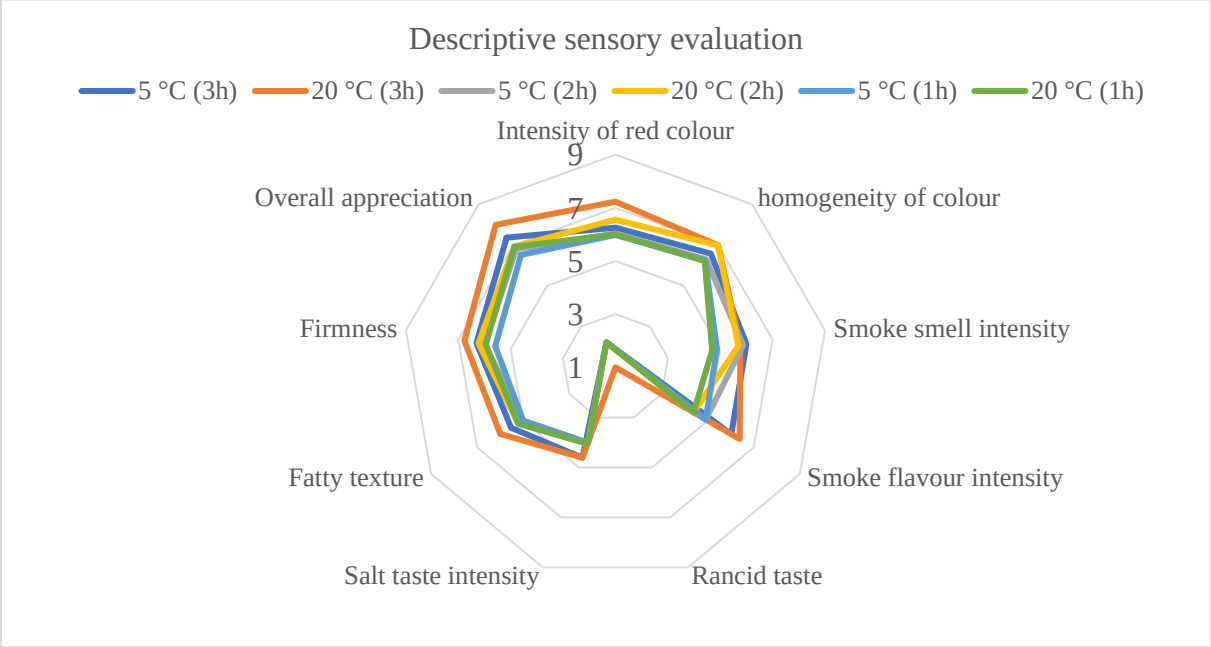


***Figure 5**. Kiviat diagram of the sensory analysis values of cryo-smoking salmon at different temperatures and times.*

## Evaluation of the effect of different raw material

The effect of the cryo-smoking process was also evaluated on two different raw materials, specifically salmons bred by the partner Nofima (Norway) in different cages fed with a traditional diet (samples M202) and an innovative one (samples C204).

Salmons were shipped under ice to the partner EMAR facilities, in which they were filleted, frozen at -45°C, vacuum-packed and kept at this temperature until processing. Before the salting and smoking procedures, salmons were thawed overnight at 4 °C.

Cryo-smoking was carried out for 3 h at 5°C, as described above, on 10 salmon fillets for each diet (N=10). For fatty acid content, three replicates were carried out considering samples pooled from 3 fillets for each.

On fresh and smoked samples, the following determinations were carried out:

- Weight loss (according to the SOP – Annex 7 to D6.1);
- Salt content titration (according to the SOP – Annex 9 to D6.1);
- Water content;
- Water activity (according to the SOP – Annex 8 to D6.1);
- Colour (according to the SOP – Annex 3 to D6.1);
- TBARS value (according to the SOP – Annex 5 to D6.1);
- Protein solubility (according to the SOP – Annex 10 to D6.1);

- Fatty acid composition and content (as methyl esters) (according to the SOP – Annex 11 to D6.1).

