AdriAquaNet

Enhancing Innovation and Sustainability in Adriatic Aquaculture

Deliverable WP5

Technical-scientific report

D 5.2.1 Technical-scientific report on a new type of farmed fish packaging for extended shelf-life

Udine, 30.06.2022

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Deliverable name:	**Technical-scientific report on a new type of farmed fish packaging for extended shelf-life with description of safety, quality and sensorial characteristics**
WP leader:	**LP (UNIUD), partners involved PP11 and PP10**
Author (s):	**G.Comi, L. Iacumin**
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European Regional Development Fund

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CONTENTS OF THE DELIVERABLE

The present Document, constituting the Deliverable of WP 5 - Activity 2.1, is divided into 2 parts:

- **PART 1**

  The first part provides details and assessment of the WP objectives related to the primary subject of the report and the implementation and results, compared to the information already provided in the different progress reports, in order to give back a cumulative illustration of what the project delivered in relation to this task.

  This part is structured as follows:

  A. Report highlights
  B. WP 5 – Activity 2.1 output and results
  C. Durability and transferability of the project and its results
  D. Capitalization of the results
  E. Partnership cooperation
  F. Target groups involvement

- **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 are also described in the final report of the Project.

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PART 1

A. REPORT HIGHLIGHTS

A cumulative short overview of the project WP task 's achievements

- **Context:**

  Increasing safety and shelf-life of fresh fish. LP and PP11 has been working together to apply different techniques and new protocols to improve fish quality after farming (P10) and during cold storage.

- **Challenge:**
  a. Evaluation of different techniques, including Modified Atmosphere, Under Vacuum packaging (MAP) and washing and Latilactobacillus sakei as a bioprotective agent,
  b. to increase the shelf-life of fresh gutted sea bass (Dicentrarchus labrax) and sea bream (Sparus aurata) stored at 6 ± 2 °C.

- **Expectations:**

  To guarantee fresh fish and fish products of excellent quality thanks to a safe packaging and longer shelf-life.

- **Solution**

  Using different techniques such as types of packaging and bioprotective culture.

- **Process:**

  We started by choosing a new packaging and starter selection. We then choose the types of packaging (Under vacuum or MAP). Finally, we choose the type of fish washing and storage at different temperature. What we achieved is that:

  1. the type of packaging is important for the shelf-life;
  2. the new packaging can increase the shelf-life;
  3. the used methods are adequate for the experiments.

  Our vision to solve the problem of the short shelf-life of fish has been explored. A new qualitative packaging has been studied that guarantees that less product goes to waste.

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B. WP PROJECT OUTPUTS AND RESULTS

The project objectives are fully achieved. WP 5.2.1 task was related to the EPO applications and specific objective as follows:

Specific 3: Increase SMEs competitiveness thanks to new high quality fresh and processed fish

The activity plan and timeframe of the WP5 were created among scientific (LP, PP1, PP2, PP3, PP5) and industrial (PP8, PP9, PP10, PP11) partners at the beginning of the project implementation and it included fuoro tasks:

1. **to produce scientific proofs on the quality including sanitary aspect and nutritive composition of the fresh farmed sea bass and sea bream and compare it with the quality of the same species from fishermen's catch;**
2. **to test possibilities of extension of the shelf-life of fresh fish, to create new products such as cold-smoked fish fillets and hamburgers made of sea bream and sea bass meat and test the new approach in extensions of shelf-life on the products;**
3. **to survey the perception of different groups of consumers** (general **consumers, consumers in catering facilities and managers of marketing facilities) in both Italy and Croatia and create marketing plans for the fish farming industry;**
4. **to promote the results of the research to stakeholders (general public, target groups among general public such as children, elder population and athletes, professionals, fish farmers and catering)**

Task 5.2 was focus on the creation of new farmed fish products and the extentions of fresh fish and farmed fish products shelf-life. Fish meat is very perishable because of indigenous and microbial enzymes, which determine spoilage and shelf life. The deterioration processes, which lead to an important, sequential and progressive modification of the initial state of freshness, are fast and depend on rearing, harvesting, slaughtering, handling and storage conditions. Usually, the shelf life of gutted fish stored at 4 ± 2 °C under vacuum packaging (VP - 1.0 bar) and modified atmosphere packaging (MAP, 70% N2, < 1% O2, 30% CO2) is approximately 9 days.

