AdriAquaNet

Enhancing Innovation and Sustainability in Adriatic Aquaculture

Deliverable WP5

D 5.2.3 Innovative products (hamburgers, fillets) Control sheet/Control Document

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CONTENT OF THE DELIVERABLE

This document has been prepared by the working group that is involved in the WP and coordinated by the WP leader or task leader.

It is divided in two parts:

- **PART 1**

A short presentation our deliverable. It is meant as a control document and a demonstrative review of the deliverables listed in Application Form in WP5 that consists of the producing an object, in this case innovative products and new types of packaging.

- **PART 2**

The second part provides the final results and a collection of data from the WP and project in relation to the General objectives at the Programme level that we will need to add to the final report.

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PART 1

1) **Introduction (objective and purpose of the deliverable)**

To increase fish consumption, two strategies are necessary:

a) **Education on nutrition and continuous information in media**, events for all population and particular in schools about the nutrition value of fish products and the transformation of processed fish into derived products. The school education is very important because the preferences formed in childhood normally continue into adulthood, and for this reason, at each level of school, meal programs must include fish because they can contribute to the formation of healthy food habits.

b) Good, safe and healthy fish products and marketing promotion.
The second strategy is more important than the first. It is well known that people sometimes give up eating fish due to the presence of fish bones and the characteristic fish odor so the research of new, easy consumable products must be done constantly. Secondly, life rhythm of the modern society has pushed the consumer to reduce cooking time, so having at disposal quick and easy to cook products are the new marketing challenges.

Therefore, **fish derivatives such as fillets and burgers can be a real strategy to increase fish consumption**. Indeed, burger production allows the elimination of fish bones and a decrease in the characteristic odor of fish, which are the main barriers to fish consumption [5], while maintaining the same high nutritional value of the whole fish. Different works have demonstrated a positive relationship between appearance and positive hedonic perception by consumers with respect to fish derivatives.

The aims of the activity work were:

- **to produce cold smoked sea bass fillets and burger made with Sea Bass and Sea Bream meat;**
- **to increase safety of cold smoked sea bass (to monitor the presence of *L. monocytogenes* in different cold-smoked fish and to use bioprotective cultures to eliminate or reduce the growth of *L. monocytogenes* intentionally inoculated into cold-smoked sea bass);**
- **to develop fish burgers made with a mix of sea bass and sea bream meat and to improve their shelf life by bioprotective cultures.**

2) **Methodology for smoked sea bass**

The three lots of sea bass used were raised in sea cages by **Orada adriatic d.o.o.(PP10) in Cres, Croatia.** They were collected, eviscerated, placed in polystyrene boxes containing ice and sent to a processing plant **of Friulitrota (PP11)** in the Friuli region within 5 hours. This company has extensive experience in the cold smoking of fish products such as both farmed and wild salmon and, especially, trout. The fish were filleted (baffe) and salted to a WPS value > or equal to 3.5%, and then they were desalted and smoked at low temperature (< 30 °C). After smoking, the fillets were vacuum-packed in plastic bags (PE/PA Niederwieser group, Italia), stored at 4 °C and transported **to the Department of Agricultural Food, Environmental and Animal Sciences of the University of Udine (LP**). Each sample weighed approximately 200 g.
The samples of each lot were divided into 10 groups of 15 samples each, as follows, and analysed in triplicate at 0, 15, 30, 45 and 60 days (until the typical deadline of the shelf life of cold-smoked fish):

1) Control samples stored as-is (not inoculated);
2) Samples with *L. monocytogenes* mix added;

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3) Samples with Sacco LAK-23 (*Lactilactobacillus sakei*) starter and a mix of *L. monocytogenes* added;
4) Samples with *Carnobacterium* spp. and mix of *L. monocytogenes* added;
5) Samples with *Lacticaseibacillus casei* (SAL 106) and a mix of *L. monocytogenes* added;
6) Samples with *Lacticaseibacillus paracasei* (SAL 211) and a mix of *L. monocytogenes* added;
7) Samples with LAK-23 (*Lactilactobacillus sakei*) added;
8) Samples with *Carnobacterium* spp. added;
9) Samples with *Lacticaseibacillus casei* (SAL 106) added; and
10) Samples with *Lacticaseibacillus paracasei* (SAL 211) added.

Inoculated samples

For each test and lot, 15 smoked sea bass samples were inoculated and analysed in triplicate at each time point: 0, 15, 30, 45 and 60 days. Fifteen samples were stored as originally packaged and represented the controls, and the others were unpackaged and inoculated with *L. monocytogenes* alone, with all starters alone and with the starters and *L. monocytogenes* mix and then repackaged according to the technique and packaging used by the facility. All control (uninoculated) and inoculated samples were stored at 6 ± 2 °C, which is the standard temperature of a supermarket refrigerator.

Physico-chemical analysis

The control samples (1) and samples inoculated with the starters (7, 8, 9, and 10) alone were also subjected to chemical-physical analyses. In particular, the pH was at 3 different points using a pH metre (Basic 20, Crison Instruments, Spain) by inserting the probe directly into the product. The water activity (Aw) was measured with an Aqua Lab 4 TE (Decagon Devices, USA). Humidity was measured according to the A.O.A.C. (1990), and NaCl and TVB-N (total volatile basic nitrogen) were measured according to Pearson (1973). WPS (water-phase salt) was determined according to the formula according to Huss et al. (1997):

$$\text{WPS (g/100 ml)} = \frac{\text{salt content (in g per 100g)}}{\text{moisture content (in ml per 100g)} \times \text{salt content (in g per 100 g)}} \times 100$$

Thiobarbituric acid-reactive substances (TBARS) were determined according to Ke et al. (1984). The pH values were detected at determined at 0,15, 30, 45 and 60 days, while the other physico-chemical parameter at the beginning (0 day) and at the end (60 day) of the shelf life. At each time point, the analyses were performed on three samples.