**Table 3.** Physicochemical parameters measured in salmons fed using traditional and innovative diets before and after cryo-smoking at 5°C for 3 h

| Parameter | Traditional (M202) | | Innovative (C204) | |
|---|---|---|---|---|
| | Fresh | Cryo-smoked | Fresh | Cryo-smoked |
| Weight loss (%) | - | 14.91±1.24 [b] | - | 17.13±0.89 [a] |
| Salt content (%) | ** | 2.51 0.88 [a] | ** | 2.41 0.43 [a] |
| Water content (%) | 69.92±0.12 [a] | 62.48±0.15 [c] | 67.69±0.02 [b] | 61.19±0.05 [c] |
| Water activity | 0.986 0.002 [a] | 0.955 0.001 [b] | 0.985 0.002 [a] | 0.960 0.006 [b] |
| L* | 45.82±0.93 [a] | 38.08±1.45 [b] | 36.81±1.58 [b] | 38.61±1.41 [b] |
| a* | 11.34±0.71 [a] | 7.15±0.42 [b] | 8.87±1.63 [b] | 8.85±1.02 [b] |
| b* | 18.05±0.80 [a] | 11.49±0.59 [c] | 11.16±1.65 [c] | 14.87±1.39 [b] |
| TBARS (mg MDA/kg) | 1.74±0.37 [c] | 2.79 0.42 [b] | 1.19±0.16 [c] | 3.98±0.39 [a] |
| Protein solubility (mg/ml) | 32.9±1.8 [b] | 29.3±7.7 [c] | 34.4±3.2 [b] | 39.9±3.2 [a] |

** Below the limit of detection

*Data are mean ± SD of 10 biological replicates in each condition. Statistical analysis was by the one-way ANOVA using Tukeys' as post-test and assuming p<0.05 as significant. Different letters indicate statistical significance.*

Comparing the two fresh raw materials, differences were observed in water content (lower in the innovative sample) and colour parameters. These differences can be attributed to the different feeding used within the project, similarly to what observed previously by other authors in organic and conventionally fed salmons [10].

However, no differences were observed between the diets in the fresh fillets for water activity. After smoking, aw was reduced to about 0.960 for both samples, in agreement with previous literature results [8].

While salt content was similar after the smoking procedure, the innovative sample underwent a higher weight loss. Ability in liquid holding capacity depends on protein functionality, but also on fatty acid composition [11]. Colour changes were observed mainly for the traditional salmon fillets, while in the innovative one only an increase of the yellow index was observed.

Lipid oxidation was increased in both samples, but more markedly in the innovative one. In both cases, values remained under the limit considered the threshold for good quality [12].

Protein solubility, which is considered an important index for protein functionality, showed a different behaviour in the 2 samples; in the traditional one it decreased after smoking, while in the innovative one it increased. This difference has to be attributed to the different protein status related to the feeding program.

The fatty acid (as methyl esters) qualitative and quantitative composition of fresh and cryo-smoked salmon are reported in **Table 4** (salmon fed the standard diet) and **5** (salmon fed the innovative diet).

