Deliverable Report

Deliverable No:	D28.4	**Delivery Month:**		29
Deliverable Title	Physical prototypes of new products from the selected species meagre, greater amberjack, wreckfish, pikeperch and grey mullet.			
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Lead Scientists preparing the Deliverable: Bou, R. (IRTA), Robles, R. (CTAQUA).

Other Scientists participating: Guerrero, L. (IRTA), Claret, A. (IRTA), Lazo, O. (IRTA), Marcos, B. (IRTA), Algarín, M. (CTAQUA), Bermúdez, L. (CTAQUA), Vélez, J. (CTAQUA), Román, C. (CTAQUA).

Objective: Physical prototypes of new products will be developed from meagre, greater amberjack, pikeperch, wreckfish and grey mullet. On the basis of their organoleptic characteristics, potential products from the specific species (up to three products of varying degree of processing incorporated per species) will be made available for further testing in WP 29. Species and product selection criteria will be decided according to technical processing limitations, fish availability and similar products availability in Markets (see WP 29), and the market potential of the new species as established in WP 27. The final outcome of WP 28 will be made available for further consumer acceptance testing in WP 29. These physical prototypes of new products from the selected species meagre, greater amberjack, pikeperch and grey mullet are defined as Deliverable D28.4.

Description: Four selected species (meagre, greater amberjack, pikeperch and grey mullet) were the raw material for new product development. A maximum of 3 physical prototypes per species of new products of varying degree of processing in commercial format (size, packaging and presentation) are delivered. The physical prototypes were developed based on: information provided by WP 27 (market potential of the new species), Task 28.1 (products concept development: technical and consumer driven), Task 29.1 (consumer value perceptions and segmentation), physicochemical characteristics of each raw material, technical properties of the products and the process, and similar product availability in the market. Guidelines to obtain new products are provided, including inputs, processing conditions, technical specifications, and troubleshooting. In addition, packaging, conservation conditions, product shelf life and consumer handling/cooking specifications are provided. These new product prototypes will be the input for consumer acceptability evaluation in WP 29.

Deviations: A 3-month delay is due to the 2-month deadline extension of a preceding deliverable, the results of which were a prerequisite for the completion of the present deliverable. The delay of this deliverable will not create further delays or affect the completion of other Tasks in the DOW.

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1. Introduction

1.1. General introduction

The quality of the execution of technological activities such as preliminary technical assessment, product development, in-house product or prototype testing, trial or pilot production, and production start-up is determining in the development of new food products (Lord, 2000). Precedent tasks focused on the market potential of the new species (WP 27) the product concept development including technical and consumer driven aspects (Task 29.1). Provided that all foods are complex biochemical and biophysical structures, various aspects such as the physical and chemical characteristics of each raw material, technical properties of the products and the process (including sensory, microbiological and safety aspects among others) will be considered here. In addition, the availability of similar products in the market will be also examined and taken into account in the development of these new products. The understanding of the market, i.e. consumer demands, together with the scientific and technological principles of foods and food processing are crucial for the successful development of new products.

1.2. Objectives

The objectives of this deliverable include:

- Selection of a number of prototypes to be developed based on the analysis of product concepts (D28.2) and fish species technological, physical and sensory characteristics (D28.3).
- Detailed information and guidelines needed to obtain the new products including inputs, processing conditions, technical specifications and troubleshooting.
- Provision of the necessary information regarding the food products packaging, conservation conditions, product shelf life and consumer handling/cooking specifications.

2. Materials and Methods

2.1. Fish species

Specimens of farmed meagre (*Argyrosomus regius*), greater amberjack (*Seriola dumerili*), pikeperch (*Sanders luciperca*), grey mullet (*Mugil cephalus*) and wild wreckfish (*Polyprion americanus*) were considered in this study. Provided that wreckfish is not available as farmed fish, the possibility to create prototypes out of this species and conduct further consumer test is not possible. Accordingly, the effort on new products development was only made on the available fish species from aquaculture.

Meagre was provided by Andromeda Group (Spain), pikeperch by Asialor (France), greater amberjack by Futuna Blue (Spain) and grey mullet by Pimsa (Spain). The characteristics of the fish used in this deliverable are shown in **Table 1**.

Table 1. Origin, farming conditions, sampling and fish size information of fish used in this deliverable

Species	Origin	Farming conditions	Sampling	Fish size
Meagre (*Argyrosomus regius*)	Aquaculture (Andromeda Group) from Burriana, Spain	floating sea cages - fed with commercial extruded feed	September 2015	Average size 60 cm and 1.8 Kg
Pikeperch (*Sanders lucioperca*)	Aquaculture (Asialor), France	Fresh water intensive farming	June 2014	Average size 54 cm and 1.4 Kg
Greater amberjack (*Seriola dumerili*)	Aquaculture (Futuna Blue), Spain	Tank culture (RAS) - fed commercial extruded feed	December 2015	Average size 49 cm and 1.4 Kg
Grey mullet (*Mugil cephalus*)	Aquaculture – polyculture (Pimsa), Spain	Ponds, natural feeding	January 2016	Average size 54 cm and 2 Kg

2.2. Selection of ideas

The selection of fish products was made after considering the different product concept positions in the ranking D28.2. This ranking position is a result of the score on 19 different criteria (healthiness, convenience, cost, etc), and on the technical feasibility of the different fish products. The first criterion was to only select those concept ideas that scored more than 95 in the final ranking of product concepts.

There were other aspects that have been considered for the selection of the different fish products and prototype elaboration. They include the simplicity of ingredients (number of ingredients and their availability in the market), ease and/or rapid preparation, maximum product acceptance (mass market or with minimum constraints due to cultural or age differences), technical feasibility and safety.

2.3. Search for similar products in the market and prototype formulation process

The existence in the market of similar fish products to the ideas described in WP28 is an important aspect to consider. These products can provide interesting information about how to formulate new products with the selected fish species and at the same time they can guarantee their familiarity and acceptance by the consumers. In this regard, various retail markets were visited to seek similar products and the Mintel GNPD database (http://www.mintel.com/global-new-products-database) was used as well to search for new fish products in Europe. The search was limited to the last 3 years and keywords such as fish, salad, fillet and burger were used to find similar products to those given in WP28.

Marketplace visits and searches in Mintel database and Internet provided altogether valuable information about different presentations, formulations and materials to be used in the prototype generation. However, in all cases it was necessary to conduct an iterative process of product formulation for new products development. As for that, different prototypes were elaborated and assessed by means of an evaluating sheet (See **Fig. 1**) in which the organoleptic characteristics of the resulting products were emphasized. With this information, new formulations and processes were examined in order to improve the quality of the product.

EVALUATION SHEET

IDEA 1: Frozen fish fillets with different recipes (recipe number 1)

APPEARENCE (Scale from 0 to 10, where 10 is the highest score)	
SUGGESTIONS FOR IMPROVEMENT:	
AROMA (Scale from 0 to 10, where 10 is the highest score)	
SUGGESTIONS FOR IMPROVEMENT:	
FLAVOUR (Scale from 0 to 10, where 10 is the highest score)	
SUGGESTIONS FOR IMPROVEMENT:	
TEXTURE (Scale from 0 to 10, where 10 is the highest score)	
SUGGESTIONS FOR IMPROVEMENT:	

OVERAL ACCEPTABILITY (Scale from 0 to 10, where 10 is the highest score)	

GENERAL REMARKS:

Figure 1. Evaluation sheet for the different fish products. New product idea 1 is shown as an example.

2.4. Determination of physicochemical properties and nutrition facts of prototypes

Physical prototypes were ground to obtain sample homogenates. The pH was determined using a conventional pH-meter on 10 g of ground sample in 100 mL of distilled water. The water activity (a_w) was determined in the homogenate by means of a water activity meter (AquaLab). The proximate composition (protein, fat, moisture and ash) of physical prototypes was determined as described in the AOAC Official Methods (AOAC, 2005). Briefly, moisture was determined gravimetrically after drying the samples in an oven at 103 °C ± 2 °C. Ash content was measured gravimetrically after drying and incinerating the samples in a muffle furnace at 550 °C to obtain white ashes. The protein content was determined based on the Dumas combustion method (N content x 6.25). The fat content was measured after acid hydrolysis with HCl in a boiling bath. The residue was filtrated and dried and subsequently the lipid content was extracted with ether by means of a Soxhlet extractor. The fatty acids were extracted and transformed into fatty acid methyl esters, which were injected into Gas Chromatograph for subsequent separation and further detection by means of a flame ionization detector. Carbohydrates were calculated by difference whereas the sugar content was determined after samples clarification with Carrez reagents. Identification and quantification was performed by means of High Performance Liquid Chromatograph equipped with a refractive index detector. The salt content (Na content x 2.5) was determined by means of an Atomic Absorption Spectroscopy in sample extracts obtained after mineralization with nitric acid and hydrogen peroxide in a microwave digester. Calories were calculated using the conversion factors for each nutrient as set out in the Regulation (EU) No 1169/2011 (European Commission).

2.5. Microbiological quality control

It is important to note that in this report we describe basic requirements and procedures for the elaboration of the different prototypes. For their production at industrial scale, the application of good hygiene practices (GHP) in combination with HACCP should be consistently applied to control the required microbiological status of raw materials and ingredients to minimize the initial contamination at manufacturing level, and/or to reduce the potential growth of microorganisms. Testing against a food safety criterion after production as we did here should be only used as a method of verifying the production process and thus the correct functioning of the GHP and HACCP–based procedures.

In this deliverable different microbiological analyses were carried out to: 1) determine the initial conditions and requirements of the raw materials, and 2) test the shelf life of the prototypes as described in the following subheading. These analyses include:

- For meagre and pikeperch prototypes, the presence of *Salmonella spp* was investigated by enrichment of 25 g of sample in Buffer peptone water (BPW, Oxoid), previously homogenized at 1/10 ratio for 1 min in a Stomacher (BagMixer, Interscience), followed by incubation at 37±1ºC for 24 h. The presence of *Salmonella* was screened by streaking a loop of the enriched broth on Selective CHROMagar Salmonella plus (Scharlab).

- For greater amberjack and grey mullet prototypes, the presence of *Salmonella spp* was investigated by enrichment of 25 g of sample in 225 ml of Buffer peptone water (Oxoid), previously homogenized at 250 rpm for 30 sec in a Stomacher (VWR). The homogenized sample was enriched with 2.5 ml of Ibisa Specific supplement (ISS). After 24 h of incubation at 41.5±1ºC, the presence of *Salmonella* was screened by streaking 10 µl of the enriched broth on IBISA selective chromogenic agar (Biomerieux). The plates were incubated at 37±1ºC for 24 h. *Salmonella spp.* was determined based on the ISO protocol 6579 (ISO, 2002).

- For meagre and pikeperch prototypes, the presence of *Listeria monocytogenes* was investigated by selective enrichment of 25 g of sample in 24 Listeria Enrichment Broth (LEB 24, Oxoid), previously homogenized at 1/10 ratio for 1 min in a Stomacher (BagMixer, Interscience), followed by incubation at 37±1ºC for 24 h. The presence of *L. monocytogenes* was screened by streaking a loop of the enriched broth on selective Brilliance™ Listeria Agar (Oxoid, formerly Oxoid Chromogenic Listeria Agar).

- For greater amberjack and grey mullet, the presence of *Listeria monocytogenes* was investigated by enrichment of 25 g of sample in 225 ml of Buffer peptone water (Oxoid), previously homogenized at 250 rpm for 30 seconds in a Stomacher (VWR). After 24h of incubation at 37±1ºC, the presence of *L. monocytogenes* was screened by streaking a 10 µl of the enriched broth on ALOA selective chromogenic agar (Biomerieux). *L. monocytogenes* was determined based on the ISO protocol 16140 (ISO, 2003).

- The presence of *Listeria spp.* was investigated by enrichment of 25 g of sample in 225 ml of Buffer peptone water (Oxoid), previously homogenized at 250 rpm for 30 seconds in a Stomacher (VWR). After 48 h of incubation at 37±1ºC, the presence of *Listeria spp.* was screened by streaking 10 µl of the enriched broth on ALOA selective chromogenic agar (Biomerieux). *Listeria spp*. was determined based on the ISO protocol 16140 (ISO, 2003).