For this reason **the first aim of our activites was focused on the Evaluation of different technique of packaging, including Modified Atmosphere and Under Vacuum packaging, washing, etc**.. To improve the shelf life and preserve the microbiological and sensory quality of farmed gutted sea bass (Dicentrarchus labrax) and sea bream (Sparus aurata), we tested different methods of packaging including VP, MAP and bioprotective culture containing Latilactobacillus sakei till 12-14 days.

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Microbiological, physico-chemical and sensory quality indices were monitored to confirm the effectiveness of biopreservation on product quality during a proper refrigerated (4 ± 2 °C) or abuse (6 ± 2 °C, simulating supermarkets and consumer fridges) storage period.
In addition, we wanted also to controll the storage and the coservation of fresh fish products proposed through all production process: from farming to selling. This part consisted in controilling the time/temperature of the delivery and the packaging. The delivery from the farm to the lab, where the products were packaged, lasted 24 hour maximum and the gutted fish were kept in boxes with flake ice. At the Lab the fish were packaged within 2 hours and kept at 4 °C. The type of packaged included under vacuum and Modified athomsphere packaged. We studied and defined the best solution of the packaging to preserve quality and and healthy safe fish to customers.

Figure 1: vacccum packed eviscerated sea bass tested for extended shelf life, UNIUD, 2021.

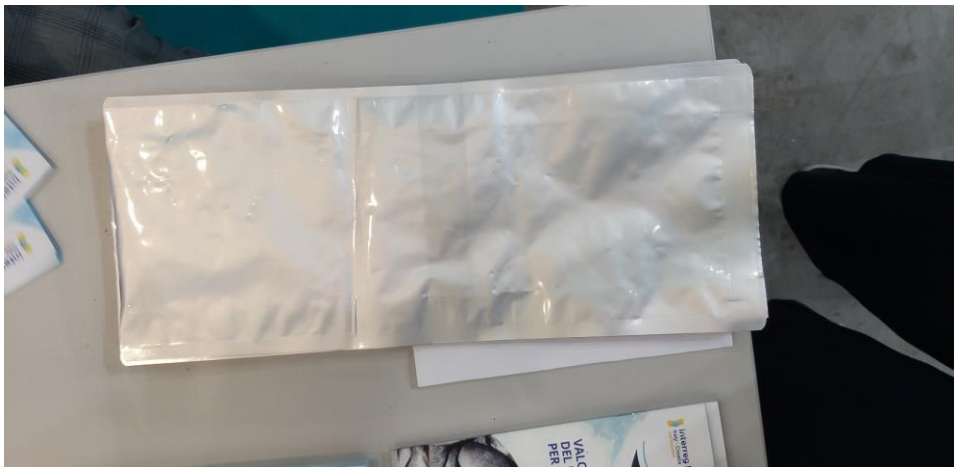

Figure 2: New packeging, presented at the training in Pordenone, May 2022

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The second aim was **to produce new farmed fish products, such as fish hamburgers and filet to increase the consumption of fa**
rmed fish species, b**ased also on the surveys and the perception of different groups of consumers** (general consumers, consumers in catering facilities and managers of marketing facilities) carried out in WP5.3 task, both Italy and Croatia. Preliminary activities were carried out by LP and the decision to develop a new product was switched from sausages to hamburger made of farmed sea bass and sea bream. Product as **smoked fillet of sea bream and the sea bass was developed in collaboration with PP10 Orada (fish provider) and PP11 Friultrota (technique provider) and their quality analysed by LP labs.**

Figure 3: New products filet and hamburgers, presented at the training in Pordenone, May 2022

On the other hand, hamburger from farmed fish can be produced by methods similar to those used to produce meat products. Since traditional techniques like canning or freezing can reduce quality of the processed fish, another objective was to increase the fish hamburger shelf life by using natural preservatives. Our testing consisted in monitoring the influence of different lactic acid starter cultures to improve quality and shelf-life of fish hamburgers. The aim was to evaluate and characterize the physico-chemical and microbial characteristics of fish hamburgers, produced by using a mix of sea bass and sea bream meat (68%), potatoes, water, rice flour, vegetable fibre, salt and to extend its shelf-life to 30 days. The hamburger was packaged in MAP (60 N2 – 40 CO2). After packaging the products were stored for 10 days at 4 °C and then at 8 °C for the rest of 20 days of storage. At the day 0, 6, 12, 18, 24 and 30, three samples were collected and microbial and physico-chemical analysis were performed. At the day 30 the products were cooked and subjected to sensory analysis.