**Table 4**. Fatty acid composition (as FAME) of the fresh and cryo-smoked salmon fed the standard diet.

| | Fresh | Cryo-smoking |
|---|---|---|
| 14:0 | 184.73±3.48[a] | 137.18±8.31[b] |
| 16:0 | 884.27±22.99[a] | 710.55±33.33[b] |
| 16:1 n-7 | 155.84±19.44[a] | 132.56±5.88[b] |
| 18:0 | 203.19±11.42[a] | 159.31±9.24[b] |
| 18:1 n-9 | 3549.12±114.05[a] | 2768.32±174.92[b] |
| 18:2 n-6 | 1200.90±35.74[a] | 942.16±60.63[b] |
| 18:3 n-3 | 470.90±16.62[a] | 368.04±23.48[b] |
| 20:1 | 371.75±31.26[a] | 277.95±24.20[b] |
| 20:4 | 64.57±3.53[a] | 50.57±3.62[b] |
| 20:5 n-3 | 180.18±8.91[a] | 142.68±3.44[b] |
| 22:5 n-3 | 63.20±4.95[a] | 45.72±3.98[b] |
| 22:6 n-3 | 541.57±51.01[a] | 401.24±5.47[b] |
| ΣSFA | 1272.19±36.43[a] | 1007.04±50.71[b] |
| ΣMUFA | 4076.71±143.80[a] | 3178.83±201.41[b] |
| ΣPUFA | 2521.32±112.48[a] | 1950.39±93.71[b] |
| ΣPUFA n-3 | 1255.86±74.27[a] | 957.67±29.73[b] |
| ΣPUFA n-6 | 1265.46±38.44[a] | 992.73±64.10[b] |
| Σ n-6/Σ n-3 | 1.01±0.03[a] | 1.04±0.04[a] |
| Total | 7870.22±280.39[a] | 6136.27±345.40[b] |

*Data are expressed as mg FAME/100 g sample, and are mean ± SD of 3 biological replicates in each condition. Different letters indicate statistical significance by the Students' t test, assuming p<0.05 as significant*

In salmon fed using standard diet, the smoking procedure significantly affected the fatty acid content and composition. The total content of fatty acid (as FAME) was lower in cryo-smoked salmon compared to the fresh product. This decrease involved almost all fatty acids, although to different extent. Overall, the cryo-smoked salmon contained a lower concentration of both saturated (SFA) and polyunsaturated fatty acids (PUFA) than the fresh one. Among PUFAs, both n-6 and n-3 significantly decreased, so the n-6/n-3 ratio did not change between products.

**Table 5**. Fatty acid composition (as FAME) of the fresh and cryo-smoked salmon fed the innovative diet

| | Fresh | Cryo-smoking |
|---|---|---|
| 14:0 | 138.33±42.78[a] | 140.48±18.29[a] |
| 16:0 | 892.99±230.80[a] | 909.96±71.98[a] |
| 16:1 n-7 | 105.74±32.82[a] | 106.15±19.83[a] |
| 18:0 | 177.19±43.18[a] | 186.17±11.16[a] |
| 18:1 n-9 | 3222.72±1021.39[a] | 3316.42±435.72[a] |
| 18:2 n-6 | 1169.07±357.46[a] | 1188.66±171.50[a] |
| 18:3 n-3 | 448.98±138.12[a] | 449.69±66.59[a] |
| 20:1 | 268.36±91.28[a] | 274.32±44.20[a] |
| 20:4 | 62.56±19.42[a] | 56.05±9.05[a] |
| 20:5 n-3 | 128.81±28.88[a] | 120.84±17.05[a] |
| 22:5 n-3 | 38.70±9.10[a] | 37.46±4.22[a] |
| 22:6 n-3 | 521.11±82.74[a] | 464.19±15.64[a] |
| ΣSFA | 1208.51±316.74[a] | 1236.61±101.42[a] |
| ΣMUFA | 3596.82±1143.76[a] | 3696.89±497.79[a] |
| ΣPUFA | 2369.23±631.42[a] | 2316.89±279.67[a] |

| | | |
|---|---|---|
| ΣPUFA n-3 | 1137.61±255.50 [a] | 1072.18±103.35 [a] |
| ΣPUFA n-6 | 1231.62±376.69 [a] | 1244.71±176.32 [a] |
| Σ n-6/Σ n-3 | 1.07±0.10 [a] | 1.16±0.06 [a] |
| Total | 7174.56±2091.06 [a] | 7250.39±877.65 [a] |

*Data are expressed as mg FAME/100 g sample and are mean ± SD of 3 biological replicates in each condition. Different letters indicate statistical significance by the Students' t test, assuming p<0.05 as significant.*

Conversely, in salmon fed the innovative diet the processing had no significant impact on the fatty acid composition.