Statistical analysis

Data were analysed using Statistica 7.0 vers. 8 software (Statsoft Inc, 2008). The values of the different parameters were compared by one-way analysis of variance and the means were then compared using Tukey's honest significance test. Differences were considered significant at $P < 0.05$.

Sensory analysis

Sensory analysis was performed by 20 nonprofessional trained tasters. Ten additional samples of treatments 1 and 7 were evaluated by tasters who were asked to evaluate the influence of the LAB starter on the organoleptic and sensory characteristics of the products. Sensory analysis was performed based on the triangle test (UNI EN ISO 4120:2004, triangle test). In short, 20 nonprofessional trained tasters were

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presented with 3 products, two of which were identical. The choice of nonprofessional tasters was mandatory because they represent typical consumers. The tasters were asked whether they understood the examined differences, and in the case of differences they were asked to specify the type, for example, colour, texture, bouquet, flavor, or smell. Among the samples with starter added, only 7 were tested, considering that the LAK-23 was the only strain inhibiting the growth of *L. monocytogenes*.

3) Methodology for burger

Sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata)* weighing approximately 474–578 g and 404–440 g, respectively, were headed, gutted, and filleted. Fillets were then minced, mixed, and divided into five equal batches. Each burger was composed of 68% minced fish (50% sea bass and 50% sea bream), 20.5% potato, 5.5% water, 3% rice flour, 2% vegetable fiber, and 1% NaCl. The first batch was formed into patties and directly packaged and used as a control (CTRL). The other batches were inoculated with starter cultures at a final concentration of 10^5 CFU/g of burgers before being formed into patties. Four batches were prepared with each commercial and F-106 starter culture. The burgers were packed in MAP, consisting of 60% N_2 and 40% CO_2, and placed inside rectangular trays made of PET/PE/EVOH/PE: PET (Polyethylenterephtalat/PE (Polyethylene)/EVOH (Ethylene vinyl alcohol)/PE (Polyethylene), ANTIFOG—EVOH (Ethylene vinyl alcohol). The trays were laminated with a top film consisting of APET/PE/EVOH/PE: Amorphous Polyethylenterephtalat/PE (Polyethylene)/EVOH (Ethylene vinyl alcohol)/PE (Polyethylene).

The packaged burgers were stored at 4 ± 2 °C for 10 days and then at 8 ± 2 °C for 20 days, according to the challenge test proposed by AFNOR NF V01-003, 2004: hygiene and safety of foodstuffs. Guidelines for the design of an ageing test protocol for the validation of a microbiological lifetime, which reports for chilled perishable goods that in case where the cold chain is not sufficiently guaranteed, two temperatures must be used: 1/3 of the shelf life at T1 (4 °C) and 2/3 at T2 (8 °C) (abuse temperatures).
Burgers were analyzed at days 0, 6, 12, 18, 24, and 30 in triplicate to test microbial growth, physico-chemical characteristics, and sensory analyses.

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Physico-Chemical and Statistical Analyses: see above

Analysis of Volatile Compounds (Volatilome)

Volatile organic compounds of samples were analyzed with gas chromatography-mass spectrometry coupled with solid-phase microextraction (SPME-GC–MS) by Montanari et al. [35]. Volatile peak identification was carried out by computer matching of mass spectral data with those of compounds contained in the libraries NIST 2005 and 2011. Data reported are means of three different burgers.

Sensory Analyses

The sensory evaluation panel consisted of 12 nonprofessional assessors. The cooked burgers were presented on white plates at room temperature. Ten burgers of the control and of each treatment were evaluated. Assessors were asked to evaluate the following descriptors: odor (fermentation, rancid, or fishy), taste (sweet, sour, pungent, or rancid), flavor (ammonia, sweet, sour, or bitter) and appearance (slime). The 12 assessors evaluated the presence or the absence of each of the nine descriptors. The results stated for each sample is the sum of the assessors who considered the presence of the descriptor out of the total of the assessors [36,37]. Then, the final score is calculated by asking the panelists to give a general evaluation of the sensory quality of the products, within a scale from 1 (excellent) to 5 (worst).

4a) Results of cold smoked sea bass fillets

Lacticaseibacillus casei 211*, *Lacticaseibacillus paracasei 106*, *Carnobacterium maltoaromaticum* and *Lactilactobacillus sakei* (LAK-23) were the bioprotective cultures used in this work against *L. monocytogenes. Their use was based on the qualified presumption of safety (QPS), which indicates the safety status of microorganisms intentionally used in food and the feed chain, certifying that they do not pose a risk to human or animal health based on the scientific literature (EFSA BIOHAZ Panel 2020).

All starter cultures and *L. monocytogenes* grew during storage when they were separately inoculated and reached 7-8 log CFU/g (data not shown). Consequently, cold-smoked fish represent a good substrate for microorganism growth. Indeed, in the control samples, CBT grew constantly during the entire period, as well as indigenous LAB. These LAB do not present a hazard to consumers, but sometimes, heterofermentative LAB can influence the quality of final products. Other analyses were also performed to confirm that anaerobes and coliforms were not present in the samples. Finally, as expected, the presence of *L. monocytogenes* was below the detection limit (Absence in 25 g).

In addition, the presence of autochthonous LAB did not influence the growth of *Listeria.*In samples in which only the pathogen was inoculated, indigenous LAB increased but did not influence the growth of *L. monocytogenes*, which reached high concentrations, as illustrated in Figure 3. Cold-smoked sea bass is indeed a suitable substrate upon which *Listeria* can grow despite the temperature of storage, presence of smoking compounds, and high salt concentration (3.5%).