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Figure 4: Different bacteriological media used for testing microbiological properties of vaccum packed fresh fish

We focused on the use bioprotective culture of microbial starter suc, h us as Lactobacillus, Pediococcus and Staphylococcus strains, or essential oils in order to check and to increase the fish meat shelf-life, considering that usually fish products are highly perishable and the refrigerated temperatures permit only a short shelf-life usually less than 7-8 days.

Among different tests performed, Latilactobacillus sakei was used as a bioprotective agent with the objective to increase the shelf-life of fresh gutted sea bass (Dicentrarchus labrax) and sea bream (Sparus aurata) stored at 6 ± 2 °C. The biprotective starter added included: BOX-57: Carnobacterium divergens, C. maltaromaticum, Latilactobacillus sakei (produttore di batteriocina), LAK-23: Latilactobacillus sakei; FP-50: Carnobacterium divergens, C. malaromaticum; Lacticaseibacillus casei 106. LAK-23 was produced and supplied by the company Sacco s.r.l. (Via Manzoni 29/A, 22071 Cadorago, CO, Italy), while the other strains came from the collection of the Department of Agri-Food, Environmental and Animal Sciences. Control samples without any starter added were also investigated in order to compare the results to the samples with starters.

The tests confirmed LAK-23 strains to be effective.The microbiological analyzes confirmed in the samples inoculated with the bioprotective starters a reduction of the spoilage population compared to the control. It is possible, as desired, to extend the shelf-life up to 12 days. The shelf-life cannot, however, be extended beyond this limit, because as it can be seen from the chemical-physical tests, especially from the TVB-N data (data presented as published in the table below), just at day 15 the hamburgers must be refused. In addition, the considerable decrease in pH highlights the occurrence of an acidification boost, validated by the 30-day tasting of the products, which were all judged to be excessively acidic.

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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	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	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	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	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}
	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: CRTL: 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 log mean ± standard deviation; Mean with different letters within each day and each character (following the columns) are significative different ($p < 0.05$).

The analysis of volatile compounds (VOCs) was carried out at days 0, 6, 18 and 30, but confirmed the previous results. At zero days only the control was considered, since at that time the profile of the VOCs was common to that of the samples inoculated with the starters. Volatile organic compounds (VOCs) are low molecular weight metabolites, produced by the spoilage and starter microorganisms during the storage of the products and are used as markers in assessing the state of freshness or spoilage of the product. Also in this case 29 molecules were identified, and divided into 5 main classes: aldehydes, esters, ketones, alcohols and acids. All the VOCs increased during the storage and at the end there were no differences among the samples independently from the presence or not of the bioprotective starter.

The control sample packaging swelled just at 12 days, and was not tested at the end of storage. Conversely the other samples were tasted at 30 days, the end of storage.

At day 30, the tasting of the samples added with bioprotective cultures (LAK-23, 106, BOX-57 and FP-50) show no significant differences in odor and flavor. The samples appeared similar at sensorial level and too acidic. Excessive acidity and astringency are characteristics obviously not appreciated by consumers. Conversely the results demonstrated that the hamburgers can have a shelf-life maximum of 12 days independently by the use of the different bioprotective cultures. Without starter the

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packaged swelled, due to the sugars coming from the potatoes mix added. According to sensorial analysis at 12-day storage, the panellists (not training assessors) preferred the products added with LAK-23 starter. However, not sensible differences were observed among the different types of hamburgers.

Considering the quality indexes represented by *Enterobacteriaceae*, total volatile nitrogen (TVB-N) and malonaldehyde concentrations (TBARS) and the sensorial analysis, the VP samples were more acceptable than the MAP fish, even though the shelf-life of the VP and MAP fish was similar and about 12 days. The bioprotective culture reduced the growth of spoilage microorganisms. Consequently, the total volatile nitrogen (TVB-N) concentration in both fish species was low (< 35 mg N/100 g). Nonprofessional and untrained evaluators confirmed the acceptability of the inoculated samples by sensorial analysis.