These results show that the diet used during salmon breeding influences the response of the raw material to processing. Further studies are needed to better relate the diet-induced characteristics of the raw material to the modification observed after the smoking procedure.

## Product packaging and storage

After the smoking process, salmon fillets were positioned on rigid paper support inserted in multilayer high barrier pouches, and packed under vacuum, as normally commercialized from the partner CS. Each fillet was packed individually as shown in **Figure 6.**

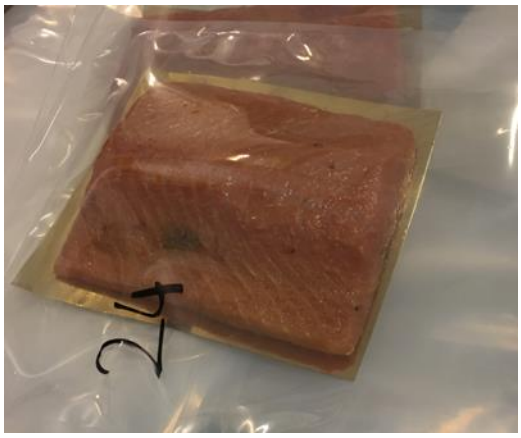

***Figure 6.*** *Images of smoked salmon fillets individually packed under vacuum*

## Shelf-life study

The shelf-life of the developed innovative products, compared to the traditional untreated one, was measured by storing the packed samples at 3 °C, analysing the main microbiological and quality features during storage.

For shelf-life, a total of 48 fillets were subjected to the smoking procedure, 24 to the conventional smoking at 25 °C and 24 to the innovative cryo-smoking one at 5 °C.

During storage, at least four packages for each sample were removed from storage and used for the analytical determinations related to:

- Microbiological analysis (SOP – Annex 2 to D6.1) for mesophiles, *Enterobacteriaceae* and psychrophiles (after 1, 2, 5, 11, 14, 16 and 21 days);
- Colour (according to the SOP – Annex 3 to D6.1) (after 1, 5, 8, 14, and 21 days), for each fillet at least two measurements were carried out;
- TBARS value (according to the SOP – Annex 5 to D6.1) (after 1, 5, 11, 14, and 21 days).

**Table 6.** Mesophiles, *Enterobacteriaceae* and Psychrophiles loads measured in the seabream fillets during refrigerated storage

| Storage day | Traditional Smoking | Cryo-smoking | ANOVA (*p* value) |
|---|---|---|---|
| Mesophiles | | | |
| 1 | 1.68±1.11 $^{aC}$ | 1.80±1.03 $^{aC}$ | 0.108308 |
| 2 | 2.73±1.88 $^{aC}$ | 2.79±2.04 $^{aB}$ | 0.241731 |
| 5 | 3.05±2.71 $^{aC}$ | 2.58±2.05 $^{bBC}$ | 0.031584 |
| 11 | 5.71±4.13 $^{aB}$ | 3.56±3.17 $^{bB}$ | 0.000296 |
| 14 | 6.25±5.52 $^{aA}$ | 5.84±4.45 $^{bA}$ | 0.002431 |
| 16 | 6.20±5.21 $^{aA}$ | 6.06±5.12 $^{aA}$ | 0.002331 |
| 21 | 6.09±5.02 $^{aAB}$ | 5.78±4.46 $^{bA}$ | 0.027909 |
| *p* value | <0.000001 | 0.000001 | |
| *Enterobacteriaceae* | | | |
| 1 | 1.67±0.98 $^{aB}$ | 1.86±0.98 $^{aB}$ | 0.060176 |
| 2 | 2.44±2.02 $^{aB}$ | 2.05±1.53 $^{bB}$ | 0.024187 |
| 5 | 2.72±1.91 $^{aB}$ | 2.29±1.40 $^{bB}$ | 0.000250 |
| 11 | 2.73±1.63 $^{aB}$ | 2.16±1.48 $^{bB}$ | 0.000006 |
| 14 | 4.20±3.63 $^{aA}$ | 3.08±2.15 $^{bB}$ | 0.000474 |
| 16 | 4.27±3.29 $^{aA}$ | 3.85±2.45 $^{bAB}$ | 0.000774 |
| 21 | 4.31±3.24 $^{aA}$ | 4.39±3.49 $^{aA}$ | 0.050143 |
| *p* value | <0.000001 | <0.000001 | |