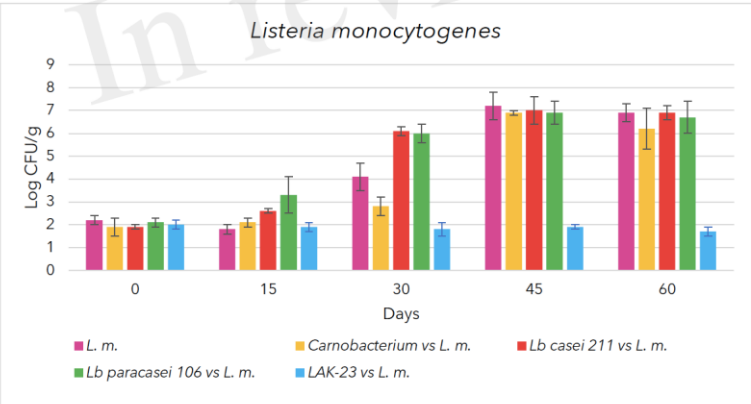

Figure 3: Evolution of *Listeria monocytogenes* intentionally inoculated in cold smoked sea bass with or without bioprotective starter added, stored at 6 ± 2 °C.

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The trends from trials of *Lacticaseibacillus casei* 211, *Lacticaseibacillus paracasei* 106 and *Carnobacterium* spp. coinoculated with *L. monocytogenes* are shown in Figures 3. Unfortunately, the coinoculated *L. monocytogenes* also grew, reaching hazardous level (8 log CFU/g). Consequently data demonstrated that of *Lacticaseibacillus casei* 211, *Lacticaseibacillus paracasei* 106 and *Carnobacterium maltoaromaticum* were not able to limit the growth of the pathogen, which could have become a serious risk to health (Figure 3).

Conversely the starter LAK-23 developed and markedly inhibited inoculated *Listeria*. In fact, the concentration of the *L. monocytogenes* mix remained almost constant over time, although slight decreases were noted at 45 and 60 days, which did not appear significant considering the large standard deviation ($p > 0.05$). Therefore, the starter used was effective, and although it did not reduce or completely eliminate the inoculated strains of *L. monocytogenes*, it still prevented their growth.
Finally, the growth of the added starters in samples inoculated with *L. monocytogenes* was confirmed by the identification of the colonies isolated from MRS and TSM agar (data not shown).

Sensory analysis of cold-smoked sea bass fillets
The samples were brought to environmental temperature immediately before the administration and checked for the presence of atypical odours and flavours, white or viscous patinas, slime, discoloration or browning. Neither the samples nor the controls were positive for the previously described parameters and consequently underwent the sensory evaluation.
The sensory acceptability of samples with or without starter was determined by the triangular test. No sensory differences were perceived between samples inoculated with LAK-23 and control samples (uninoculated). In fact, neither group of samples was recognized as different. From the comparison of the samples under analysis, it emerged that they belonged to a single sample. Therefore, the starter did not profoundly modify the sensory characteristics of the product to which it was added.

Conclusions
The results obtained confirm that *L. monocytogenes* can be isolated from cold-smoked fish products. These products are characterized by a physico-chemical composition capable of supporting its growth. They have pH values greater than 5.8 units, Aw values greater than or equal to 0.97 and humidity values equal to 59-60%. Usually, initial contamination, which is derived from the raw materials, humans and the processing environment, is limited to a few cells per g of product. Processing technology based on salting, cold smoking, vacuum packaging and refrigeration is not able to eliminate the hazard represented by *L. monocytogenes*. The results obtained show that the Aw values of cold-smoked sea bass were never lower than 0.97 and that the pH did not drop below 5.0 units. Consequently, the product can support the growth of *L. monocytogenes*. To remedy this, the use of bioprotective starters was suggested. The LAB contained in the starter grew throughout the storage period and did not block the growth of the pathogen. Only LAK-23 was able to stop the increase in the *L. monocytogenes* load, which remained at the inoculum level until the end of storage. Thus, its use as bioprotective agent is suggested. Therefore, although cold-smoked sea bass does not have a pH ≤ 4.4 and Aw ≤ 0.92 or pH ≤ 5.0 and Aw ≤ 0.94, as stated in EC Regulation 2073/2005, the results scientifically affirm that these products with added bioprotective starters are not a favourable medium for the growth of *L. monocytogenes*. Consequently, they could easily fall into category 1.3 (ready-to-eat foods that do not constitute a favourable medium for the growth of *L. monocytogenes*, other than those intended

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for infants and special medical purposes), in which a maximum concentration of *L. monocytogenes* of 100 CFU/g is allowed.

4b) Results of fish burgers

Microbial and Physico-Chemical Characteristics

To evaluate the capability of the different starter cultures to compete with the autochthonous microbial flora of the burgers, viable counts were performed during storage. The results of viable counts of total bacterial count (CBT), Enterobacteriaceae, and lactic acid bacteria (LAB) are reported in Table 1. In all samples, CBT increased until 12 days of storage, reaching values between 5.32 and 6.47 Log CFU/g, and then decreased; at the end, it was between 3.01 and 3.58 Log CFU/g.

At the beginning of storage, the LAB counts of the inoculated samples ranged between 5.09 and 5.68 Log CFU/g, corresponding to the added amount of starter, and the control samples had 3.42 Log CFU/g. Lactic acid bacteria counts increased rapidly during the first 12 days and reached more than 7 Log CFU/g in all the samples. Thereafter, the growth continued, and at the end of the storage, the counts in the various samples reached maxima, which ranged from 8.62 to 9.18 Log CFU/g. In all the samples, the Enterobacteriaceae population increased progressively until Day 12, after which it decreased. No significant differences in counts ($p > 0.05$) of Enterobacteriaceae between the control and inoculated samples were recorded from Day 0 to Day 18 of storage. Therefore, burgers inoculated with LAK-23 presented low population amounts compared to the other samples. Listeria monocytogenes and Salmonella spp. were not detected in any of the samples. All pH profiles demonstrated clear acidification as a function of time, both in the control and when a starter culture was added. The initial pH of the burgers ranged from 6.17 to 6.31. On Day 12 of storage, the pH values decreased in all the samples, but the acidification in the inoculated samples was higher (Table 1). In fact, the pH of the control samples was 5.56, which was significantly higher ($p < 0.05$) than that of the inoculated samples, which had pH values between 4.67 and 5.09. In contrast, at Day 18, the control, together with FP-50, had lower pH values. Subsequently, the pH values of all the samples remained practically unchanged until the end of storage (4.31–4.45). The water activity (a_w) of the burgers at Day 0 was 0.9836. Total volatile basic nitrogen (TVB-N) is known as a product of bacterial spoilage and endogenous enzyme action, and its level is often used as an index to evaluate fish quality. The levels of these compounds, which increase with the onset of microbial spoilage, are primarily responsible for the fishy odors, which increase as spoilage proceeds (Table 1). At the onset of storage, the TVB-N value of burgers was 25.60 mg N/100 g. TVB-N increased with storage time. Significant differences ($p < 0.05$) between the samples were observed from Day 18. The increase in TVB-N values was lower in the samples inoculated with FP-50 starter culture. At the end of storage (30 days), the control samples had a higher value of TVB-N. The TVB-N content of all the samples exceeded the maximum level for acceptability for marine fish (i.e., 35 mg/100 g [38]) at Day 12. The pouches were examined daily for swelling during storage. A large portion of the uninoculated burgers (40%) started swelling at 12 days. In contrast, the inoculated burgers did not become swollen during the overall storage period (Figure 1).