- For greater amberjack and grey mullet, the presence of *Shigella spp.* was investigated by enrichment of 25 g of sample in 225 ml of Buffer peptone water (Oxoid), previously homogenized at 250 rpm for 30 sec in a Stomacher (VWR). After 24h of incubation at 37±1ºC, the presence of *Shigella spp.* was screened by streaking 10 µl of the enriched broth on XLD agar (Biomerieux). *Shigella spp.* was determined based on the ISO protocol 21567 (ISO 2004).

- Enterobacteriaceae counts were measured based on the ISO protocol 16140 (ISO, 2003). Counts of all the reddish colonies in Rebecca agar incubated at 37±1ºC during 24 hours were conducted.

- Mesophilic bacteria counts were determined based on the ISO protocol 4833 (ISO, 2013). Total bacterial counts were conducted in PCA agar incubated at 30±1 ºC during 72 hours.

- For meagre and pikeperch prototypes, psycrophylic bacteria counts were determined as described in (NMKL, 2006). Total bacterial counts were conducted in Long & Hammer agar incubated at 15±1ºC for 5 days.

- For greater amberjack and grey mullet, psycrophylic bacteria count was investigated by enrichment of 25 g of sample in 225 ml Buffer peptone water (Oxoid), previously homogenized at 250 rpm for 30 seconds in a Stomacher (VWR). Total bacterial counts were conducted in Rose Bengali Agar (OXOID) incubated at 15±1ºC for 5 days.

- Lactic acid bacteria counts were determined based on the ISO protocol 15214 (ISO, 1998). Total bacterial counts were conducted in MRS agar, incubated under anaerobic conditions at 30±1ºC during 72 h.

In all cases, the microbiological analyses were performed in 5 different samples. Note that Regulation (EC) No 2073/2005 indicates that food business operators shall ensure that foodstuffs comply with the relevant microbiological criteria and refer to the shelf life studies that they also shall conduct in order to investigate compliance (European Commission). In particular, this criterion applies to ready to eat foods that are able to support the growth of *L. monocytogenes* and that may pose a *L. monocytogenes* risk for public health. In this regard, products with pH $\leq$ 4.4 or $a_w \leq 0.92$ and products with pH $\leq$ 5.0 and $a_w \leq 0.94$ were considered as safe for *L. monocytogenes.* It also indicates the content in histamine as a food safety criterion in fishery products from fish species associated with a high amount of histidine. For this, the histamine content was also measured in frozen fillets by means of:

- A competitive direct ELISA kit (Veratox 9505 and 9506; Neogen Europe, Auchincruive, UK).

With respect to safety of chilled foods that are vacuum and modified atmosphere packaged (VP and MAP, respectively), the Institute of Food Research (IFR) made a number of recommendations (Peck, Goodburn, Betts, & Stringer, 2006). These include: 1) the storage at temperatures below 3 °C, 2) a heat treatment of 90 °C for 10 min or equivalent lethality combined with storage at chilled temperature, 3) a pH ≤ 5.0 throughout the food, combined with storage at chilled temperature, 4) s salt concentration ≥ 3.5% throughout the food, combined with storage at chilled temperature, 5) an $a_w \leq 0.97$ throughout the food, combined with storage at chilled temperature, 6) combinations of heat treatment and other preservative factors which can be shown consistently to prevent growth and toxin production by *Clostridium botulinum*.

Notwithstanding these analyses and recommendations, in order to guarantee the microbiological safety of any product, the use of predictive microbiology and/or microbiological challenge tests assessing a growth potential in the foreseeable conditions that might be expected to occur throughout the shelf life period is recommended.

2.6. Shelf life testing

The identification of relevant pathogens is critical for the successful assessment of a safe shelf life. However, the shelf life of a product is not only determined by microbial factors as other factors such as oxidative and enzymatic reactions, physical defects (e.g. phase separation, crystallization) may greatly contribute to product quality loss. In general, perishable foods undergo microbial or enzymatic spoilage whereas preserved foods undergo chemical or physical degradation reactions. However, this statement is a generalization and, in consequence, it is necessary to identify these changes in every food product to determine the limiting factor and the acceptability limits and, in consequence, its shelf life (Man & Jones, 1994; Nicoli, 2012). An appropriate shelf life assessment should therefore consider the product safety (i.e. microbial analyses) and the maintenance of its desired sensory, chemical, physical, microbiological and functional characteristics until consumption.

The storage for significant periods is required to determine the shelf life of a new product, and includes samples from development stages (Nicoli, 2012). Accordingly, preliminary studies about the shelf life of the different prototypes are presented in this deliverable. Valuable information can be also obtained from the literature. In this regard, it is widely known that frozen products can last for several months (3-18) and, given that the time for developing the prototypes reported in this deliverable is not enough for a proper assessment of the shelf life of frozen products, their shelf life has been estimated based on the literature (Erickson & Hung, 1997). In frozen fish products, lipid oxidation (e.g. TBARS), formation of dimethylamine and protein aggregation are expected to influence the sensory properties depending on a variety of processing and compositional factors such as fish tissue fat content (Aubourg, Rodriguez, & Gallardo, 2005; Hall, 1997; Ozogul & Ucar, 2013; Pereira de Abreu, Losada, Maroto, & Cruz, 2010). Therefore, in addition to sensory analysis it is recommended to examine those mentioned chemical and physical parameters in further studies to set the shelf life for a specific frozen product.

The observed changes (quality loss) during the storage of the prototypes at chilling temperatures, as well as that in other precedent failed prototypes, serves to determine the shelf life of the different products. As stated previously, the growth and effects of spoilage bacteria are more likely to be responsible for the product loss of quality and thus serve for the identification of acceptability limits in chilled foods. However, it is worth to mention that Regulation (EC) No. 2073/2005 sets out the microbiological criteria for *L. monocytogenes* in ready-to-eat foods (European Commission) and the existence of a technical guidance document for conducting shelf life studies on *L. monocytogenes* in such products (EURL, 2014). There are also guides regarding the evaluation of product shelf life for chilled foods (Betts, Brown, & Everis, 2004) and the safe

production of VP and MAP chilled foods with respect to non-proteolytic *Clostridium botulinum* (FSA, 2008; Peck et al., 2006). According to these recommendations we assessed the shelf life of refrigerated products by storing them at 4 °C for one third of the total shelf life of the product. This temperature is set as the maximum that needs to be ensured during manufacture and until arrival to the display cabinet. Therefore, these storage conditions mimic those before the product can be sold. Subsequently, the prototypes were stored at 8 °C for two thirds of the total shelf life to mimic maximum temperature conditions at retail and consumer storage (Betts et al., 2004). However, this temperature needs to be justified by detailed information and ensure the 75th percentile of the observations for the country where the stage of the cold chain is located. Otherwise, it is recommended to use 12 °C instead of 8 °C.

The prototypes reported in this deliverable need to be scaled-up for industrial production and thus the results reported here should be considered as an estimation of the potentially marketed product. In general, variations due to the utilization of different equipment, ingredients, materials, etc. may contribute to bias in products' shelf life and thus needs to be re-assessed when these changes occur. Therefore, the shelf life of foods needs to be assessed in the final product elaborated under the same conditions that it is sold. This deliverable therefore aims to provide suitable information about the potential shelf life of the different selected product ideas defined in precedent deliverables. It is also important to highlight that, based on HACCP principles together with the implementation of good hygiene practice, food business operators are required to ensure the following:

(a) that the supply, handling and processing of raw materials and foodstuffs under their control are carried out in such a way that the process hygiene criteria are met,
(b) that the food safety criteria applicable throughout the shelf life of the products can be met under reasonably foreseeable conditions of distribution, storage and use.

In relation to sensory properties, this was evaluated by trained panellists (ASTM, 1981; ISO, 1993, 1994) with a minimum of 5 years of experience in fish descriptive analysis. Key attributes of appearance, aroma, flavour, and texture for each product were selected and measured by consensus at different time points by means of a 3 point scale defined based on their expertise with similar products ranging from 0 (similar to fresh product) to 2 (unacceptable). Product prototypes showing scores equal to 2 in one of the evaluated attributes were considered to reach the shelf life acceptability limit. Accordingly, a primary characterization of the product has been set for each product and the evaluation of changes in the described attributes over the course of the storage has been also considered for their shelf life assessment.

The products used to elaborate the prototypes in this deliverable are farmed in aquaculture conditions. An important aspect that has been investigated since quite some years in fish production is the influence of the harvesting method on the quality of the fish fillet. Pre-slaughtering and slaughtering practices used during fish production have a direct impact on fish stress. Quality changes start with changes in blood parameters such as decrease of glucose, increase of cortisol, increase of lactic acid, decrease of energy levels, (ATP mainly) and increase of HUFA oxidation (Borderias & Sanchez-Alonso, 2011; Rasco, Down, & Ovissipour, 2015). Thus, the different conditions applied during the harvesting and slaughtering of the fish have direct influence on the preservation potential of the fish fillet, shelf life and its physicochemical properties. Although it is not the objective of this deliverable, this aspect of fish production should be a concern for the producers since it has a direct impact on the product to be offered to the market and on the response of the consumer to that product. Aquaculture is a growing industry and it has already started looking in the direction to reduce stress on harvested fish and there are already (few) laws and regulations in order to increase fish welfare during harvesting and slaughtering procedures. Maintaining good husbandry conditions to alleviate stress is not only an ethical practice, but a good business practice as well.

3. Results

The selection of ideas for the physical prototypes reported in this document was made considering the different fish species and their technical compatibility. Accordingly, the ideas 1, 6 and 4 were selected for meagre; the ideas 21, 30 and 9 were selected for pikeperch; the ideas 2, 33 and 21 for grey mullet, and the ideas 13, 30 and 34 for greater amberjack.

3.1. Idea 1: Frozen fish fillets with different recipes

Description of the product concept:

Frozen fish fillets divided in double portions; each packaging includes three or four 2-person portions from the same or different fish species packaged separately. The product is environmentally sustainable (containing ASC label). It is labelled as a premium product; the country of origin is EU. The product is included in transparent vacuum packed bags (one for each 2-persons' portion) made of recyclable material where fish fillets are laid; each bag can be divided easily from the other; each 2-portion bag has a different recipe from the others within the same package; a picture of the prepared dish is included on each 2-portion bag. It is created, based on the frozen fish fillet (D28.1) by removing seasoning or marinating but adding different fillets and recipes to create added value. The aim is to make it more attractive to traditional consumers that like to be involved in cooking and to allow a longer shelf life.

Reasons for its selection and existence of similar products in the market:

This fish product is an additional concept generated within the D28.2 and occupies the 10th position in the ranking of product concepts (D28.2; score 102.1500). Meagre occupies the first position in the list of technical compatibility in the group of ideas involving the development of frozen fish fillet products without further processing (ideas 1, 13, 18, 19 and 25). This is mainly due to the low fat content of this product which makes it appropriate for long term frozen storage (D28.3) (Monfort, 2010).

This is a well-defined and detailed idea; however, there are other similar products in the European market that can serve as references of this concept. Some of them are described as follows:

Description: Deutschesee Saiblings-Filets (Char Fillets) are now available. The ASC-certified product retails in a 220 g pack. Germany. Frozen product.

Description: Atlantic Filetti di Merluzzo Nordico (Nordic Cod Fillets) have been peeled and glazed. This product can be cooked in a pot in eight minutes or in a pan in six to eight minutes and retails in a 375 g pack, containing three separated units and featuring MSC logo for sustainable fishing. Italy. Frozen product.

Description: Fiskemannen Laksefilet (Salmon Fillets) has been repackaged in a redesigned XL-sized 560 g pack containing 4 x 140 g portions and bearing the Key Hole and the Gront Punkt logos. The product is described as simple and tasty and can be prepared from frozen by cooking in slightly salted water, or grilled, fried in the oven or on the frying pan. The salmon is Norwegian-produced and comes without skin and bones, containing a high amount of omega-3 fatty acids. Norway. Frozen product.

Description: Coop Laksefilet (Salmon Fillet) is natural and without skin. The fillets are high in omega-3 fatty acids and suitable for frying, boiling, cooking in the oven or in the microwave. This product retails in a 250 g pack, containing two 125 g units and featuring the Green Keyhole logo. Denmark. Frozen product.