| Psychrophiles | | | |
|---|---|---|---|
| 1 | 1.85±0.33 $^{aC}$ | 1.89±1.04 $^{aB}$ | 0.244379 |
| 2 | 2.77±1.46 $^{aC}$ | 2.49±1.78 $^{bB}$ | 0.000145 |
| 5 | 2.70±1.77 $^{aC}$ | 2.47±1.27 $^{bB}$ | 0.000496 |
| 11 | 5.54±4.45 $^{aB}$ | 3.61±2.13 $^{bB}$ | 0.000072 |
| 14 | 6.65±5.21 $^{aA}$ | 5.87±4.74 $^{bA}$ | 0.020089 |
| 16 | 6.32±5.12 $^{aAB}$ | 5.46±4.21 $^{bA}$ | 0.034704 |
| 21 | 6.37±5.48 $^{aAB}$ | 5.66±4.34 $^{bA}$ | 0.008875 |
| *p* value | 0.000010 | 0.001795 | |

*Data are expressed as log CFU/g sample and are mean ± SD of 2 biological replicates and 2 technical replicates (n =4) in each condition. Statistical analysis was done by the one-way ANOVA using Tukeys' as post-hoc test and assuming p<0.05 as significant. Different lowercase letters indicate statistical significance within a row of values; Different uppercase letters indicate statistical significance within a column of a microbial group.*

Table 6 reports the microbial loads (log CFU/g) of the mesophiles, *Enterobacteriaceae* and psychrophiles bacteria in salmon fillets during storage. At the beginning of the storage mesophiles was around 1.7 log CFU/g, but without differences between the samples.

The end of the shelf-life was considered as the time necessary to reach the microbiological load of 6 log CFU/g (Colony Forming Unit/g) of mesophiles and of 4 Log10 CFU/g of *Enterobacteriaceae,* according to standard references [13, 14, 15].

In the sample subjected to cryo-smoking, this value was reached for mesophiles between the 16$^{th}$ and 21$^{th}$ day while for *Enterobacteriaceae* between the 11$^{th}$ and 14$^{th}$ day of storage. On the other hand, when subjected to traditional cold smoking at 20 °C, the limit for mesophile was reached on 16$^{th}$ day while for *Enterobacteriaceae*, it was reached between 5$^{th}$ and 11$^{th}$ day.

Therefore, the use of innovative cryo-smoking at 5°C increased the shelf-life of smoked salmon fillets by about 20% compared to those smoked at 20°C. This result might be due to the difference in the temperature during the process. Even if for a short time (3h), conventional cold smoked samples were exposed to a higher temperature (20°C) compared to the cryo-smoked ones (5°C). This might have resulted in a faster microbial growth.