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Figure 1. Pictures of the uninoculated (**A**) and inoculated (**B**) fish burgers at 12 days of storage.

Table 1. Fate of physico-chemical and microbial characteristics of hamburgers made with a mix of sea bass and sea bream meat.

		Days					
		0	6	12	18	24	30
	Starter	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Total bacterial count (Log CFU/g)	CTRL	4.76 ± 0.12 ^{a}	4.89 ± 0.47 ^{a}	6.47 ± 0.63 ^{b}	3.58 ± 0.17 ^{a}	3.68 ± 0.67 ^{a}	3.26 ± 0.19 ^{a}
	LAK-23	4.71 ± 0.08 ^{a}	5.00 ± 0.59 ^{a}	6.03 ± 0.37 ^{ab}	4.06 ± 0.77 ^{a}	3.20 ± 0.36 ^{a}	3.49 ± 0.20 ^{a}
	F-106	4.89 ± 0.09 ^{a}	4.73 ± 0.06 ^{a}	6.33 ± 0.13 ^{b}	4.33 ± 0.49 ^{a}	3.94 ± 0.44 ^{a}	3.58 ± 0.07 ^{a}
	FP-50	4.85 ± 0.09 ^{a}	5.19 ± 0.44 ^{a}	5.32 ± 0.20 ^{a}	4.00 ± 0.67 ^{a}	3.01 ± 0.05 ^{a}	3.27 ± 0.43 ^{a}
	BOX-57	5.22 ± 0.20 ^{b}	5.80 ± 0.18 ^{a}	5.70 ± 0.17 ^{ab}	3.78 ± 0.30 ^{a}	3.00 ± 0.17 ^{a}	3.01 ± 0.15 ^{a}
Lactic acid bacteria (Log CFU/g)	CTRL	3.42 ± 0.14 ^{a}	4.44 ± 1.34 ^{a}	7.55 ± 0.16 ^{a}	8.73 ± 1.15 ^{a}	7.83 ± 0.22 ^{a}	9.18 ± 0.11 ^{b}
	LAK-23	5.13 ± 0.16 ^{b}	6.94 ± 0.90 ^{b}	8.79 ± 0.07 ^{b}	8.58 ± 0.36 ^{a}	9.15 ± 0.37 ^{b}	9.07 ± 0.04 ^{b}
	F-106	5.09 ± 0.09 ^{b}	5.90 ± 0.89 ^{ab}	8.17 ± 0.15 ^{ab}	8.98 ± 0.21 ^{a}	8.38 ± 0.23 ^{ab}	8.62 ± 0.08 ^{a}
	FP-50	5.68 ± 0.15 ^{c}	5.64 ± 0.27 ^{ab}	7.98 ± 0.66 ^{ab}	9.48 ± 0.79 ^{a}	8.76 ± 0.67 ^{ab}	9.11 ± 0.16 ^{b}
	BOX-57	5.39 ± 0.08 ^{bc}	6.35 ± 0.86 ^{ab}	8.36 ± 0.22 ^{ab}	9.01 ± 0.17 ^{a}	8.77 ± 0.26 ^{ab}	8.99 ± 0.16 ^{b}
Enterobacteriaceae (Log CFU/g)	CTRL	2.74 ± 0.14 ^{a}	4.67 ± 0.27 ^{a}	4.58 ± 1.02 ^{a}	4.01 ± 0.27 ^{a}	2.39 ± 0.41 ^{bc}	1.44 ± 0.36 ^{b}
	LAK-23	2.83 ± 0.18 ^{a}	4.40 ± 0.71 ^{a}	5.45 ± 0.32 ^{a}	3.55 ± 0.47 ^{a}	0.52 ± 0.15 ^{a}	0.48 ± 0.01 ^{a}
	F-106	2.58 ± 0.12 ^{a}	3.55 ± 1.55 ^{a}	5.26 ± 1.13 ^{a}	3.62 ± 0.32 ^{a}	2.34 ± 0.38 ^{bc}	3.00 ± 0.30 ^{c}
	FP-50	2.63 ± 0.29 ^{a}	4.31 ± 0.22 ^{a}	3.97 ± 0.11 ^{a}	4.08 ± 0.59 ^{a}	1.48 ± 0.00 ^{b}	2.40 ± 0.09 ^{c}
	BOX-57	2.75 ± 0.06 ^{a}	4.48 ± 0.27 ^{a}	4.27 ± 0.77 ^{a}	3.66 ± 0.37 ^{a}	2.61 ± 0.62 ^{c}	1.20 ± 0.22 ^{b}
pH	CTRL	6.23 ± 0.03 ^{ab}	6.25 ± 0.06 ^{a}	5.56 ± 0.34 ^{b}	4.36 ± 0.03 ^{a}	4.30 ± 0.04 ^{a}	4.31 ± 0.00 ^{a}
	LAK-23	6.31 ± 0.03 ^{b}	6.30 ± 0.11 ^{a}	4.89 ± 0.01 ^{a}	4.48 ± 0.02 ^{b}	4.32 ± 0.08 ^{a}	4.34 ± 0.07 ^{ab}
	F-106	6.27 ± 0.03 ^{ab}	6.31 ± 0.06 ^{a}	5.09 ± 0.14 ^{a}	4.52 ± 0.09 ^{b}	4.39 ± 0.05 ^{a}	4.38 ± 0.02 ^{ab}
	FP-50	6.29 ± 0.05 ^{b}	5.99 ± 0.15 ^{a}	4.67 ± 0.01 ^{a}	4.36 ± 0.01 ^{a}	4.23 ± 0.06 ^{a}	4.37 ± 0.06 ^{ab}
	BOX-57	6.17 ± 0.04 ^{a}	6.00 ± 0.19 ^{a}	4.83 ± 0.05 ^{a}	4.57 ± 0.02 ^{b}	4.40 ± 0.07 ^{a}	4.45 ± 0.05 ^{b}
TVB-N (mg N/100 g)	CTRL	25.60 ± 3.33 ^{a}	32.80 ± 2.75 ^{a}	40.03 ± 1.46 ^{a}	47.27 ± 0.80 ^{b}	60.93 ± 1.60 ^{bc}	88.63 ± 0.96 ^{d}
	LAK-23	25.60 ± 3.33 ^{a}	31.33 ± 2.91 ^{a}	38.87 ± 1.70 ^{a}	45.23 ± 2.58 ^{ab}	55.13 ± 1.10 ^{a}	74.37 ± 0.81 ^{b}