The protocol used to produce scientific proofs on the quality including sanitary aspect and nutritive composition of the fresh farmed sea bass and sea bream and compare it with the quality of the same species from fishermen's catch was developed, as well as the procedure to test possibilities of extension of the shelf-life of fresh fish:

- **To compare VP and MAP samples stored at 4 ± 2 °C**, analyses were performed on days 0, 6, and 12. At each time point and for each packaging condition and fish species, three samples were collected and analysed. Each lot and each type of packaging included 9 farmed sea bass and 9 farmed sea bream samples. The results demonstrated that the under vacuum packaging was better than MAP.
- **To study the microbial and physicochemical development of both fish stored in VP at 6 ± 2 °C** (simulating abuse temperatures), analyses were performed on days 0, 3, 6, 9, and 12. At each time point and for each fish species, three samples were collected and analysed. Each lot included 15 sea bass and 15 sea bream samples.
- **To prolong the shelf life of both fish samples stored at 6 ± 2 °C** (simulating abuse temperatures) with or without supplementation a bioprotective starter consisting of *Latilactobacillus sakei* (LAK-23, Sacco s.r.l., Via Alessandro Manzoni 29/A, 22071 Cadorago, CO, Italy). The strain was selected and isolated from meat products and tested for its genetic and phenotypic characteristics. Then its use was proposed as starter for meats fermentation and as bioprotective agent, being bacteriocin producer, versus *L. monocytogenes* and spoilage microorganisms in meat and fish products. Three samples were collected and analysed on days 0, 7 and 14.

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Figure 5: Lab tests at University of Udine

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Reasons of discrepancies between planned and realized outputs (if any): No discrepancies

Impact of outputs underachievement on project results: No underachievement

Additional results (was the project able to reach additional outputs /results besides those foreseen in AF? No additional results**.**

The research work performed was published in two international papers during the project activities:

- Iacumin, L., Cappellari, G., Pellegrini, M., Basso, M., Comi, G. (2021) **Analysis of the bioprotective potential of different lactic acid bacteria against *Listeria monocytogenes* in cold-smoked sea bass, a new product packaged under vacuum and stored at 6 ± 2 °C.** Frontiers in Microbiol., December,2021, https://doi.org/10.3389/fmicb.2021.796655
- Iacumin, L., Jayasinghe, A.S., Pellegrini, M., Comi, G. (2022) **Evaluation of Different Techniques, including Modified Atmosphere, under Vacuum Packaging, Washing, and *Latilactobacillus sakei* as a Bioprotective Agent, to Increase the Shelf-Life of Fresh Gutted Sea Bass (*Dicentrarchus labrax*) and Sea Bream** (*Sparus aurata*) Stored at 6 ± 2 °C. Biology, 11, 217. January, 2022 https://doi.org/10.3390/biology11020217.

The results about the study of hamburgers and the cold smoked sea bass were subject of two papers that were published in Agust 2022:

- Iacumin, L.; Pellegrini, M.; Sist, A.; Tabanelli, G.; Montanari, C.; Bernardi, C.; Comi, G. (2022) **Improving theShelf-Life of Fish Burgers Made witha Mix of Sea Bass and Sea BreamMeat by Bioprotective Cultures.** Microorganisms 2022,10, https://www.mdpi.com/2076-2607/10/9/1786
- Physico-chemical and microbial characterization of cold smoked sea bass stored at refrigerated temperature. (still waiting for the approval of publishing).

C. DURABILITY AND TRANSFERABILITY OF THE PROJECT AND ITS RESULTS

How will the outputs and results be maintained and developed further after project end?

The results of the project allow to use the protocols and products elaborated during the project, contribute to the evaluation of sustainable farming, testing of a new approach to feeding, feeding with new feed formulation and emphasizing preventive approach in disease management and mitigation. Following the practices proposed, a SMEs can produce an impact during the production cycle and check on the high-quality fresh fish and fish products. Cross-border cooperation engaged the most appropriate research facilities with extensive experience in creating and sanitary control of fish products (LP), fish quality (PP1) and marketing and promotion of healthy nutrition (PP5) with industries that participated and applied a different approach to farming. These experiences can be

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applicable on different structures in marine aquaculture, food processing, catering facilities and use on a larger scale, with a positive impact on the nutrition and health of the general population.
The results will be maintained thanks to the publications, flyers, videos realised during the project life time and shown and distributed to the fish producers during the 9 cycles of the trainings.
The new packaging produced was shown during the training and numerous events to the participants. The materials show and explain the food process and how the use of bioprotective cultures permit to prolong the shelf-life and the acceptability of the fish. Indeed, also new types of packaging either film composition or atmosphere must be investigated.