Description: Deutsche See Fischmanufaktur Bio Lachs-Filets (Organic Salmon Fillets) are juicy and tender with a fine balanced taste. The product, from ecological aquaculture in the Fjords of Norway, retails in a 300 g pack with two boneless fillets without skin, bearing the EU Green Leaf logo. Germany. Frozen product.

Description: Fregat Kuller Filet (Haddock Fillets) are line caught in the Faroe Islands. This skinned and deboned fish is a lean, low in fat and rich in selenium, and contains no additives. The product is said to be easy and straightforward to prepare, and retails in a 250 g pack featuring the Green Keyhole logo. Denmark. Frozen.

Description: Findus Torskrygg Ryggfiléer (Cod Loin Fillets) has been repackaged and now comes with a new design. The product is said to be the best part of the fish and described as the beef fillet of the ocean. It is caught in the northern ice sea between the Norwegian Sea and the Barents Sea, and has juicy meat with a clean, mellow flavour. The fillets retail in a 450 g recyclable pack, containing three units and featuring the Green Keyhole and MSC logos and a Facebook link. Sweden. Frozen product.

Description: Findus Dos de Cabillaud (Cod Fillets) has been repackaged. The fish for this product have been sourced from the Northern Pacific Ocean respecting the marine resources. The fillets have been selected for their firm meat and delicate taste. This microwaveable product retails in a newly designed 440 g pack with four 110 g individually wrapped units. France. Frozen product.

Overall, there are various fish products in the EU market that are similar to idea 1. These products usually contain salmon and cod fillets although other fish species are also sold. Skin and vacuum packaging seem to be widely used for this concept idea.

Technical approach for the final prototype production:

The elaboration of this product is relatively simple, therefore it is recommended to freeze meagre fillets under conventional processing conditions (e.g. air-blast freezers, plate freezers) and then vacuum package (Hall, 1997) as vacuum-skin packaging technology (or simply referred as skin packaging hereafter) can be more limiting. Glazing is an alternative but it is not necessary in vacuum products. In some of these products

the skin has been removed; however, the presence of skin in meagre fillets is not considered to be a major issue for its purchasing motivations.

Ingredients:

Meagre fillets with skin (1200 g) in 3 separated units (each containing 2 fillets) each averaging 400 g. However, it is possible that the proposed 2-portions fillet weight results relatively high in comparison to other marketed products. In that case, it may be therefore reasonable to reduce the 2 fillets weight to 300 g or less in order to satisfy consumer demands.

Manufacturing information:

The product can be stored on ice until processing. Fish are de-scaled, headed and eviscerated as soon as possible at refrigeration temperatures. The resulting fish fillets are immediately frozen by using a conventional freezer (e.g. air-blast, plate, fluidized bed, liquid nitrogen) to reach an internal temperature of -20 °C, vacuum packaged and stored at this temperature in a cold storage room.

Product packaging and retail market prototype:

Packaging system:	vacuum
Packaging equipment:	vacuum packaging machine, EV-13 Tecnotrip (Barcelona, Spain).
Design:	bag divided by thermo-sealing in three sections of 200 mm that contain 2 fish portions each.
Packaging Material:	90 µm polyamide/polyethylene COMBIVAC bag (Niederwieser; Modena, Italy)
Size:	400 mm x 800 mm
Recyclable:	yes

By following these instructions the prototype should be as shown in **Fig 2**.

Figure 2. Physical prototype of the "*frozen meagre fillets with different recipes*" product concept.

Physicochemical properties:

Moisture (%): 76.6; water activity: 0.992; pH: 6.42

Nutrition facts:

Expressed in 100 g of product:

Energy: 96 Kcal or 407 KJ

Protein (g): 20.67

Lipids (g): 1.49 of which saturated (g): 0.4

Carbohydrates (g): 0.02 of which sugars (g): not detected

Salt (mg): 104.5

Potential allergens:

Fish is the only ingredient.

Storage conditions:

Frozen storage at -20 °C ± 2.

Microbiological and sensory shelf life assessment:

The evaluation of the shelf life takes into account different quality aspects. In fishery products, the content in histamine is quite often determined because it is not only an indicator of spoilage and microbial growth but also a foodborne chemical intoxication. The histamine levels were 8 ± 0.6 mg/kg and thus quite below to those reported in the EU regulation 2073/2005 (European Commission). Meagre fillets from previous studies were vacuum packaged in aluminium bags and frozen for 17 months and no detrimental effects were observed (personal data). Moreover, the shelf life of this product has to be similar to that of other frozen lean fish (Man & Jones, 1994). Therefore, this is expected to be 9 months or higher as meagre fish has a low fat content and it is vacuum-packed (Giogios, Grigorakis, & Kalogeropoulos, 2013; Rodriguez, Cruz, Paseiro-Losada, & Aubourg, 2012; Rodriguez et al., 2015).

Consumer handling/cooking specifications:

The fish is recommended to be thawed in the refrigerator before its use. When finished it can be cooked following the suggested recipes that are showed on the same package.

Different recipes were selected from kitchen books, Internet and own recipes (**Table 2**). This selection was done taking into account that they are intended to an international mass market. Some additional criteria were the number and common use of ingredients, simplicity of preparation and fast cooking, and healthiness.

Table 2. Description of the assessed recipes used to prepare fish meals from frozen meagre fillets

Recipe and Ingredients	**Preparation**
"Fish fillet citrus sauce": 1 fish fillet 1 orange 1 tangerine 3 lemons 100 ml of white wine 60 g of sugar 20 ml of edible (olive) oil 1 pinch of salt 1 pinch of pepper	For 1 person: Add the tangerine juice, orange, lemon and white wine in a saucepan. Remove the peel of these fruits without the white part with a knife and add them to the saucepan. Add sugar, stir and cook for 25 minutes. It is cooked when the sauce is thick. You can add salt and pepper if necessary. Season (salt and pepper) the fillets and cook in a skillet with oil for 3 minutes per side, until golden brown. Serve fish fillets with the citrus sauce, previously strained, on top or at a side.
"Baked fish": 2 meagre fillets 1 white onion 1 tomato 1 yellow pepper 2 potatoes 1 teaspoon mayonnaise or garlic dressing 1 pinch salt and pepper 1 pinch of thyme	For 2 people: Wash and peel the potatoes and cut into thin slices and place them in a baking dish. Place the fish fillet and sliced peppers over potatoes. Then place a layer of tomatoes and season with salt and pepper, mayonnaise (or lemon garlic dressing) and thyme to taste. Bake the meagre fillet with vegetables for 45 minutes at 170^{o} C and serve.
"Fish with tomato (microwave)": Fish fillets 500 g 1/2 onion, chopped 6 tablespoons tomato sauce 4 tablespoons olive oil 1 cup white wine 2 cloves garlic, minced 1 pinch of chopped parsley 1 pinch of white sugar 1 pinch of salt	For four people: Place in a microwave-safe bowl: oil, onion, garlic, salt and sugar. Cover the bowl and cook on high power for 3 minutes. Incorporate and stir the tomato sauce, add the white wine and frozen fish fillets. Fish fillets have to be covered by the sauce. Place the fish well-covered again in the oven on high power for 7 minutes. After 7 minutes, carefully place the fish pieces that were in the outer sides to the centre and move the centre to the edges, so that they cook evenly. Cover fish fillets with tomato sauce and cover again. Cook on high power for 5 minutes. Let stand a few minutes, covered, and finally sprinkle the chopped parsley.
"Fish fillet in green sauce": 4 slices of fish fillets 250 g of clams	For 1 person: Clean the fish and clams. Heat the fish broth until warm and cook the eggs. Chop the garlic finely and fry over medium heat in a skillet for a few minutes.

150 ml fish broth 60 ml white wine 4 garlic cloves 100 g chopped parsley 30 g of flour 2 eggs oil salt	Add the flour and wine, then broth and stir until blended. Add the parsley and place the slices previously salted fish fillet. Leave a few minutes shaking the pan slightly so that the sauce has more consistency. Turn over the fish fillets and add the clams, when opened add chopped boiled eggs.
"Fish fillet with yogurt sauce and mushrooms": 2 fish fillets 1 plain yogurt 100 ml cream 200 g mushroom 1 onion 1 clove garlic edible (olive) oil parsley salt	For two people: Wash the mushrooms and cut them into slices. Finely chop onion and garlic (you can also add and remove entire later). Add a little olive oil in skillet over medium heat, when hot add the onion and garlic and fry until onion is transparent. Add the sliced mushrooms, leave a few minutes until they lose water and add cream and yogurt. Add a pinch of salt, mix well and let cook for about 5 minutes. Remove and reserve the sauce. Put a little oil in a separate pan and put over medium heat. When hot, put the fish fillets, leave about five minutes, turning halfway, to be made on both sides. Serve the fillets with the sauce. This dish may be accompanied by roasted potatoes or a salad.
"Baked fish with tomato sauce": 1 fillet 1 potato 2 tablespoons tomato sauce 1 tablespoon oil 1 teaspoon chopped parsley Salt	For one person: Preheat oven to 180 °C. Put water to boil in a saucepan with a pinch of salt. Peel the potatoes and cut in half, boil for 15 min approx. (depending on size). Drain and cut into thin slices. Spread a casserole (or other oven-resistant material) with olive oil and place the potatoes in the bottom forming a base. Place the fish fillets on top and sprinkle with tomato sauce and a tablespoon of olive oil, and finally sprinkle over some chopped parsley. Bake at 180 °C for about fifteen minutes.
"Fish fillet with orange": 8 fish fillets 4 oranges 125 g of butter or margarine 75 g of chopped almonds 5 cherry tomatoes Salt	For 6-7 people: Season the fish fillets with salt and pepper and then turn them in wheat flour. Fry the fish fillets on both sides until they are golden brown. In a saucepan, pour water and add the grated peel of oranges, butter or margarine and chopped almonds. Bring to medium-high heat and stir occasionally. Before coming to the boil, take it out of the heat and add it over the fish

pepper olive oil flour	fillets. Add tomatoes and bake it at 200 °C for about 15 minutes to broil.
"Fish fillet with garlic and parsley": fish fillet 3 cloves garlic A sprig of parsley ½ cup extra virgin olive oil	For one person: Mix the garlic, parsley and oil in blender and put aside. Place fish fillets in the pan, skin side up, over high heat. Add a splash of dressing and leave three minutes, turning and letting it cook another three minutes on the other side.
"Fish fillet with vegetables": 1 fillet 100 g fresh green beans 1 large onion 2 ripe tomatoes 4 tablespoons cheese mixture to grill 3 tablespoons extra virgin olive oil salt pepper	For one person: Season the fish and keep it for later. Wash the beans and cut into thin strips lengthwise. Blanch in boiling salted water for 3 minutes. This will get the beans become crispy. Peel and cut the onion into thin strips and cover the bottom of a baking dish with 1 tablespoon oil. Drain the beans and place them on the fish. Wash and cut the tomatoes into thin slices. Place the tomatoes and sprinkle with the remaining oil. Cover with cheese and bring to a preheated oven at 200 °C for about 25 minutes.
"Three cheeses fish fillets": 4 fillets of 150 g each, approximately 250 g of goat cheese 250 g of manchego type cheese (or hard ripened) 250 g of creamy cheese 10 g of corn-starch 10 g of butter 1/2 l of fish broth (or water) 30 ml of milk steamed vegetables salt pepper	For four people: Season the fillets and cook until browned on both sides. Mix the three cheeses in a blender with a little milk. In a saucepan melt the butter and add the flour. When it turns to golden brown, add the fish broth or water, which already began to boil, add our cheese sauce and mix very well. Season to taste and let it warm but avoid boiling because it will make cream. Serve over freshly cooked fish and accompanied by steamed vegetables.