**Table 7.** Colorimetric parameters measured in the smoked salmon fillets during refrigerated storage

| Storage day | Traditional smoking | Cryo-smoking | Traditional smoking | Cryo-smoking | Traditional smoking | Cryo-smoking |
|---|---|---|---|---|---|---|
| | L* | | a* | | b* | |
| 1 | 37.37±0.36$^{a}$ | 32.13±0.36$^{b}$ | 10.93±0.65$^{a}$ | 8.79±0.65$^{b}$ | 9.61±0.17$^{a}$ | 9.61±0.17$^{a}$ |
| 5 | 34.00±0.96$^{a}$ | 31.81±1.50$^{b}$ | 8.61±0.94$^{a}$ | 8.63±1.04$^{a}$ | 12.06±1.15$^{a}$ | 8.50±0.63$^{b}$ |
| 11 | 37.54±0.99$^{a}$ | 38.52±0.83$^{a}$ | 8.86±0.36$^{a}$ | 9.82±0.54$^{a}$ | 12.23±0.98$^{a}$ | 9.66±0.55 |
| 15 | 37.73±2.53$^{a}$ | 40.05±2.58$^{a}$ | 9.45±0.77$^{a}$ | 9.94±0.52$^{a}$ | 12.07±1.62$^{a}$ | 11.73±1.93$^{a}$ |
| 17 | 37.73±0.90$^{b}$ | 40.05±0.62$^{a}$ | 10.40±0.54$^{a}$ | 11.66±0.95$^{a}$ | 14.98±0.54$^{a}$ | 14.16±0.44$^{a}$ |
| 19 | 34.59±0.75$^{a}$ | 35.79±0.92$^{a}$ | 10.23±0.76$^{a}$ | 12.56±1.15$^{a}$ | 12.07±0.77$^{a}$ | 13.62±0.77$^{a}$ |

*Data are mean ± SD of 8 replicates in each condition. Different letters between parameters at the same sampling time indicate statistical significance by the Students' t test, assuming p<0.05 as significant.*

Table 7 reports the colorimetric parameters of L*, a* and b* measured in the smoked almon fillets during storage. After the smoking process, cryo-smoked salmon resulted characterized by lower L* and a* values. However, during storage these differences were lost as, after 11 days, values were almost completely the same for the three parameters considered, for both the smoked samples.

These results indicate that the different temperature used during smoking process generally does not affect in a significant way the visual quality of the salmon.

Table 8 reports the TBARS values measured in smoked salmon fillets during storage. The use of the lower processing temperature (cryo-smoking) resulted in a lower value of the considered oxidation parameter, until the 15$^{th}$ day of storage.

**Table 8.** TBARS value measured in the smoked salmon fillets during refrigerated storage

| Storage day | Traditional smoking | Cryo-smoking |
|---|---|---|
| 1 | 4.09±1.10$^{a}$ | 1.98±0.68$^{b}$ |
| 5 | 4.54±0.87$^{a}$ | 2.01±0.35$^{b}$ |
| 11 | 5.12±0.65$^{a}$ | 2.34±0.23$^{b}$ |
| 15 | 5.29±1.48$^{a}$ | 2.74±1.48$^{b}$ |
| 17 | 5.43±1.05$^{a}$ | 6.46±0.72$^{a}$ |
| 19 | 3.83±0.33$^{b}$ | 5.01±0.85$^{a}$ |

*Data are expressed as* log mg MDA/kg *sample and are mean ± SD of 4 biological replicates in each condition. Different letters between parameters at the same sampling time indicate statistical significance by the Students' t test, assuming p<0.05 as significant.*

## Conclusion

The smoking process was optimized according to the sensorial test and the time of 3 hours was chosen based on the higher acceptability score.

Different raw materials were tested, and results highlighted that feeding is of paramount importance in determining the effect of processing. Indeed, in salmon fed the standard diet cryo-smoking significantly reduced the concentration of all fatty acids, including EPA and DHA, compared to the fresh salmon. Conversely, cryo-smoking of salmon fed the innovative diet did not modify the fatty acid composition of the final product, which kept its nutritional value similar to the fresh one.

Our results evidence the interactions between feeding system and processing in the production of processed fishes, further underlining that the preparation of a final product with high nutritional value starts from a valuable raw material, but it can be strongly modified as a consequence of processing procedures.

Although from a sensorial and visual quality point of view no differences were observed between the samples smoked at the 2 different temperatures, a 20% increase of the microbiological shelf-life and a lower lipid oxidation index highlighted how the use of the cryo-smoking can increase the value of the smoked salmon compared to traditional cold smoked one.

Therefore, the product obtained by the application of cryo-smoking at 5°C was considered as the prototype of the innovative smoked salmon product, and it has been subjected to a deeper characterization in comparison with the traditional product subjected to smoking at 20 °C.