Figure 6: prof. Comi and the products shown during Aquafarm fair on the project stand, May, 2022

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How has the availability of project results and outputs for general public and other stakeholders been ensured during the project life and eventually after the project end?

The results of WP 5.2 activities were presented in several trainings, in particular in Padua (November, 19th, 2021), in Pordenone (Aquafarm, May 25th, 2022), in Ostuni (May 7th, 2022), Zadar on 2nd and final events in Zadar on 3rd June 2022 and in Udine on 20th June, 2022. The availability of the results for general public and other stakeholders on international level will be ensured by International publications. Besides, we have produced a large number of publications such as **"The Manual of European Sea Bass and Gilthead Sea Bream Safety, Quality and Health Benefits." (WP 5.1, LP and PP1 work).** This Manual was distributed in different workshops and Congress to stakeholders. Several brochures, publications and video promoting material have been prepared **(WP 5.3, PP5 and PP2) and presented during 39 project events**. In addition to these public events students of different university programs were reached with results of the research carried out in the WP5; not only students of the faculties of project partners but also students of the Faculty of Food Technology, Biotechnology and Nutrition and Veterinary Faculty, University of Zagreb, Faculty of Food Technology, the University of J.J. Strossmayer in Osijek and Faculty of Agronomy and Food Technology, University of Mostar, Bosnia and Herzegovina, etc.

Figure 7: AAM produced and shown during AAN events.

The results will be showed during future seminars and tutorials after project's end.

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D. CAPITALISATION OF RESULTS

Please provide information about capitalisation:

Was the project able to capitalise or influence future calls or other projects? Please specify main results or output to be considered for future capitalisation action.

The technological results including the use of new packaging, the type of packaging and the bioprotective cultures could possibly be implemented for improvement of safety and shelf-life of the fish and fish products and represent a challenge for the sustainability of the entire production chain. It's a topic of extreme interest even for future capitalization actions as it was disused also during the last partners' meeting. All the observed techniques could be considered for future actions and present a new upgrade actions to future Italy – Croatia programme.

Are there any obstacles of legal or administrative nature that the project has encountered and which hampered cooperation? Is there any room to solve these obstacles?

No obstacles of legal or administrative nature have been encountered during the project that have limit the cooperation among people and institutions.

E. PARTNERSHIP COOPERATION

Which Partners were active in your WP and the activities related to the report? Were all the Partners involved also active?

Giuseppe Comi and Lucilla Iacumin (LP team in the activity 5.2.1 also task leader), M. Gumiero (PP10 Orada Adriatic d.o.o.) and M. Pighin (PP11 Friultrota coordinator) were directly involved in the research activities. Also Ittica Caldoli (PP9) was involved in some activities of the tests.

LP, PP1, PP2 and PP5 researchers (Snježana Zrnčić, Emilio Tibaldi, Marco Galeotti, Nada Vahčić, Jelka Pleadin, Greta Krešić, Sabina Passamonti, Paola Sist) were involved in fish farming, harvesting and transforming tests. All colleagues encouraged the experiments and supervised them.

Were they all able to attract other local/regional actors and involve them in the project activities?

Yes, all partners were involved to promote and disseminate the research activities and results. Ittica Caldoli (PP9), Orada Adriatic d.o.o.(PP10) and Friultrota (PP11 Friultrota) are established company with a solid relationship with the territory; therefore, it will be able to effectively spread the present outcomes to other stakeholders of the aquaculture sector even after the end of the project.

Morover, Andrea Fabris, director of API - Associazione Piscicoltori italiani was directly involved in the project activities (events, trainings, deliverables production) and he promoted the project outcomes to the stakeholders and fish producers and organized meetings.

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What was the added value given by the cooperation?

The added values consist in:

- the dissemination of the results to the producers;
- the cooperation allowed the publication of three scientific articles.
- new networking.

Moreover, it resulted in strengthened knowledge on these topics and could be the base of future research projects.

Which were the main problems encountered?