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	F-106	25.60 ± 3.33 [a]	33.00 ± 2.95 [a]	40.40 ± 1.13 [a]	47.80 ± 0.60 [b]	63.50 ± 2.69 [c]	69.07 ± 1.25 [a]
	FP-50	25.60 ± 3.33 [a]	31.40 ± 0.96 [a]	39.73 ± 1.39 [a]	42.50 ± 1.32 [a]	54.63 ± 2.01 [a]	70.50 ± 1.11 [a]
	BOX-57	25.60 ± 3.33 [a]	32.73 ± 3.49 [a]	39.43 ± 0.61 [a]	45.63 ± 0.68 [ab]	56.37 ± 1.59 [ab]	80.53 ± 0.70 [c]

***Legend**: CRT, control non inoculated; Starter added (LAK-23), Lactobacillus sakei bacteriocin producer; F-106, Lacticaseibacillus casei; FP-50, Carnobacterium divergens, C. maltoaromaticum; Box 57 Carnobacterium divergens, C. maltoaromaticum, L. sakei bacteriocin producer. Data mean ± standard deviation; mean with different letters within each day and each character (following the columns) are significantly different ($p < 0.05$).*

The TBARS content changed slightly during storage. However, either at time 0 or at 30 days of storage, no significant differences were observed in any of the samples regardless of the treatment (with or without starters added). At 0 days, the TBARS means were at the level of 1.2 ± 0.3 nmol malondialdehyde/g in all the burgers, and then they slightly increased, reaching acceptable levels at 30 days (2.1± 0.5 nmol malondialdehyde/g).

Volatile Compound Characteristics (VOCS)

The compounds are grouped according to their chemical structure, into aldehydes, ketones, alcohols, acids, and esters. The results are expressed as the ratio between the peak area of each molecule and the peak area of the standard (4-methyl-2-pentanol).

Alcohols, and in particular 1-propanol, isopropyl alcohol, and 1-penten-3-ol, were detected in higher amounts in the control at time 0. The second group was represented by aldehydes, among which hexanal, nonanal, and pentanal were the major contributors. All these aliphatic aldehydes derive mainly from lipid oxidation; they are responsible for “green notes”, but at higher concentrations, they can result in rancid perception.

After 6 days, aldehydes demonstrated a small decrease in the control and in the fish burgers containing the culture F-106, while an increase was observed with BOX-57, FP-50, and LAK-23 samples. Alcohols were characterized by relevant increases, especially in the samples containing bioprotective cultures. These latter were also responsible for high concentrations of ketones, such as diacetyl and acetoin, especially in BOX-57, FP-50, and LAK-23. The same three bioprotective cultures were characterized by a higher accumulation of acetic acid, while the culture F-106 seems to confirm its slower metabolism under the conditions considered here.

Sensory Characteristics

Table 3 provides the results of a sensory evaluation performed at Day 12 of storage. Inoculated burgers were also evaluated on Days 18, 24, and 30 (supplementary material). Instead, control samples were evaluated only on Day 12, as the packages subsequently swelled. Sensory analysis results presented slight changes with the progress of storage. However, on Day 30, at the opening of packages, there was a strong ammonia odor, except for the burgers inoculated with LAK-23, which had the best sensory analysis scores during the overall storage period. The inoculation of LAB starter cultures improved the sensory attributes of fish burgers. Indeed, the bioprotective cultures reduced the spoiler activities, such as TVB-N production; consequently, the sensory attributes of sea bass and sea bream burgers were acceptable for up to 12 days of storage (10 days at 4 °C and 20 days at 8 °C). Finally, inoculated burgers did not present odors, flavors, or the sticky white slime indicative of deterioration. In contrast, a sticky, white slime was observed in some uninoculated burgers.

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Table 3. The sensory panel scores of cooked fish burgers.