## Prototype characterization

The product was characterized in terms of physicochemical characteristics (water content, pH, colour and textural parameters), oxidation status and sensorial acceptability, and for nutritional composition (nutritional fact label) compared to the traditional untreated product at the beginning of the storage (T0) and at the end of the microbiological shelf-life (Tf) considered as 14 days for the traditional cold smoked products and 18 for the innovative cryo-smoked one.

### Physicochemical properties

pH, colour and salt content were determined according to the SOPs attached in D6.1.

Water content was determined gravimetrically after drying in an oven at 105°C until constant weight.

Texture was measured with a Texture Analyser mod. TA.HDi 500 (Stable Micro Systems, Godalming, UK) equipped with the Warner-Bratzler (WB) device with a load cell of 5 kg and a descent speed of 3 mm/s. The WB device is a V-shaped blade with 60° angles with a thickness of about 2 mm. Results were expressed as Shear Force (N), considered as the maximum peak obtained from the diagram of force versus time.

**Table 9.** Physicochemical characteristics of the innovative cryo-smoking salmon product compared to the traditional one

| | Conventional smoking | Cryo-smoking | Conventional smoking | Cryo-smoking |
|---|---|---|---|---|
| | T0 | | Tf | |
| Water content (%) | 59.99 ± 2.86$^{a}$ | 58.77 ± 1.31$^{a}$ | 61.68 ± 2.19$^{a}$ | 60.3 ± 2.55$^{a}$ |
| pH | 5.79 ± 0.04$^{a}$ | 5.92 ± 0.06$^{b}$ | 5.70 ± 0.09$^{a}$ | 5.58 ± 0.06$^{a}$ |
| L* | 37.37 ± 0.36$^{b}$ | 32.13 ±0.35$^{a}$ | 37.73 ± 2.53$^{a}$ | 40.05 ± 0.62$^{a}$ |
| a* | 10.93 ± 0.65$^{a}$ | 8.79 ± 0.97$^{a}$ | 9.45 ± 0.77$^{a}$ | 11.6 ± 0.95$^{a}$ |
| b* | 12.06 ± 1.23$^{a}$ | 8.50 ±0.45$^{b}$ | 12.07 ± 1.62$^{a}$ | 14.16 ± 0.44$^{a}$ |
| Shear force (N) | 14.37 ± 1.23$^{a}$ | 13.39 ± 1.30$^{a}$ | 20.76 ± 1.91$^{a}$ | 19.1 ± 0.64$^{a}$ |

*Data are mean ± SD of 4 biological replicates in each condition. Different letters indicate significant difference ($p<0.05$) between the products for each parameter at the same sampling time, by the Students' t test, assuming $p<0.05$ as significant.*

As reported in **Table 9**, significant differences were observed for the parameters of pH, L* and b* values. Specifically, the salmon subjected to cryo-smoking (5°C) showed a slightly higher pH and lower values of luminosity and yellow index. However, these differences were not perceived by a sensorial test, conducted on untrained panellists with a triangular test (results not reported).

Nevertheless, at the end of the shelf-life, no significant differences were observed for the two products.

## Oxidation status and sensorial acceptability

As the same time, in order to evaluate the quality of the product, a lipid oxidation index was measured (TBARS value, according to the SOP – Annex 5 to D6.1) and a sensorial analysis was conducted.

Sensory evaluation was carried out according to the method used for processing optimization and described previously in this deliverable. The sensory score reported in Table 3 corresponds to the overall acceptability parameter.

**Table 10**. TBARS values and sensory score determined in cryo-smoking salmon samples (traditional one – 5°C and innovative one – 20 °C) after the smoking process (T0) and at the end of the shelf-life (Tf).

| | Conventional smoking | Cryo-smoking |
|---|---|---|
| | T0 | |
| TBARS (mg MDA/kg) | 4.09 ± 1.09[a] | 1.98± 0.68[b] |
| Sensory score | 8.5 | 8.4 |
| | Tf | |
| TBARS (mg MDA/kg) | 5.28 ± 1.48[a] | **6.30** ± 0.58[b] |
| Sensory score | 7.2 | 7.5 |

*Data are mean ± SD of 4 biological replicates in each condition. Different letters indicate significant difference ($p<0.05$) between the products for each parameter at the same sampling time, by the Students' t test, assuming $p<0.05$ as significant.*

As shown in **Table 10**, compared to the traditional sample (20 °C), in the innovative one (5 °C), an increase of lipid oxidation was observed during storage leading to different values at the end of the shelf-life.