These recipes were sensory evaluated by at least 3 experienced panel members using the score sheets described in the material and methods section (**Fig. 1**). The overall scores for the different recipes were as

follows: "*Fish fillet citrus sauce"*: 8.0; "*Baked fish"*: 7.0; "*Fish with tomato (microwave)":* 7.5*; "Fish fillet in green sauce":* 8.0*; "Fish fillet with orange":* 8.0*; "Fish fillet with garlic and parsley":* 8.5*; "Fish fillet with vegetables":* 8.5*; "Three cheeses fish fillets":* 8.0. Overall, the recipes that obtained the top 5 highest scores were selected and reformulated for improvement according to panel comments. However, the following considerations were made: Firstly, the "*Fish fillet with garlic and parsley"* recipe may be appropriate for the healthy seasoning. The intensity of this seasoning can be used to mask undesirable flavours and consumers can use it depending on their preferences. Secondly, the "*Fish fillet citrus sauce"* recipe was considered to be sour in excess for a mass-marketed suggested recipe. Therefore, the finally selected recipes were as follows: "*Three cheeses fish fillets", "fish fillet with yogurt sauce and mushrooms", "fish fillet with vegetables", "baked fish with tomato sauce"* and *"fish fillet with orange"*.

Suggested final presentation:

Frozen meagre fish was cooked following the suggested recipes as shown in **Fig. 3-7**.

Figure 3. Suggested presentation of the prototype after following the "*Three cheeses fish fillets*" recipe

Figure 4. Suggested presentation of the prototype after following the "*Fish fillet with yogurt sauce and mushrooms"* recipe

Figure 5. Suggested presentation of the prototype after following the "*Fish fillet with vegetables"* recipe

Figure 6. Suggested presentation of the prototype after following the "*Baked fish with tomato sauce"* recipe

Figure 7. Suggested presentation of the prototype after following the "*Fish fillet with orange"* recipe

3.2. Idea 2: Thin smoked fillets

Description of the product concept:

Fresh thin smoked fillets from the same (or different) fish species, which can be used as a starter or incorporated within a sandwich/salad. The product is sustainably produced (containing ASC label). It is labelled as a premium product; the country of origin is EU. The packaging is a plastic tray where the fillets are laid covered with a transparent plastic, which allows visibility of the fillets and VP or MAP is used for shelf life prolongation. Ideas concerning the different uses of the fillets are included on the product's sleeve. This idea tries to assimilate classic smoked fillet products with the need of consumer for convenience.

Reasons for its selection and existence of similar products in the market:

This fish product is an additional concept generated within the D28.2 and occupies the 10th position as well as idea 1 as they obtained the same score (D28.2; score 102.1500). Grey mullet occupies the first position in the list of technical compatibility mainly because of its high fat content.

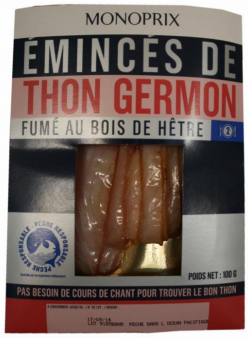
In the European market we can find the following similar presentations of smoked fish products:

Description: Golden Seafood Gerookte Wilde Zalm (Smoked Wild Salmon) has been relaunched with a new brand, previously known as Prestige. The product is wild caught in Alaska and retails in a 150 g pack bearing the MSC logo. The Netherlands. Stored under refrigeration.

Ingredients: Sockeye salmon (contains *Oncorhynchus nerka*) (contains fish), salt, sugar, smoke

Description: Lerøy Skivad Kallrökt Lax (Sliced Cold Smoked Salmon) is now available in a new smart packaging. This product is farmed, prepared and packaged in Leroy's facilities. It contains omega-3, features the Green Keyhole logo and retails in a 180 g pack. Sweden. Stored under refrigeration.

Ingredients: Salmon (*Salmo salar*) from aquaculture, salt



Description: Monoprix Emincés de Thon Germon (Smoked Albacore Tuna Slices) are now available. This product has been smoked with beech wood and retails in a 100 g pack providing two portions. The manufacturer respects sustainable fishing standards. France. Stored under refrigeration.

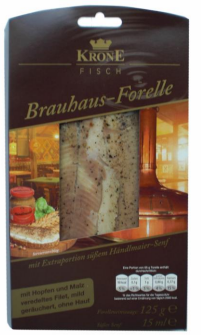
Ingredients: Albacore tuna (97%) (*Thunnus alalunga*), salt

Description: Marque Repère Ronde Des Mers Emincés Haddock églefin Fumé (Smoked Haddock Slices) have been repackaged in a newly designed 100 g recyclable pack that contains between six and 12 slices. The product is coloured with annatto natural pigments, beech wood smoked, and is described to be soft and ideal in potato salads. France. Stored under refrigeration.

Ingredients: Haddock (*Melanogrammus aeglefinus*) (97%), salt, colouring (annatto)

Description: Lerøy Najadlaks (Smoked & Cured Salmon) has been sliced for buffets. The Norwegian salmon retails in a 175 g pack featuring a Facebook link. Norway. Stored under refrigeration.

Ingredients: Salmon from aquaculture, salt, sugar, white pepper, dill



Description: Krone Fisch Brauhaus Forellen-Filets (Brewery Trout Fillets) are now available. The lightly smoked product comes without the skin and is refined with pepper, hops and malt, and has an additional sweet mustard sauce. It is filleted by hand and retails in a pack containing 125 g fish and 15 ml mustard sauce. Germany. Stored under refrigeration.

Ingredients: Rainbow trout (*Oncorhynchus mykiss*), table salt, black pepper, white pepper, red pepper, barley malt extract, hops extract, wood smoke

Description: Levevis Økologisk Varmrøget Ørredfilet (Organic Smoked Trout Fillets) is now available. The product is sourced from organic aquacultures in Denmark and retails in 100 g pack featuring the Green Keyhole logo. Denmark. Stored under refrigeration.

Ingredients: Skinless trout (97%), salt

Description: Aquafood Pesce Spada al Naturale per Carpacci (Swordfish Carpaccio) has been processed in a way that is natural and preservative free and obtained by selecting the best swordfish caught in the waters of the Pacific. The delicious non-smoked fish is said to be ideal for light meals and retails in a 100 g pack. Italy. Stored under refrigeration.

Ingredients: Swordfish (*Xiphias gladius*), salt, brown sugar

Description: Anfele Anchoa Ahumada (Smoked Anchovy) is now available. The product retails in an 80 g pack featuring a QR code. Spain. Stored under refrigeration.

Ingredients: Anchovies (*Engraulis encrasicolus, Engraulis anchoita*), salt, natural smoke

Description: Ricardo Fuentes e Hijos Salazones El Auténtico Tapeo Mediterráneo Atún Ahumado (Smoked Tuna) is part of a new range that features authentic Mediterranean flavours. This sea-caught product represents a quick way to enjoy Mediterranean flavours, and retails in a 0.050 kg pack. Spain. Stored under refrigeration.

Ingredients: Tuna (*Thunnus albacares*), salt, natural smoke, vegetable extracts (acerola, lettuce, chard, spinach, carrot), natural spice extracts (rosemary, oregano), dextrose

Overall, the majority of these products are made of salmon; however, there are other retailed fish species as reported above. In these products we can find the two packaging options, VP and under MAP. Therefore, both options can be suitable for the smoked grey mullet product.

Technical approach for the final prototype production:

Grey mullet is commonly sold as a whole in a range of size from 300 g up to 2 kg. The bigger specimens are used to elaborate the product known as “bottarga”, which is the roe salted and dried. Grey mullet fillet flesh has a pinkish coloration and variable lipid content (up to 12.6 %) (El-sebaiy, Metwalli, & Khalil, 1987),

which makes it a suitable product for smoking. Smoking is one of the most traditional, almost ancient method to preserve fish. Most probably it dates back to the times after the possibility of cooking over a fire was discovered (Sampels, 2015a).

Smoking is not only used as preservation method but is also a way to create new products with special organoleptic characteristics. Most of the smoked products present in the markets are from salmon; however other fish species are also used such as herring, eels, trout, mackerel and gilthead seabream (Arvanitoyannis & Kotsanopoulos, 2012).

The compounds present in smoke are very diverse, i.e. organic acids, alcohols, hydrocarbons and phenols. These compounds are produced during the pyrolysis of the wood compounds and are responsible for the preserving and antimicrobial effect of the smoke (Hall, 2011). The phenolic compounds of the smoke have antioxidative effects due to their ring structure with conjugated double bonds, which are able to build stable radicals. Smoking has also a drying effect and increases the inhibition of bacterial growth. The dried surface of smoked fish is a barrier against microbes. Moreover, smoking includes salting and drying processes, which add to the preserving effect and the sensory attributes as well.

Different types of wood can be used for smoking (maple, oak, alder, hickory and fruitwoods). The type of wood and the smoking technique modulate the typical taste of the final product. Two very different techniques are used for smoking: cold and hot smoking. During cold smoking temperature does not exceed 30-35ºC so cold smoked products are not sufficiently protected against microorganisms and would need further cooking before consumption (Sampels, 2015a).

Hot smoking is done using ovens, traditional or high-tech equipment with different degree of automation. Although other methods are in use, heat is usually produced by electricity. Nowadays wood beams, sawdust or wood chips are used as raw material instead of whole wood (used for very traditional smoking in northern countries). Burning the whole wood generates more heat and less smoke compared to sawdust, which burns slowly and at lower temperatures, creating more flavours.

Martinez et al. (2012), have evaluated an alternative to traditional smoking. They found that dry salting with addition of sugar before immersion in a liquid smoke flavouring resulted in a product with lower oxidation, lower hardness and elasticity values and therefore a higher quality product compared to liquid smoked fillets that were only brine salted without sugar. Hot smoking results in ready-to-eat products and longer shelf life compared to cold smoking.

For the preparation of this prototype for Idea 2: thin smoked grey mullet fillets skin-on have been used. Hot smoking with dry salting and addition of sugar was the procedure followed for the preparation of the product.

An important constraint for the production of all the prototypes based on grey mullet has been to find the right amount to complete the elaboration of the three grey mullet products. For the preparation of smoked grey mullet not enough products could be elaborated to complete the nutritional analysis. Microbiological and sensorial analyses are completed.

Ingredients:

Salting mixture: prepare a mixture of pure sea salt and sugar in a proportion of 3:1.

Raw smoking material: pure oak chips.

Fish fillet: pin-boned portions of grey mullet fillet skin on.

Manufacturing information:

Fish fillets have been smoked using hot smoking method. An electrical oven has been used with oak chips as raw material for smoke production.

Fish fillets can be stored on ice until processing. Fish are washed to remove slime, de-scaled, gutted and fileted. It is very important to clean well the belly cavity to remove any traces of blood and any black belly-wall lining. Fillets are then trimmed. After a careful rinsing, the fish fillets are further prepared for the smoking process by carefully removing the remaining spines in the fillet and obtaining a well pin-boned portion. At this moment, the fillet should be cut in portion sizes.

Instead of a brining, a drying salting mixture has been used. Previous work of the partner with this species has proven that the use of a drying salting mixture instead of brining provides better results in terms of flavor and appearance of the final product.

The drying salting mixture is prepared with pure sea salt and sugar in a proportion of 3:1. A kitchen tray bottom is covered with a layer of 2 cm of the salting mixture. The fish fillets are placed on this layer and covered with another layer of 2-3 cm of the salting mixture. Fish fillets should remain in the salting tray for 2.5 h. After this time, the fillets should be rinsed thoroughly under tap water to remove any trace of the salting mixture. Once the excess of water has been removed, the fish fillets are ready for smoking.

The smoking is done at 60ºC for 40 minutes. Smoked fillets are left to cool down at room temperature. Once the smoked fillets portions are cooled down, they are vacuum packed per three, two or individual portions (**Fig. 8**).

Figure 8. Prototype of smoked grey mullet fillets, vacuum packed ready for consumption.

Grey mullet fillets with skin on can be used in 1 package of 3 units (660 g) or 3 packages of 1 unit. Average weight per unit was 210 g, which is considered adequate for one person meal. The 3 units per package can be reasonable for a two people meal or even a small family meal in which fish is not the main course.