The main problems encountered during the project development were operative and due to the restrictions caused by the sanitary emergency experienced with the COVID-19 pandemic. Travelling, collection of samples and access to laboratories resulted impossible with a consequent obligation of suspending the experimental tests. The extension of the project allowed us to restore all the scheduled activities and achieve the project objectives without any limitations.

Was the project able to create links with other projects?

Yes, several links with the other projects were established during the international and national events, such as Aquafarm 2019 and 2022, **Advanced Course on Application of Epidemiology in Aquatic Animal Health**, OYSTER workshop PIRAN in 2020, Perform Fish, many other EU and Horizon project presented during the 19th and 20th Conference on Diseases and Shelf fish in 2019 and 2022 and 12th Nordic Nutrition Conference Programme in 2022, etc.

Will the PPs cooperate in the future even without funding (if yes explain the main aims of this cooperation)?

Yes, we are going to cooperate for future project with or without funding from the Italy Croatia programme. The cooperation of Udine University allowed strengthening the group of researchers dealing with the theme, with possible future exchanges and participation in research projects.

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F. TARGET GROUPS INVOLVEMENT

Please list the main target groups that benefited from your WP project's achievements as inserted in the relevant Report Section in SIU that you will find on the left (the numbers are our project numbers). In few word provide further details on how they were able to make use of the outputs/ results of the project.

TARGET GROUPS	***Description***
SMEs (50)	Two fish farms PP10 and PP11 were directly involved in the experimentation and two fish farms PP8 and PP9 were involved by participating to the trainings. 18 enterprises (Fanin, Bluefarm, Gas Clima Service, Skretting, RINA Service, INmare, rehomare srl Gallipoli, Ittinsect srl, Panittica Italia Società Agricola srl, Acquacoltura Jonica, Azienda Agr. Eredi di B., Aciris Sardegna, Acqua soc. Agr., Mattinatese Maricoltura, Cromaris, COISPA, Bluefarm, Aquarium Pula, Acquaazzurra, participated directly to trainings in Padua, Ostuni, Pordenone and Zadar.
Universities, technology transfer institutions, research institutions (10)	*Scientific collaboration with different researches: Uniud (dr. M. Pellegrini, E. Orecchia,* S. Jaysinghe, A. Colautti), Unimi (prof. C. Bernardi), Unibo (C. Montanari, G. Tabanelli, F. Gardini) for microbial, physico-chemical, volatilome analysis. University from Bari, University from Salento,
NGOs, associations, innovation agencies, business incubators, cluster management bodies and networks (5)	API – Associazione Italiana Piscicoltori (more then 500 associates) Klaster Maricluster PP7 (80 producers)
Centers of R excellence (5)	Rappresentatives ns of Centro di Ricerche per la Pesca e l'Acquacoltura, OISPA Tecnologia & Ricerca scrl, Stazione sperimentale per lo studio delle Risorse del Mare were involved in trainings in Ostuni.
Local, regional and national public authorities (10)	Representatives from Puglia Region, FVG Region, Veneto Region, MIPAAF, Ministry of Health, Interreg Programme Italy and Croatia, Croatian Chamber of Commerce, ASL LECCE, ASL Bari, Ordine Veterinari Bari,
General public (1000)	Dissemination of results on the AAN project website and through radio or tv involved more than 3 million persons

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PART 2

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 5.2.1 will increase the shell-life and safety of fresh fish and consequently the economic value of marine aquaculture sector and 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 on 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 5.2.1 contributes sustainable aquaculture and sustainable use of new packaging and bioprotective cultures in order to improve the safety and the shelf-life of fish.

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.

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

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(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.

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***

Our project is based on *output indicators*

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CO01 – 24 enterprises received support,
CO02 – 4 enterprises received grants,
CO04 – 20 enterprises received non-financial support,
CO42 – 7 research institutions participated in cross-border, transnational or interregional research projects,
CO44 – 578 participants involved in joint local employment initiatives and joint training)

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 techniques and protocols implemented during the project within the task 5.2.1 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.

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.

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TANGIBLE IMPACTS

Tangible impacts	Example/ quantitative information
Improved access to services	N.A.
Cost savings	The new packaging can reduce the costs of fish production
Time savings	The new packaging can reduce the time of fish production
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

Intangible impacts	Example/quantitative information
Building institutional capacity	N.A.

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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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