The limit of acceptability correlated to the TBARS value is not clear and while some authors state that the perception of rancid occurs when this value is over 4 mg MDA/kg [12], other reports that the acceptability limit is 8 mg MDA/kg. This probably depends in the specific type of fish considered.

Despite the high fat content of salmon, the lipid oxidation index always remained below 6.5mg MDA/Kg, probably due to the characteristics of the prototype used (use of nitrogen as carrier gas, thus absence of oxygen).

No differences were detected between control and treated samples with regard to the sensorial characteristics. At the end of the microbiological shelf-life, both samples were found to be of good quality (A) by the panel, as shown in Table 4.

This indicate that notwithstanding an induction of lipid oxidation was observed, it remained modest and under the limit of detection of potential consumers.

## Nutritional characteristics

*Nutrition facts label*

Analyses were performed using accredited methods:

- Total lipids: UNI ISO 1443:1991
- Total carbohydrates: MIC 039 Rev.00 2013
- Total sugars: MIC 041 Rev.00 2013
- Proteins: UNI ISO 937:1991
- Salt: ISTISAN 96/34 page 124
- Humidity: ISTISAN 96/34 page 124
- Ashes: UNI 10590:1997

Energy was calculated according to EU Regulation N. 1169/2011.

The "nutrition facts" label of conventional and innovative smoked salmon is reported in **Table 11.**

**Table 11.** Nutrition facts of traditionally smoked and cryo-smoked salmon.

| | Conventional smoking | Cryo-smoking |
|---|---|---|
| **Energy** (kcal/100g) | 214±4[b] | 236±4[a] |
| **Total lipids** (g/100g) | 12.87±0.45[b] | 15.47±0.51[a] |
| **Total carbohydrates** (g/100g) | 0.49±0.25[a] | 0.69±0.28[a] |
| **Sugars** (g/100g) | 0.43±0.21[a] | 0.61±0.26[a] |
| **Proteins** (g/100g) | 23.90±0.67[a] | 23.40±0.42[a] |
| **Salt** (g/100g) | 3.45±0.22[a] | 3.38±0.17[a] |
| **Humidity** (g/100g) | 57.13±0.70[a] | 54.80±0.20[b] |
| **Ash** (g/100g) | 5.61±0.24[a] | 5.64±0.25[a] |

*Data are mean ± SD of 3 biological replicates in each condition. Different letters indicate significant difference ($p<0.05$) between the products for each parameter at the same sampling time, by the Students' t test, assuming $p<0.05$ as significant.*

The energy content of cryo-smoked salmon was higher than conventionally smoked salmon. This was related to a higher content of total lipids and to the lower residual humidity after cryo-smoking.

These results differ from the ones showed in Table 5 for total lipids, and in Table 8 for water content, attributable to the different analytical protocols and to a natural variability of the different salmon fillets.

## Final remarks

The innovative process of cryo-smoking showed great promise in producing a high-quality smoked salmon product with high nutritional properties.

From a technological point of view, cryo-smoked products physicochemical and sensory quality are similar to products already on the market. The longer shelf-life observed in cryo-smoked salmon was probably because the cold chain is only slightly interrupted (as was not in the case of salmon smoked at 20°C). A 20% longer shelf-life is an important achievement for a highly perishable product, and it can increase market potential, facilitate distribution and reduce waste.

Finally, our results evidenced that the quality of the raw material is very important and influences the response to processing. Therefore, the selection of the breeding conditions, mainly the diet used for feeding fish, should be carefully considered to maximise the quality and nutritional value of the final product.

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