Product packaging and retail market prototype:

Packaging system:	vacuum
Packaging equipment:	vacuum packaging machine, EDESA VAC-40DT
Design:	vacuum bag sealed by thermo sealing, including the desired number of portions.
Packaging material:	90 µm polyamide/polyethylene bag (Orved, Italy)
Size:	30 mm x 20 mm
Recyclable:	yes

Physicochemical properties:

With respect to fish:

Moisture = 80.11%; pH = 5.90

Potential allergens:

Fish is the only ingredient

Storage conditions:

Refrigerated (2-4ºC)

Microbiological and sensory shelf life assessment:

For the shelf life assessment the product was stored at 4ºC. **Table 3** shows the microbial stability of the smoked grey mullet fillets throughout the storage period.

Table 3. Shelf life assessment of the smoked grey mullet fillets: microbial counts over the shelf life assessment period[1].

	Day 1 Log (CFU/g)	Day 21 Log (CFU/g)	Day 31 Log (CFU/g)
Mesophilic bacteria	<1.00*	<1.00*	3,62
Enterobacteriaceae	<1.00*	<1.00*	<1.00*
E.coli	<1.00*	<1.00*	<1.00*
Psycrophilic bacteria	<1.00*	<1.00*	<1.00*
Salmonella	A	A	A
Listeria monocytogenes	A	A	A
Listeria spp.	A	A	A
Shigella	A	A	A

[1]Samples were stored at 4ºC during 31 days. Results are averages of 3 replicate samples. The presence of microorganisms is determined in 25 g of sample. Letter "A" stands for absence and the asterisk indicates that is below the limit of detection. For more information see material and methods section.

The microorganisms *Salmonella, Listeria spp*., *Listeria monocytogenes* and *Shigella* were not detected in any sample tested. The mesophilic bacteria had significantly higher counts at day 31 compared to the beginning of the shelf life assessment. In consequence, the limit of this product is less than 31 days of storage.

With respect to sensory attributes, changes in the quality parameters of the smoked grey mullet fillets were evaluated during the same storage sampling points (day 1, day 21 and day 31) as during the microbial assessment. **Table 4** shows the different attributes that are expected to decay in this product according to the literature (Betts et al., 2004; Man & Jones, 1994; Robertson, 2009). According to the results, 31 days can be considered as the limit of acceptability for this product.

Table 4. Sensory evaluation of grey mullet fillet attributes which may change during storage[1]

Quality parameter	Attributes and score	Day 1	Day 21	Day 31
	0: totally homogeneous	0	1	**1**
Appearance	1: appearance of some grey/oxidized areas			
	2: totally heterogeneous			
	0: fresh	0	1	**2**
Odour	1: mold			
	2: rotten taint			
	0: fresh	0	1	**1**
Flavour	1: mold			
	2: rotten taint			
	0: firm and not slimy	2	2	**2**
Texture	1: a little soft and slimy			
	2: very soft and slimy			

[1]Samples were stored at 4ºC during 31 days. Results are averages of 3 replicate samples. The attributes and scores reached at the end of the storage period are highlighted in bold.

Consumer handling/cooking specifications:

Smoked fish fillets are recommended to be kept in the refrigerator until consumption.

The product can be consumed directly on a toast or as part of a salad or even as main course of a meal served with smashed potatoes or boiled rice. Grey mullet is a fish species that is known only in limited areas of Europe. In the areas where pond aquaculture is a common practice, the species is well recognized by consumers and it is included in the local restaurants of the regions. Nowadays there is a new generation of chefs trying to implement the consumption of grey mullet due to the association of this species with pond culture in areas with high natural value and in some cases, using culture practices in accordance with respect to the surrounding ecosystem. This is the case of the farm Veta La Palma (Pimsa), located at the limits of the Natural Park of Doñana, South of Spain. Grey mullet benefits from the high productivity and special features of this ecosystem.

Suggested final presentation:

Smoked grey mullet can be included in a salad preparation or stand alone in a meal preparation. As an example, a recipe awarded during a cooking contest in the South of Spain, where grey mullet is well known as a fish meal preparation, is shown in **Fig. 9**. The preparation is smoked grey mullet with Jimena's mushrooms (cook León Griffioen).

Figure 9. Presentation of smoked grey mullet with Jimena's mushrooms.

3.3. Idea 4: Ready to eat meal: salad with fish

Description of the product concept:

Fresh ready to eat salad that includes fish as well as an accompanying sauce; fish and sauce are separately packed and included within the original package. The fish included is either a smoked fillet (provided in slices), or vinegar-cooked, or alternatively bottarga; thus, the dish can be eaten cold. The product is produced in an environmentally sustainable way (containing ASC label). It is labelled as a premium product; the country of origin is EU. The packaging (MAP) is composed by a bowl where the salad is placed; the fish pieces and the sauce are provided in separate transparent accompanying packages incorporated with the original bowl package. A transparent lid exists on the top to allow product visibility and the packaging has the picture of the ready meal on it. Ready to eat meals are generated in an attempt of improving / changing idea 8 from D28.1 by creating other ready to eat meals utilizing fish without having the weaknesses pointed out by the experts in the aforementioned deliverable.

Reasons for its selection and existence of similar products in the market:

This idea of fish product is an additional concept generated within the D28.2 and occupies the 15th position in the ranking of product concepts (D28.2; score 100.6667). Grey mullet, meagre, amberjack, wreckfish, halibut and pikeperch have been reported to be of technical compatibility with ready to eat ideas (ideas 3, 4, 5, 26, 41).

Meagre was found to have appropriate sensory attributes (D28.3) which makes it an interesting candidate for the salad. In fact, meagre fish is used in the elaboration of dishes such as Peruvian Ceviche in which this is marinated in acid (lemon juice) and served at room temperature. In this regard, a vinegar-cooked presentation can be a suitable alternative whereas its low fat content suggests that is not suited for a smoking process.

There are different products in the European market that can be used as a reference for this idea:

Description: Galeria Gourmet Salat Thunfisch (Tuna Fish Salad) has been added to the range. The product consists of a blend of lettuces and vegetables topped with tuna fish. It retails in a 340 g pack. Germany. Stored under refrigeration.

Ingredients: salad and vegetable mix in varying proportions (53%) (endive lettuce, carrots, radicchio lettuce), tuna fish (15%) (tuna fish, water), peppers, cooked and peeled eggs (cooked eggs, water, spirit vinegar, salt), tomatoes, cucumber, marinated pepperoni (pepperoni, table salt, colouring (E101), sweetener (E954)), lamb's lettuce, black olives (olives, water, salt, stabilizer (E579)).

Description: Galeria Gourmet Mischsalat mit Thunfisch, Ei & Joghurtdressing (Salad Mix with Tuna, Egg & Yogurt Dressing) has been relaunched in a new pack and now also features a yogurt dressing. The product comes with tomatoes and retails in a 215 g pack, which includes a fork. Germany. Stored under refrigeration.

Ingredients: yogurt dressing (35%) (mayonnaise (rapeseed oil (80%), egg yolk, water, spirit vinegar, mustard seeds, sugar, table salt, seasoning), skimmed milk yogurt (40%) (contains fat in the dry matter (0.1%)), natural flavour, invert sugar syrup, mustard (water, mustard seeds, spirit vinegar, salt, seasoning), garlic powder, acidifier (E296, E330, E334), preservative (E202)), salad and vegetable mix (32%) (contains lettuce and vegetables in varying proportions (frisee, lamb's lettuce, chicory, beetroot)), tuna fish in sunflower oil (12%) (tuna fish, sunflower oil, salt), cooked and peeled egg (12%) (cooked egg, water, spirit vinegar, salt), tomatoes (9%).

Description: Havita Ready To Eat Frischer Salat Nizza Kidneybohnen Thunfisch (Fresh Nice Salad with Kidney Beans & Tuna) comes with French dressing. The product retails in a 260 g pack containing 130 g mixed salad, 40 g tuna and 30 g kidney beans in separate compartments, a 60 ml French dressing pouch and a fork. Germany. Stored under refrigeration.

Ingredients: Salad mix (romaine lettuce, red Salanova lettuce), French dressing (23%) (water, rapeseed oil, mustard (6%) (water, mustard seeds, spirit vinegar, table salt, spices, spice extracts), sugar, cream, egg yolk, spirit vinegar, table salt, modified starch, thickener (carob bean gum, guar gum), sodium caseinate, spices, herbs, acidifier (citric acid, tartaric acid, malic acid)), tuna (15%) (tuna, sunflower oil, table salt), kidney beans (11%), cherry tomatoes, onions.

Description: Daunat Tout Simplement! Surimi Pâtes Crudités Sauce Citron Fromage Blanc (Surimi, Pasta & Vegetable Salad with Creamy Lemon Dressing) is now available. The convenient product retails in a 240 g pack that includes a plastic fork. France. Stored under refrigeration.

Ingredients: Mixed salad (88%) (cooked cereal speciality (30%) (water, durum wheat semolina, egg white, salt), lettuce (21%) (iceberg, frisee, treviso), surimi based preparation (14%) (surimi (6%) (fish (5.6%), sugar, stabilisers (sorbitol (wheat), E450, E451)), water, rehydrated egg white powder, starches (wheat), rapeseed oil, flavourings (fish, shellfish), salt, flavour enhancer (E621), colouring (paprika extract)), sweetcorn (12%), carrot (6%), cherry tomato (3%), rapeseed oil), creamy lemon dressing (12%) (water, rapeseed oil, fromage blanc (2.4%), dijon mustard (water, mustard seeds, alcohol vinegar, salt, acidifier (E330)), natural flavouring, sugar, lemon juice concentrate (0.1%), salt, alcohol vinegar, modified corn starch, white pepper, thickener (xanthan gum)).

Description: Argal Ensaladas Mediterráneas Pasta al Dente con Marisco (Pasta Salad with Seafood) has been repackaged. This natural product has been made with olive oil, is free from preservatives, and retails in a 200 g pack containing a fork. Spain. Stored under refrigeration.

Ingredients: Fusilli pasta (37%) (durum wheat semolina, fish broth (water, salt, flavour enhancer (E621)), lactose, fish, onion, aromatic herbs, spices, soy sauce (soy, wheat, salt), celery seeds, colourant (E150c), dressing (water, sunflower oil, vinegar, olive oil (1%), salt, sugar, mustard, stabilizers (guar gum, bean gum, xanthan gum, carrageenan), starch, flavours), carrot, corn, peas, seafood (16%) (surimi (fish, stabilizers (sorbitol, E452i), water, egg white powder, starch, rapeseed oil, wheat starch, flavours, salt, sugar, flavour enhancers (E621, E635), colourants (cochineal carmine, capsanthin)), squid, shrimp).

Description: Pierre Martinet Penne, Surimi, Salade, Tomate Cérise (Penne, Surimi, Lettuce and Cherry Tomato Salad) contains a pot of balsamic vinaigrette, a sachet of croutons and a plastic fork. The product retails in a 300 g pack. France. Stored under refrigeration.

Ingredients: Pasta salad (57%) (penne pasta (76%) (wheat semolina, egg white), pepper, water, lemon juice from concentrate, rapeseed oil, spices, alcohol vinegar, double concentrate tomato purée, salt, sugar, modified starch, thickener (xanthan gum)), surimi based preparation (13%) (water, surimi (35%) (fish, sugar, stabilisers (E451, E450)), wheat starch, modified starch, sugar, salt, sunflower oil, egg white, yeast extract, flavouring (crab), flavour enhancer (E631), colourings (calcium carbonate, paprika extract)), green salad (13%), cherry tomato (7%), balsamic vinegar sauce (7%) (balsamic vinegar (28%), rapeseed oil, water, extra virgin olive oil, lemon juice, salt, pepper), croutons (3%) (wheat flour, sunflower oil, salt, yeast).

Description: Verdifresh Mil Islas (Thousand Island Salad) features a mix of iceberg lettuce, curly endive, carrots, corn, white cabbage, crab sticks and pink sauce. The product retails in a 330 g pack including a fork. Spain. Stored under refrigeration.

Ingredients: Crab sticks (30%) (surimi (fish), water, sunflower oil, cephalopod (mollusc), corn starch, modified potato starch, crab extract (crustacean), salt, egg albumin, vegetable protein, flavour enhancer (E621), white wine extract, sugar, natural colourants (E120, E160c)), pink sauce (24%) (tomato, sunflower oil, sugar, alcohol vinegar, glucose and fructose syrup, egg yolk, mustard (water, mustard grains, vinegar, salt, acidulant (E330), preservative (potassium metabisulfite), salt, modified corn starch, stabilizers (E412, E415), preservatives (E211, E202), antioxidant (E385), colourant (E160a))), carrots (11%), iceberg lettuce (10%), corn (9%) (sweet corn, sugar, salt, antioxidant (E330)), white cabbage (9%), endive (7%).