Sensory Attributes	Day 12				
	CTRL	BOX-57	F-106	FP-50	LAK-23
Fermentation	8/12	6/12	4/12	5/12	3/12
Rancid	7/12	4/12	3/12	3/12	2/12
Sweet	2/12	1/12	1/12	1/12	1/12
Pungent	7/12	2/12	3/12	3/12	2/12
Fish	12/12	12/12	12/12	12/12	12/12
Sour	4/12	5/12	5/12	4/12	3/12
Bitter	6/12	4/12	2/12	2/12	2/12
Ammonia	4/12	3/12	1/12	2/12	1/12
Slimes	2/12	0/12	0/12	1/12	0/12
Final scores [a]	5	4	2	3	1

Legend: *CTRL, no starter; BOX-57 (C. maltaromaticum, C. divergens, and L. sakei); F-106 (Lacticaseibacillus casei); FP-50 (C. maltaromaticum, C. divergens); LAK-23 (L. sakei). Data: sum of the assessors' identifiers of the presence of the descriptor out of the total of the assessors;* [a] *Final scores, the assessors were requested to ranked the products within a scale from 1 (excellent) to 5 (worst).*

4.b Conclusions

The results of this study indicated that the shelf life of sea bream and sea bass burgers, as determined by the sensory scores and physico-chemical and microbiological data, was 12 days.

The inoculation of different starter cultures did not have a particular effect on the microbial populations and physico-chemical characteristics of the burgers.

The volatilome changed in the different treatments, and in particular, important differences were induced by the presence of bioprotective cultures. The samples supplemented with BOX-57, FP-50, and LAK-23 presented a profile that described the rapid growth and colonization of fish burgers by LAB, with the production of typical molecules derived from their metabolism.

The sensory attributes of burgers were affected by the presence of the bioprotective cultures, as the odors, flavors, and sticky, white slime indicative of deterioration were not observed. Additionally, inoculated burgers did not demonstrate bloating spoilage. The bioprotective cultures evaluated in this study can potentially extend the shelf life and improve the sensory properties of fish burgers, contributing to the reduction of food waste in the fish supply chain.

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5) Annexes are not available

6) List of references with links where possible

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List of equipment used during project testing:

Ref.	Image (photo with the project label on)	Description (name of the equipment and short description of the object)	Station Town and PP where is places
1		Incubator – To maintain standard temperature for bacteria growth	Di4a
2		Conventional PCR thermocycler To test genes and identify microorganisms	Di4a
3		Minicentrifuge Used for centrifugation of small quantities of ingredients used in molecular techniques	Di4a
4		Orved VM53 vacuum machine Used to package fish and fish products	Di4a
5		Autoclave Used to sterilize broths and agars	Di4a
6		Microscope Used to observe the morphology of the microorganisms.	Di4a

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- **PART 2**

The second part provides the final results and a collection of data from the WP and project in relation to the General objectives at the Programme level that we will need to add to the final report.

A. CONTRIBUTION TO EUSAIR

Please provide a description of the project contribution to the EUSAIR in terms of synergy with the Strategy's pillars and alignment of implemented project's activities with the Action Plans and labelled projects.

Project contributes to the EUSAIR Strategy's pillar "blue growth" and through innovation and development of the sustainability of aquaculture in the Adriatic Sea establishes a basis for the development of aquaculture in the whole EUSAIR region. In particular, a network of academia and industry worked together in the enhancing profitable, high-quality and sustainable aquaculture production which is capable to contributes to job creation and economic growth of rural and outlying island communities as well as to supply of healthy food products, respecting the EU and international rules. The results of task 5.2.3 will increase the number of fish products considering the aim was to produce, to prolong shelf-life and to improve new fishery products using different technologies. The results can be easily transferred to other territories of the EUSAIR especially those missing specialised research centres as well as other Mediterranean areas.

B. CONTRIBUTION TO HORIZONTAL PRINCIPLES

Please provide a description of the project contribution to the horizontal principles of equality between men and women, non-discrimination and sustainable development.

The project gathered different experts based on the skills regardless of race, nationality, ethnic origin, religion, disability, age or sexual orientation. In particular, it provides a description of the project contribution to the horizontal principles of equality between men and women, non-discrimination and sustainable development. The focus was the promotion of a healthy and sustainable product from the Adriatic regions, bringing together farmers, scientists, consumers, veterinarians and experts in the field. In particular, task 5.2.3. contributes sustainable aquaculture and sustainable production of new products improving their safety and shelf-life with bioprotective cultures.

C. COMMUNICATION ACTIVITIES

Please refer to the Final Communication Report template and provide a summary on the main achievements trying also to identify which were the most successful communication tools in reaching general public/decision makers/other target groups.

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All activities were disseminated through different media channels (social media such as Facebook, Twitter, LinkedIn), project website, international and national journals and portal sand through different virtual conferences and face to face conferences. Many experts were reached through virtual and online workshops organised to disseminate the project results. However, the most important events were press conference organised in Rijeka that presented the project outputs in 2019, as well as the press conference immediately before the final conference in Zadar on 3rd June 2022, in Udine (June, 21rst, 2022), in Padua (November, 19th, 2021), in Pordenone (Aquafarm, May 25th, 2022), in Ostuni (May 7th, 2022). These press conferences raised a huge interest of journalists and reached huge number of general public.

prof. Comi and the products shown during Aquafarm fair on the project stand, May, 2022

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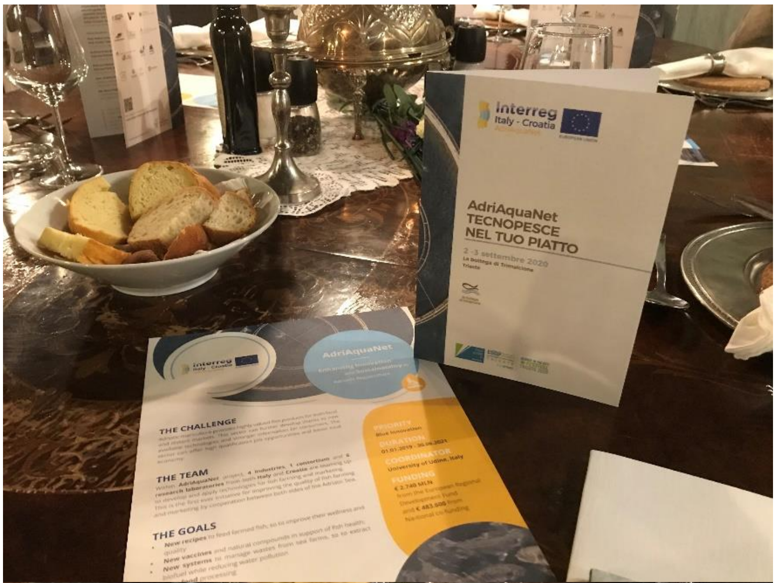
The farmed fresh fish and the fish products were also tested by focus groups of the WP5 task:

1) **FISHTECH in your DISH event inserted in TRIESTE NEXT festival in Trieste, September 30, 2020**



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2) **Final event in Udine, June 2022, social dinner with testing of new products filets and hamburgers. Questionnaires related to the sensory analysis were distributed to the partners and participants.**

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UNIVERSITA' DEGLI STUDI DI UDINE
DIP. DI SCIENZE AGROALIMENTARI, AMBIENTALI E ANIMALI (DI4A)
Sezione di Scienze Animali e Veterinarie (SAV)

PROGETTO/PROJECT AdriAquaNet

Scheda di valutazione sensoriale / Evaluation test on

Filetto di branzino affumicato / sea bass smoked fillet

Colore/Colour	Decisamente Pallido/ Definitely pale ☐	Leggermente Pallido/ Slightly Pale ☐	Ideale, va bene così/ Ideal, that's okay ☐	Leggermente marcato/ Slightly marked ☐	Decisamente Marcato/Definitely marked ☐
Odore/Smell	Troppo Debole/too weak ☐	Leggermente debole/ Slightly weak ☐	Ideale, va bene così/ Ideal, that's okay ☐	Leggermente forte/ Slightly strong ☐	Troppo Forte/too much strong ☐
Gusto/Aroma/ Flavour	Troppo Debole/ too weak ☐	Leggermente debole/ Slightly weak ☐	Ideale, va bene così/ Ideal, that's okay ☐	Leggermente intenso/ Slightly intense ☐	Troppo Intenso/too much intense ☐
Consistenza/ Texture	Troppo Tenero/too soft ☐	Leggermente tenero/slightly soft ☐	Ideale, va bene così/ Ideal, that's okay ☐	Leggermente duro/slightly hard ☐	Troppo Duro/too much hard ☐

☹ **Gradimento complessivo / overall judgment** 😋

Estremamente sgradevole / Extremely unpleasant /	**Molto sgradevole/ Very unpleasant /**	**Sgradevole/unpleasant**	**Leggermente sgradevole/slightly unpleasant**	**Né gradevole, né sgradevole/ Neither pleasant nor unpleasant**	**Leggermente gradevole / Slightly pleasant**	**Gradevole/ pleasant**	**Molto gradevole/ very pleasant**	**Estremamente gradevole/ Extremely pleasant**
☐	☐	☐	☐	☐	☐	☐	☐	☐

Comments, observations on the fillet: ______________________________

____

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Hamburger di pesce / Fish Hamburger

Colore/Colour	Decisamente Pallido/ Definitely pale □	Leggermente Pallido/ Slightly Pale □	Ideale, va bene così/ Ideal, that's okay □	Leggermente marcato/ Slightly marked □	Decisamente Marcato/Definitely marked □
Odore/Smell	Troppo Debole/too weak □	Leggermente debole/ Slightly weak □	Ideale, va bene così/ Ideal, that's okay □	Leggermente forte/ Slightly strong □	Troppo Forte/too much strong □
Gusto/Aroma/ Flavour	Troppo Debole/ too weak □	Leggermente debole/ Slightly weak □	Ideale, va bene così/ Ideal, that's okay □	Leggermente intenso/ Slightly intense □	Troppo Intenso/too much intense □
Consistenza/ Texture	Troppo Tenero/too soft □	Leggermente tenero/slightly soft □	Ideale, va bene così/ Ideal, that's okay □	Leggermente duro/slightly hard □	Troppo Duro/too much hard □

Gradimento complessivo / overall judgment
Gradimento complessivo / overall judgment

Estremamente sgradevole / Extremely unpleasant /	Molto sgradevole/ Very unpleasant /	Sgradevole/unpleasant	Leggermente sgradevole/slightly unpleasant	Né gradevole, né sgradevole/ Neither pleasant nor unpleasant	Leggermente gradevole / Slightly pleasant	Gradevole/ pleasant	Molto gradevole/ very pleasant	Estremamente gradevole/ Extremely pleasant
□	□	□	□	□	□	□	□	□

Comments, observations on hamburger: ________________________________

Questionario

1) Genere/Genre M □ F □
2) Anno di nascita /Year of birth(aaaa) _ _ _ _
3) Provincia (o Stato se non è italiano) di residenza/Province (or State if not Italian) of residence

____

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4) Indichi il suo titolo di studio più alto
4) Indicate your highest educational qualification

Nessuno/frequenza scuola elementare/ None / elementary school attendance	1☐
Licenza elementare/ Primary school diploma	2☐
Diploma di scuola media inferiore/ Primary school diploma	3☐
Diploma di scuola media superiore/ High school diploma	4☐
Laurea/ Degree	5☐
Titolo post-laurea/ Post-graduate degree	6☐

5) Indichi la sua occupazione attuale
5) Indicate your current occupation

Casalinga / Household worker	1☐
Studente /Student	2☐
Pensionato / Retired	3☐
Disoccupato/ Unemployed	4☐
Inabile al lavoro/ Unable to work	5☐
Agricoltore/Pescatore/Allevatore/ Farmer / Fisherman / Breeder	6☐
Libero professionista / Freelance	7☐
Commerciante, artigiano o altro lavoro indip./ Merchant, craftsman or other indep)	8☐
Impiegato/Operaio/Lavoratore dipendente / Clerk / Worker / Employee	9☐
Tecnico specializzato / Specialized technician	10☐
Prestaz. d'opera occasionale/Co.co.co-pro/ Performance occasional work	11☐
Docente/Insegnante / Lecturer / Teacher	12☐
Altro (specificare)/ Other (specify)	13☐

6) Lavora nel settore ittico (pesca, trasformazione o vendita)? /Do you work in the seafood sector (fishing, processing or sales)?