Description: Verdifresh Ensalada César con Pollo, queso y Picatostes (Chicken Caesar Salad with Cheese and Croutons) is a gourmet mixed salad. The product retails in a 210 g pack complete with a fork. Spain. Stored under refrigeration.

Ingredients: Caesar's salad dressing (23.3%) (water, sunflower oil, spirit vinegar, sugar, Parmesan cheese, salt, stabilisers (E415, E412) parsley , acidifier (E270), emulsifier (E435), garlic, pepper, preservatives (E211, E202), antioxidant (E385)) curly endive, cheese (16.7%) (pasteurised cow´s milk, salt, rennet, potato starch, lysozyme), chicken (14.3%) (chicken meat (92%), stabilisers (E451, E407), preservative (E327, E263), salt, chicken meat extract, fibre, aroma, antioxidant (E301), spices, colouring (caramel)), croutons (11.9%) (wheat flour, dextrose, sugar, animal fats, yeast, wheat gluten, salt, whey , malt extract, antioxidant (E300)), lambs lettuce, radicchio.

In general, we can find different salads with tuna and surimi. Some of these salads also include pasta as ingredient. The product weight normally ranges between 200-340 g. In some cases part of the ingredients are placed in different compartments.

Technical approach for the final prototype production:

The muscle fat of farmed meagre is relatively low and ranging from 0.73-2.93% (Giogios et al., 2013; Poli et al., 2003). In consequence, it can be more appropriate to cook meagre fish in vinegar than conduct a smoking process, which is more favourable in species with higher fat contents. In this regard, different concentrations of pure acetic acid and vinegars were studied. First, relatively high concentrations of acetic acid were used according to the literature (Ozden, 2005; Mariusz Szymczak, 2011). However, it was necessary to adjust and reduce the concentration of the acid given the fact that the assayed concentrations conferred a strong acid taste even after marinating at 2 and 3% concentrations overnight. As for this, vinegars from wine, cider and rice were also studied. Cider vinegar was chosen for having a smoother flavour and being more available than rice vinegar, which also conferred a mild flavour. After that, the concentration and period of marinating was adjusted to reach a pH below 4.4 to avoid microbial growth, in particular *Listeria monocytogenes,* during its storage at refrigeration temperatures (**Table 5**). According to the results, the minimum required concentration of vinegar was 70% and requires 3 hours to arrive to the desired pH. The mustard vinaigrette containing honey was found to combine with the fish as it reduces the tangy flavour of the salad.

Table 5. Effect of different concentrations of cider vinegar on the pH of meagre after different times of marinating at 4 °C[1]

Vinegar	Initial pH	pH 30 min	pH 60 min	pH 120 min	pH 180 min
30%	6.64	6.45	5.72	5.2	5.1
40%	6.64	6.24	5.52	5.16	4.90
50%	6.64	5.99	5.10	5.06	4.87
60%	6.64	5.79	4.75	4.54	4.45
70%	6.64	5.34	4.56	4.35	4.2

[1] Meagre was cut in cubes of approximately 1.5 cm and marinated at 1:1 weight ratio with the different vinegar solutions containing 1% NaCl. The pH was measured in well-drained samples, which were homogenized in 10 volumes of distilled water.

Ingredients:

Mixed vegetables "mesclun" (75 g): the proposed mix consists of romaine lettuce, endive lettuce, lamb's lettuce and radicchio in similar ratios.

Cherry tomatoes (35 g).

Vinegar-cooked meagre (45g): meagre fillets, cider vinegar, water.

Croutons (10 g): crunchy bread cubes made of wheat flour, vegetable oils (sunflower and palm), yeast, salt and malt wheat.

Mustard vinaigrette (35 g): Dijon mustard (water, mustard seeds, alcohol vinegar, salt, citric acid, potassium metabisulfite), oregano, honey, olive oil, lemon (juice and zest), black pepper.

Manufacturing information:

This product contains the following components: salad, marinated meagre, vinaigrette sauce, croutons and a plastic fork (**Fig. 10**). This type of product is normally produced and assembled in a clean-room environment and packaged in MAP. Given that the salad ingredients and croutons (in a separate bag) are commonly used in this type of product, we only considered the production of the particular ingredients: marinated meagre and the vinaigrette sauce.

Regarding the production of the marinated meagre, the EFSA states that it is not possible to identify which farmed fish species do not present a health hazard with respect to the presence of parasites (EFSA, 2010). Provided that many traditional marinating and cold smoking methods are not sufficient to kill *Anisakis* larvae, it is necessary to freeze meagre fish for not less than 24 hours before processing. Thereafter, meagre fish without skin is cut in dices of 1.5x1.5 cm approximately. Salt (1 g/100 g fish weight) and diluted apple cider vinegar (70:30, v/v) is added until completely covering the product (fish to vinegar ratio is approximately 1:1, w/v) and stored for 3 hours at 3-4 °C to achieve a pH below 4.5. After this period, the liquid was removed and the fish dices were allowed to drain. Marinated fish pieces (approx. 2.5 g each) were distributed in different bags and sealed.

The mustard vinaigrette is produced as follows: in a conventional blender, 1 g lemon zest (1.6%), 7.5 g lemon juice (11.9%), 7.5 g extra virgin olive oil (11.9%), 20 g of Dijon mustard (31.8%), 1.5 g of oregano (2.4%), 25 g of honey (39.7%), 0.3 g of salt (0.5%) and 0.15 g pepper (0.2%) were mixed. Special attention must be given to pepper and oregano as a microbial contamination source. The microbial quality of these ingredients needs to be guaranteed before their use (e.g. irradiated). The mix is distributed in different bags and sealed.

Figure 10. Components of the "*ready to eat meal: salad with fish"* physical prototype.

Product packaging and retail market prototype:

Packaging system:	modified atmosphere packaging (MAP: 7% O_2, 8% CO_2, 85% N_2).
Packaging equipment:	MAP heat sealing machine, C26S COMPAC Srl. (Canavaccio, Italy)
Design:	the vegetable mixture is packed in a transparent tray. Vinegar-cooked fish, mustard vinaigrette and croutons are individually packed in plastic sachets and included in the tray. A plastic fork is also included in the tray.
Packaging Materials:	tray: amorphous polyethylene terephthalate (A-PET), GB 85 GT, COMPAC srl. Sealing film: PET/ PET-AF with antifog properties, B260TAPBX, COMPAC srl. Sachets: PA/PE film
Size:	tray: 190 x 137 x 85 mm (1500 ml). Sachets: 100 x 130 mm
Recyclable:	yes

The different components of the "*salad with fish"* physical prototype and its packaging in MAP is shown in **Fig. 11-13**.

Figure 11. Physical prototype of the "*salad with fish*" before its packaging in MAP.

Figure 12. Packaging of the "*salad with fish*" physical prototype.

Figure 13. Physical prototype of the "*salad with fish*" packaged in MAP.

Physicochemical properties:

Moisture = 80.11%; water activity = 0.988; pH = 4.2

Nutrition facts:

Expressed in 100 g of product:

Energy: 93 Kcal or 388 KJ

Protein (g): 4.41

Lipids (g): 4.17 of which saturated (g): 0.67

Carbohydrates (g): 8.44 of which sugars (g): 6.0

Fiber (g): 1.9

Salt (mg): 295

Potential allergens:

Gluten and mustard

Storage conditions:

Under refrigeration conditions (2-4 °C).

Microbiological and sensory shelf life assessment:

The vegetable mix and croutons were not considered in this deliverable. The shelf life of croutons should be given by the provider and it should be longer than that given to the rest of the ingredients. However, in this case it should be borne in mind that they can become rancid or loose crunchiness with time. The product should be used time before these detrimental effects may occur. With respect to the salad, we understand that this needs to be processed in the facilities of conventional producers of this type of products in order to ensure product's safety and standard shelf life, which is up to 8 days.

The shelf life assessment of the vinegar-cooked meagre fish and the mustard vinaigrette was carried out according to the given recommendations (EURL, 2014; European Commission). These ingredients were stored at 4 °C for the first 2 days and at an abuse temperature of 8 °C till the end of the period (8 days). Results are shown in **Table 6**.

Table 6. Shelf life assessment of the vinegar-cooked meagre and the mustard vinaigrette of the "*salad with fish*" prototype: microbial counts over the shelf life assessment period (8 days). [1]

		Day 1 Log (ufc/g)	Day 5 Log (ufc/g)	Day 8 Log (ufc/g)
Vinegar-cooked meagre	Lactic acid bacteria	<1.00*	<1.00*	<1.00*
	Mesophilic bacteria	1.10	1.45	0.86
	Enterobacteriaceae	<1.00*	<1.00*	<1.00*
	E.coli	<1.00*	<1.00*	<1.00*
	Psycrophilic bacteria	<2.00*	<2.00*	<2.00*
	Salmonella	A	A	A
	Listeria monocytogenes	A	A	A
Mustard vinaigrette	Mesophilic bacteria	4.06	4.22	4.33
	Enterobacteriaceae	<1.00*	<1.00*	<1.00*
	Salmonella	A	A	A
	Listeria monocytogenes	A	A	A

[1] Samples were stored at 4 °C during the two first days and the rest of the estimated period were stored at an abuse temperature of 8 °C. Results are averages of 5 different samples. The presence of *Listeria* and *Salmonella* is determined in 25 g of sample and the rest in 10 g. Letter "A" stands for absence and the asterisk indicates that is below the limit of detection. For more information see material and methods section.

As expected, the pH of the studied components is determining for microbial growth. Accordingly, minimal changes were recorded in the shelf life test. This test was conducted up to 8 days because this was the acceptability limit of the salad. The stability of these components (mustard vinaigrette and vinegar-cooked meagre) is longer than the studied shelf life (limited by that of the salad). However, this extended shelf life should be evaluated in case it is required.

In order to determine the shelf life, initial characteristics and changes in the quality of vinegar-cooked fish and the mustard vinaigrette were evaluated at different storage points (day 1, day 5 and day 8) as in the microbial assessment (two thirds of the storage period at abuse temperature). As shown in **Table 7**, various attributes are expected to decay in these food components according to the literature (Kilinc, 2009; M. Szymczak, Szymczak, Koronkiewicz, Felisiak, & Bednarek, 2013). Few minor changes were observed throughout the storage period. In consequence, the limit of this product is, as expected, determined by the vegetables that may present various defects such as limpness after 8-9 days of storage.

Table 7. Sensory evaluation of the characteristic attributes which may change during the storage of the vinegar-cooked fish and vinaigrette components of the "*salad with fish*" idea[1]

Quality parameter by components		Attributes and score
Appearance	Fish colour	**0: totally homogeneous**
		1: appearance of some grey/oxidized areas
		2: totally heterogeneous
	Seasoning colour	0: light yellow green
		1: intermediate
		2: darkish yellow brown
Odour	Fish	**0: fresh**
		1: mold
		2: rotten taint
	Seasoning	0: balanced sweet, acid and fresh herbs
		1: decrease of odour intensity
		2: unbalanced and appearance of off-flavours
Flavour	Fish	**0: fresh**
		1: mold
		2: rotten taint
	Seasoning	0: balanced sweet, acid and fresh herbs
		1: decrease of flavour intensity
		2: unbalanced and appearance of off-flavours
Texture	Fish	**0: firm and not slimy**
		1: a little soft and slimy
		2: very soft and slimy

[1] Samples were stored at 4 °C during the two first days and the rest of the estimated period were stored at an abuse temperature of 8 °C. The attributes and scores reached at the end of the storage period are highlighted in bold.

Overall, microbiological and sensory results indicate that the shelf life of the vinegar-cooked meagre and mustard vinaigrette are longer than the expected shelf life of the green vegetables "mesclun" that determine the limiting factor of this product idea.

Consumer handling/cooking specifications:

The product needs to be stored under refrigeration until its consumption but it can be removed from the fridge few minutes before if desired. The product can be consumed after mixing all the ingredients in the same tray or alternatively serve it in a plate.