SI/YES☐ NO☐

7) Chi fa normalmente la spesa alimentare?/ Who normally does the grocery shopping?

Io stesso /I do shopping☐ Altri /others☐

8) Con quale frequenza **consuma** pesce (di ogni tipo) mediamente? / How often do you consume fish (of all kinds) on average?

1 volta al mese o meno/1 time per month or less	1 ☐
2-3 volte al mese/2-3 times a month	2 ☐
1 volta alla settimana/ Once a week	3 ☐
2-3 volte alla settimana/2-3 times a week	4 ☐
4-5 volte alla settimana/4-5 times a week	5 ☐

9) Dove **acquista** solitamente il pesce?/ Where do you usually buy fish?

Non lo acquisto/ I don't buy it	1 ☐
Non lo acquisto, lo pesco io stesso/ I don't buy it, I fish it myself	2 ☐
Direttamente dal pescatore / Directly from the fisherman	3 ☐
Direttamente dall'allevatore/ Directly from the breeder	4 ☐
In pescheria o alla bancarella del mercato/ At the fish market or at the market stall	5 ☐
Al supermercato / At the supermarket	6 ☐

10) Come acquista SOLITAMENTE, in prevalenza, il pesce? (è possibile anche più di una risposta) / How do you usually buy fish? (more than one answer is also possible)

Fresco /Fresh	1☐
Surgelato/ Frozen	2☐
Trasformato (inscatolato, salmone affumicato, calamari ripieni, polpettine, spiedini di gamberetti ecc..)/ Processed (canned, smoked salmon, stuffed squid, meatballs, shrimp skewers, etc.)	3☐

Grazie per la collaborazione! / Thank you for your collaboration!

3

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D. NATURA 2000

Please describe, if it is the case, measures foreseen and implemented by the project:

a)	**In case the project involved Natura 2000 sites, describe what measure the project envisaged and implemented to avoid any negative impact:** No Natura 2000 sites are involved.
b)	**In case the project had a positive effect on Natura 2000 sites, please describe which measure the project has foreseen and implemented in order to reach a direct or indirect positive impact:** No Natura 2000 sites are involved.

E. TYPES OF ACTIONS ADDRESSED (as defined in the Cooperation Programme)

These are our primary objective's types of actions, that we addressed by the Project:

Specific Objectives	***Types of action***	***the most relevant one within the SO addressed by your project***
1.1 Enhance the framework conditions for innovation in the relevant sectors of the blue economy within the cooperation area	*Joint projects and actions aimed at creating platforms, networks and at supporting exchange of good practices in order to enhance the knowledge transfer and capitalization of achieved results in the field of blue economy*	***X***
	Actions aimed at cluster cooperation, joint pilot initiatives in order to boost the creation of marketable innovative processes and products, in the field of blue economy	***X***

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F. TYPES OF OUTPUTS PRODUCED

Specify the types of outputs generated by your activity that are reported here and provide a brief description

Output typology	Description
Trainings	9 cycles of AAN courses either in Italy or in Croatia regarding the safety of the fish have been performed during the project.
Monitoring systems	N.A.
SMEs clusters	Potential collaboration and exchange of work and resources among enterprises involved in the aquaculture business chain, such as fish farms and industries for fish end fish products aquafeeds producing and waste recycling were established. The innovative products developed during the project within the task 5.2.3 can be applied in other Italian and/or Croatian fish farms and facilities. The cross border production chain that involves Italian hatcheries, which grow sea bass and sea bream fingerlings and juveniles, and Croatian on-growing sea cages-based farms, which than exported the fish to the Italian market, was implemented thanks to the project training courses and events.
New networks	New collaborations among project partners and researchers of Udine University were developed during the project in order to achieve the task 5.2.1 objectives. Moreover, an active cooperation among researchers of LP and fish farmers was developed so as to improve the interest of entrepreneurs for R&D and innovation as well as allow the project to respond to their needs.
Platforms	N.A.
Adaptation plan	N.A.
Building renovation	N.A.
Others (please specify)	N.A.

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G. TYPOLOGY OF IMPACTS

Please indicate what type of impact(s) your project has had. You can choose more than one answer. For each tangible impact selected, please provide a concrete example from your project, where possible supported by quantitative information.

TANGIBLE IMPACTS

Tangible impacts	Example/ quantitative information
Improved access to services	N.A.
Cost savings	The new products can reduce costs of fish production.
Time savings	N.A.
Reduced energy consumption	N.A.
Reduced environmental impact	The application permits indirectly to reduce energy in fish production, less waste.
(Man-made, natural) risk reduction	N.A.
Business development	In sea bass/bream intensive farms in the Adriatic area will ensure a better productivity and more eco-compatible productions that will be more appreciated by the consumers, increasing the profitability of the mariculture sector.
Job creation	New and permanent employment opportunities to costal populations of both sides of the Adriatic Sea can increase thanks to the knowledge transfer and skills.
Improved competitiveness	New packaging and new products of farmed sea bass/bream on intensive farms in the Adriatic area can improve and ensure an increased competitiveness of SMEs on regional and international markets.
Other tangible impacts (specify)	N.A.

INTANGIBLE IMPACTS

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Intangible impacts	Example/quantitative information
Building institutional capacity	N.A.
Raising awareness	The project has stimulated the attention of fish farmers and fish product producers in particular the topics related to the production improvement, with less waste and the use of new marketing techniques.
Changing attitudes and behaviour	New trend of production (new products) and new marketing materials can change the attitudes and behaviour of the consumers and improve the nutritional habits of the population.
Influencing policies	N.A.
Improving social cohesion	N.A.
Leveraging synergies	The project lead to the strengthening of relations between Italian and Croatian research groups, as well as between universities or centres of excellence and fish farmers. The project provides to fish farmers new techniques and protocols for the safe and healthy fish production that can be applied in hatcheries and sea plants, so to improve the sustainability of Mediterranean aquaculture and consequently the competitiveness of sector.
Other intangible impacts (Specify)	N.A.

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