Suggested final presentation:

The suggested presentation of the physical prototype is shown in **Fig. 14**.

Figure 14. Suggested final presentation of the "*salad with fish*" physical prototype.

3.4. Idea 6: Fish burgers shaped as fish

Description of the product concept:

Frozen fish burgers shaped as fish. The burgers are ready to cook and prepared with a mild seasoning and can be incorporated in a sandwich or prepared as a part of a meal. Among the advantages of this product is the absence of bones and the attractive shape for children. The product is produced in an environmentally sustainable way (containing ASC label). It is labelled as a premium product; the country of origin is EU. The product is included in a transparent vacuum-packed bag or in a plastic tray with transparent plastic on the top. Information on fish for educative purposes (children) and playful gifts (e.g. sticker) are included in the packaging. This idea was based on the idea of fish sausages and hamburgers that were discarded in D28.1 (not successful), by trying to improve its weaknesses. The comments of "good for kids", but "not new" provided by the experts of D28.1 were those used to improve the initial idea.

Reasons for its selection and existence of similar products in the market:

This fish product is an additional concept generated within the D28.2 and occupies the 14th position in the ranking of product concepts (D28.2; score 100.7333). Meagre is a very interesting fish species for this product idea for various reasons. Firstly, meagre has a higher proportion of discarded muscle, which can be used for that purpose (D28.2). Secondly, due to its low fat content it is particularly indicated for frozen products. Finally, it presents low chewiness values and thus it may be indicated for children, which would, in their majority, prefer less "chewy" fish (D28.3).

As reported, similar presentations (not breaded burgers and retailed in similar packagings) can be found in the European market:

Description: Campos Atún Burguer (Tuna Burger) is a source of omega 3 and can be enjoyed with sauce, salads or in sandwiches. The product retails in a 160 g pack featuring the Dolphin Safe logo and containing two pieces. Spain. Frozen.

Ingredients: Tuna (60%), fried tomato (tomato, vegetable oil, glucose syrup, water, modified corn starch, salt, acidulant (citric acid), vegetables), egg, soy, bread crumbs (wheatflour, yeast (*Saccharomyces cerevisiae*), salt, spices), onion, lactose, salt, parsley.

Description: iFish Fish Burger de Salmón Nata y Cebollino (Salmon Fish Burger with Cream and Chive) is now available. The deep-frozen product retails in an 80 g pack. Spain. Frozen

Ingredients: Salmon from Norway, onion, cream (3%), salt, chive (1%), pepper

Description: Lofoten 80% Grov Fiskeburger med Torsk og Sei (Coarse Fish Burger with Cod and Coalfish) is free from gluten and milk. The 80% fish product is made with Spanish olive oil and retails in a 500 g pack featuring the Green Keyhole logos, which represents healthy eating, and also the Green Point logo. Norway. Stored under refrigeration.

Ingredients: Fish (80%) (haddock (29%), pollock (25%), cod (16%), white salmon (10%)), rice milk, potato starch, olive oil, onion, potato flakes, dijon mustard, salt, garlic, celery salt, mace, white pepper.

Description: Dalmare Atelier Burger di Pesce alla Mediterranea (Mediterranean Style Fish Burger) features 85% fish content and is made with fresh fish fillets. It contains no additives and is said to be ready in five minutes. The product retails in a recyclable pack containing 2 x 90 g units. Italy. Stored under refrigeration.

Ingredients: Fish (85%) (fillet of rainbow trout reared in Italy, fillet of Nile perch *Lates niloticus* caught in freshwater in Tanzania, fillet of salmon reared in Norway), sun dried tomatoes (8%) (sun dried tomatoes, sunflower oil, salt, garlic, oregano), potato flakes, vegetable fibre, edible salt, herbs, pepper.

Description: Dagens Fangst Grov Fiskekarbonade (Coarse Fish Burger) is made with 82% of first-class fish. This product retails in a 300 g pack, containing two units. Norway. Stored under refrigeration.

Ingredients: Haddock fillet (58%), pollock filet (24%), milk, potato starch, rapeseed oil, salt, spices, onion, leek, paprika.

Description: Lofoten Fiskeburger med Sei og Purre (Fish Burger with Pollock and Leek) is said to combine the best ingredients of the sea with healthy and exciting flavours. This product is made from 56% fish, contains 4.7% fat, and is said to be a perfect starting point for a healthy, easy and good dinner. The product retails in a 440 g pack with 2 x 2 burgers and features the Green Keyhole logo. Norway. Stored under refrigeration.

Ingredients: Fish (56%) (pollock (42%), haddock (14%)), milk, leek, potato flour, butter, lactose, nutmeg flower, white pepper, salt, celery salt, stabilizer (locust bean gum).

Description: Kløver Mini Fiskeburger (Mini Fish Burger) is now available. This product is precooked and easy to prepare, and is recommended heated on the grill and served in a hamburger bun. The product retails in a 360 g pack. Norway. Stored under refrigeration.

Ingredients: Haddock fillet (75.8%), water, rapeseed oil, salt, stabilizer (E461), dextrose, stabilizer (E451), spices (black pepper, garlic, onion).

To summarize, these products are either refrigerated or stored frozen. Some of them include other additional ingredients or mild seasonings including pepper. However, this latter ingredient was considered to be inadequate in a product destined for children.

Technical approach for the final prototype production:

There were two important concerns in this product idea. The first one was to select a seasoning or mild ingredient suitable for children and, if possible, help to improve the intake of fish in children. In this regard, the addition of cheese may be a suitable strategy for masking the fish flavour of this product and somehow resemble to conventional cheeseburgers. The second concern was, the integrity of the sample, especially the fish tail, when cooking. In addition, it was found that the texture of the product was soft and easy to disaggregate. Taking these aspects into consideration, we assessed two strategies: the formation of calcium-alginate gels and transglutaminase crosslinking. The first one provided a slimy texture when eating whereas the second one formed a fish burger with a hard texture, which was decided to be more appropriate. After that, different proportions of cheese and microbial transglutaminase enzyme were assessed to adjust the formulation of the final product. It should be mentioned that transglutaminase is a processing aid and as such it is not necessary to declare it as ingredient.

Ingredients:

Meagre fish meat, Emmental shredded cheese (milk, salt, bacterial culture, microbial enzyme), salt and black olives (water, olives, salt, thyme and oregano aroma, and stabilizer (ferrous gluconate)).

Manufacturing information:

Formulation for 100 g of product:

85 g of meagre chopped and 15 g of Emmental cheese in a mixer (Braun minipimer MQ 5000) for 10 seconds to obtain a grinded product but not purée (small fish chunks). Then 0.1 g of transglutaminase (Activa GS, Ajinomoto, Tokyo, Japan) dissolved in 5 ml of water at a temperature between 10-15 °C was added immediately to the fish mixture; salt and white pepper were added and evenly mixed. Thereafter, within 20 minutes 100 g of the product were filled into the fish-shaped mold and a vacuum cycle was conducted (Tecnotrip, Terrassa, Barcelona) to compact the sample, thus allowing a homogenous crosslinking. The fish burger was stored at 0 – 2 °C overnight (8-10 hours) before freezing in a conventional freezer (i.e. plate freezer). After that, the fish burger was placed on a tray and an olive was placed to simulate a fish eye and then it was packaged with a skin pack.

Product packaging and retail market prototype:

Packaging system:	vacuum skin packaging
Packaging equipment:	thermosealing machine, SMART 500, ULMA Packaging, S. Coop (Oñati, Spain)
Packaging Materials:	tray: EOST 1523-30, CRYOVAC (Sealed Air; Charlotte, USA). Sealing film: 150 µm thick, VST 0280
Size:	tray: 147 (width) x 132 mm (length) x 30 mm (height)
Recyclable:	yes

By following the instructions the physical prototype should be as illustrated in **Fig. 15**.

Figure 15. Physical prototype of the "*fish burgers shaped as fish*" product concept.

Physicochemical properties:

Moisture = 71.37%; water activity = 0.992; pH = 6.45

Nutrition facts:

Expressed in 100 g of product:

Energy: 137 Kcal; 574 KJ

Protein (g): 20.86

Lipids (g): 5.9 of which saturated (g): 3.26

Carbohydrates (g): 0.09 of which sugars (g): not detected

Salt (mg): 503.8

Potential allergens:

Milk proteins

Storage conditions:

Frozen storage at -20 ^{o}C ± 2.

Microbiological and sensory shelf life assessment

Histamine was determined because of its health effects and as indicator of spoilage and microbial growth before freezing the product. Prototype histamine levels (9 ± 0.3 mg/kg) are far below the limits reported in the EU regulation 2073/2005 (EU, 2005). The shelf life of this product has to be similar to other related products that have been processed and under the same storage conditions and, in consequence, expected to be of 9 months or higher (Gimenez, Gomez-Guillen, Perez-Mateos, Montero, & Marquez-Ruiz, 2011; Man & Jones, 1994).

After production, the product was characterized and its quality loss was evaluated by assessing those sensory attributes that are expected to decay in this product as shown in **Table 8**. Note that the evaluation of cooked fish burgers was only carried out after 2 months of storage since there was not more time to conduct the shelf life study for extended periods. Results showed that there is no limiting factor for the sensory characteristics of burgers at this storage time.

Table 8. Characteristic attributes of the "*fish burger shaped as fish*" product concept[1]

Attribute	Scores
Overall appearance and colour	**0 relatively homogeneous colour/ fish shaped**
	1 not homogeneous
	2 very inhomogeneous/black spots/broken apart
Odour	**0 notes of fish and cheese**
	1 mild fish and cheese notes
	2 cheesy/fishy/rancid
Flavour	**0 notes of fish and cheese**
	1 mild fish and cheese notes
	2 cheesy/fishy/rancid
Texture	**0 relatively hard / not breaking in small pieces**
	1 not hard/ beaks into pieces
	2 breaks in small pieces/soft/chewy

[1] The attributes and scores reached after 2 months of frozen storage at -20 °C are highlighted in bold.

To conclude, based on previous experiments and the existing literature (Erickson & Hung, 1997) the shelf life of this product is expected to be as much as or higher than 9 months when stored at -20 °C. However, further studies are necessary to determine the shelf life of this product idea.

Consumer handling/cooking specifications:

Fish burger is recommended to be thawed in the refrigerator before its use. Once the product is thawed it may be fried on the pan with a little of oil on it. It is recommend not to overcook it. It can be served accompanied by mashed potatoes, vegetables, etc. or in hamburger bread.

Suggested final presentation:

Final presentations of the cooked prototype are shown in **Fig. 16** and **17**.

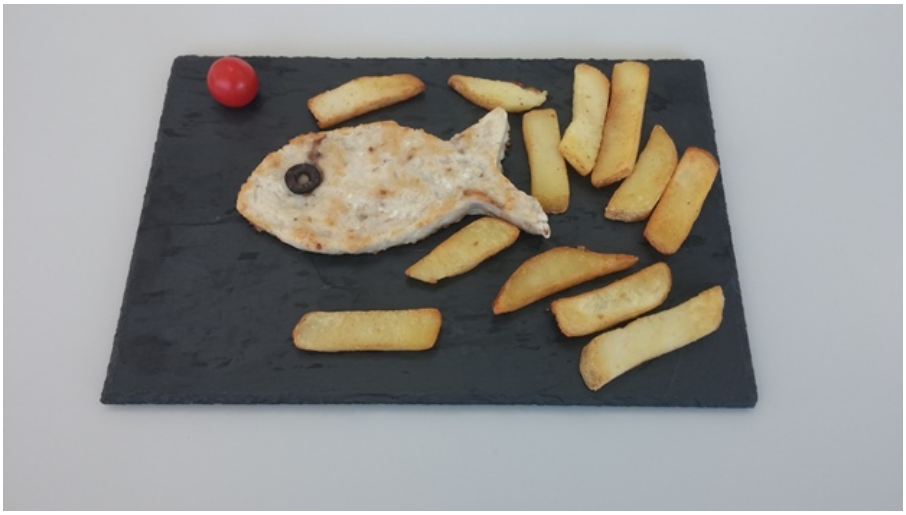


Figure 16. Suggested final presentation of the "*fish burger shaped as fish*" physical prototype.

Figure 17. Suggested final presentation of the "*fish burger shaped as fish*" physical prototype

3.5. Idea 9: Fish spreads / pate

Description of the product concept:

Fish pate / spreads prepared using different recipes. Can be used as starter or incorporated in a sandwich. The product is sustainably produced (containing ASC label). It is labelled as a premium product; the country of origin is EU. The product is included in a tube to facilitate use, extraction of right amount of product as well as prolong shelf life (only outer part of the product will come in contact with air in each use). This idea was an attempt to utilize raw materials that are considered of less value or losses to create added value. Consumer convenience and existence of similar non-fish products were considered. Not something similar has been provided by the focus groups.

Reasons for its selection and existence of similar products in the market:

This fish product idea is an additional concept generated within the D28.2 and occupies the 19th position in the ranking of product concepts (D28.2; score 99.9167). Pikeperch was suggested for this idea due to its technical compatibility. This fish species is characterized by the presence of "earthy" odour and flavour, which can be masked by adding other ingredients. In this regard, the employment of various ingredients in the formulation of the fish paste may help to overcome this issue.

Description: Speca Szardellapaszta Olivaolajban (Anchovy Paste) is made with olive oil. This fish paste retails in a 60 g pack. Hungary. Stored under refrigeration.
Ingredients: Anchovy (*Engraulis encrasicolus*) (71%), olive oil (14%), salt.

Description: Biffi Salsa Tonné Ricetta Classica (Tuna Sauce) has been relaunched with a new brand name, previously Biffi Le Specialià di Paolo Biffi, and is now available in a newly designed 150 ml pack. The classic recipe sauce is ideal on veal, eggs and other white meats. The product is free form gluten and GM ingredients. Italy. Stored at room temperature.
Ingredients: Sunflower oil, drained tuna (16%), fresh pasteurized egg yolk, wine vinegar, salt, sugar, capers (1%), anchovies, lemon juice, flavourings, acidity regulator (lactic acid).

Description: Ostsee Fisch Kaviar Creme aus Heringsrogen mit Würziger Senf-Note is a caviar cream, made from herring eggs. The product is flavoured with Mustard and is available in a 75 g pack. Hungary. Stored under refrigeration.
Ingredients: Salted herring eggs (42%), vegetable oil, mustard (12%), salt, sugar, sweet cream, aroma, guar flour, xanthan, preservative (E210).

Description: Pingo Doce Aquele Paté de Sardinha (Sardine Pâté) is a new product that retails as 4 x 22 g individually wrapped portions in an 88 g recyclable pack. Portugal. Stored at room temperature.
Ingredients: sardine (55%) (sardine, pilchard), vegetable oil (sunflower oil), water, tomato, onion, margarine (oils, vegetable fats, water, salt (2.9%), emulsifiers (lecithin, mono and diglycerides of fatty acids), preservative (potassium sorbate), acidity regulator (citric acid), flavours, natural colourings (beta carotene)), soy protein, mustard, red pepper, salt, spices.

Description: Giana Lososový Krém (Salmon Paté) is now available. This sterilized product retails in an 80 g pack. Czech Republic. Stored at room temperature.
Ingredients: Crushed salmon (*Oncorhynchus gorbuscha*) (44.6%), sunflower oil, drinking water, celery (8.4%), onion (8.4%), thickening agent (modified tapioca starch (E1442), cellulose, modified corn starch (E1440), xanthan gum), salt, ground white pepper, colour (E171), yeast extract, flavour and aroma enhancer (E621).

Description: The Petit Navire tuna mousse has been reformulated and is now claimed to be smoother. 2 x 80 g ring-pull cans are packaged in a board sleeve. France. Stored at room temperature.
Ingredients: Tuna (38%), water, colza oil, pea fibre, egg powder, milk powder, modified maize starch, flavour, salt, thickeners: guar gum, xanthan gum.

To summarize, there are different fish spreads and pates all of them with added oil in their formulations. They are sold in different packagings, also included in a tube. Fish spreads in a tube can be stored at room temperature and under refrigeration.

Technical approach for the final prototype production:

The rheological properties of the product are crucial for its intended application and success. Given that pikeperch is a lean fish, it is necessary to add a reasonable amount of lipids (as in pâté) to obtain the desired texture and spreadability. However, the emulsifying properties of fish proteins are limited and even so more when the product is submitted to a thermal treatment (Pasteurization or sterilization). Therefore, it is necessary to obtain a thermally stable emulsion in which the lipid phase is dispersed in a continuous matrix of proteins. Therefore, different proteins with good emulsifying properties were considered and evaluated (e.g. caseinate and other milk, egg proteins). We did not find a tube resistant to a sterilization process so it is necessary to first conduct the thermal treatment and then fill into the tube under aseptic conditions or fill the product into the tube and then pasteurize the product and store at refrigeration temperatures. It is worth to mention that many of the assayed formulations suffered from phase separation (oiling out) after thermal treatments. Therefore, it was necessary to first stabilize a thermally resistant oil-in-water emulsion, which was then mixed with the other food ingredients. Note that for this product idea we used the whole fillet, however, as this is of high quality and may be used in other product ideas (i.e. idea 2) it may be wise to use discarded pikeperch muscle for this idea. Therefore, this idea can maximize yields, add value and complement other ideas.

Ingredients:

100 g of cooked pikeperch (64.00%), 55 g of emulsion (described below) (35.20%), 1 g of salt (0.64%), 0.15 g of garlic powder (0.10%), 0.1 g of cayenne pepper (0.06%).

Manufacturing information:

The emulsion is elaborated as follows (100 g): Dissolve 5.9 g of caseinate in 47 g hot water (70 °C approx.) until its complete solubilization. Prepare an oil-in-water emulsion by gradually adding the sunflower oil (47

g) in the caseinate aqueous solution while mixing in a blender (Braun minipimer MQ 5000) at high speed. A homogenizer is recommended for improved emulsion stability.

Pikeperch fillets have to be free of skin and fish bones. Fish is scalded for 5 minutes as follows: in a boiling pot the fish that is already placed in sealed cooking bags is introduced into the pot (100 °C) and left there for 6 minutes while the stove is turned off. Afterwards, the cooked fish is mixed together with the emulsion and the remaining ingredients (garlic, cayenne and salt). Here it is important to note that texture and other rheological properties of the product depend on the degree of fish comminution. Overall, the higher the particle reduction the lower the viscosity. Therefore, the ability to flow and spread can be controlled by the level of grinding. In this regard, we preferred a relatively low degree of grinding as the viscosity was quite high and the observation of small protein fibres and noticeable presence in the mouth feeling is considered to be desirable in this kind of product. However, if the presence of small fibres and fish characteristic texture is considered to be undesirable the product can be grinded to a smaller particle size (higher degree of comminution) and the viscosity, if desired, can be increased by the addition of ι-carrageenan (0.5%) and other thickening agents.

With respect to non-proteolytic *C. botulinum*, a heat treatment of 90 °C for 10 min or equivalent lethality is required for a 6-log reduction. For this, it is recommended to pasteurize (or even sterilize) the product first and then conduct an aseptic packaging of the product under appropriate facilities and thus represent a limiting aspect. As an alternative, in this deliverable we conducted the thermal treatment (95 °C for 20 min, 100% humidity) in a conventional oven (Rational AG, SCC101 model, Landsberg am Lech, Germany) after filling and sealing the tubes with the product. As shown in the **Fig. 18**, this thermal process was enough to give a 6D for nonproteolytic *C. botulinum*.

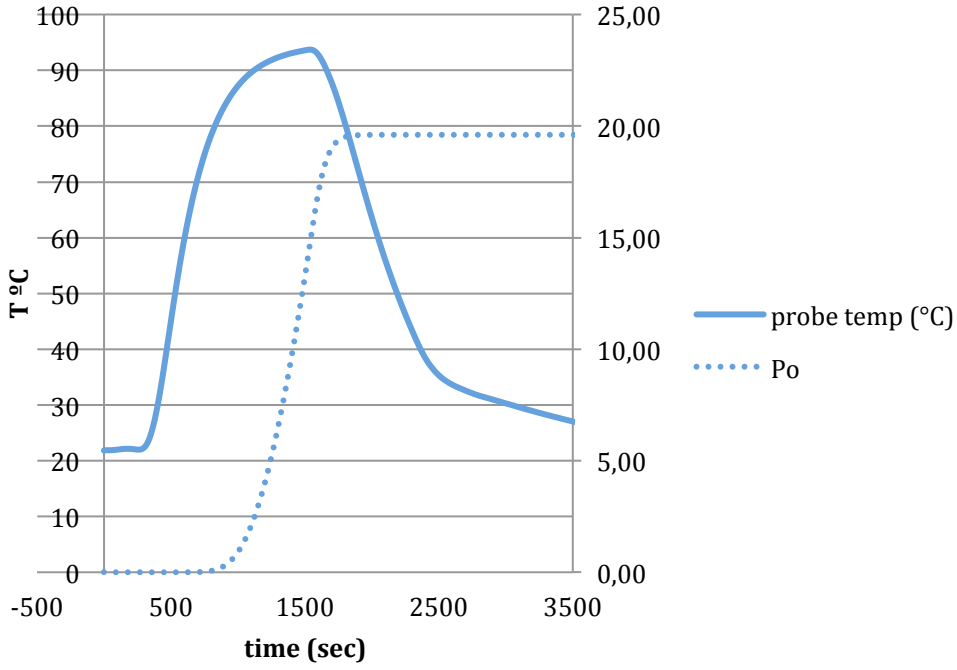


Figure 18. Fish product internal temperature during the thermal treatment (95 °C for 20 min)

Product packaging and retail market prototype:

Packaging system: recommended to pasteurize followed by aseptic filling

Packaging equipment: in this case manually but can be automatized

Packaging Materials: white tube: Aluminium, Witte y Solá, S.A. White cap: high density polyethylene, Witte y Solá, S.A.

Size: 30 mm diameter, 160 mm height, 60 ml volume

Recyclable: yes

By following these instructions the prototype should be as shown in **Fig. 19 and 20**.

Figure 19. Physical prototype of the "*fish spreads/pate*".

Figure 20. Physical prototype of the "*fish spreads/pate*" with the product coming up from the tube.

Physicochemical properties:

Moisture = 64.89%; water activity = 0.988; pH = 6.69

Nutrition facts:

Expressed in 100 g of product:

Energy: 221 Kcal; 919 KJ

Protein (g): 16.37

Lipids (g): 17.32 of which saturated (g): 1.92

Carbohydrates (g): 0.02 of which sugars (g): not detected

Salt (mg): 824

Potential allergens:

Milk proteins

Storage conditions:

Stored under refrigeration (2-4 °C).

Microbiological and sensory shelf life assessment:

The shelf life assessment of the fish pate was carried out according to the given recommendations (EURL, 2014; European Commission). Pikeperch fish pate was stored at 4°C the first 10 days and at an abuse temperature of 8°C till the end of the period. Taking into account the results shown in **Table 9**, it seems possible to obtain similar products with a shelf life of about 1 month under refrigeration conditions.

Table 9. Shelf life assessment of the pate: microbial counts over the shelf life assessment period[1]

	Day 1 Log (ufc/g)	Day 7 Log (ufc/g)	Day 14 Log (ufc/g)	Day 20 Log (ufc/g)	Day 29 Log (ufc/g)
Lactic acid bacteria	<1.00*	<1.00*	<1.00*	<1.00*	<1.00*
Mesophilic bacteria	4.00	4.18	4.09	4.39	6.37
Enterobacteriaceae	<1.00*	<1.00*	<1.00*	<1.00*	<1.00*
Psycrophilic bacteria	3.51	3.30	3.47	3.61	6.30
Salmonella	A	A	A	A	A
Listeria monocytogenes	A	A	A	A	A

[1] Samples were stored at 4 °C during the 10 first days and the rest of the estimated period were stored at an abuse temperature of 8 °C. Results are averages of 5 different samples. The presence of *Listeria* and *Salmonella* is determined in 25 g of sample and the rest in 10 g. Letter "A" stands for absence and the asterisk indicates that is below the limit of detection. For more information see material and methods section.

It is estimated that after opening the product should be consumed within the 3 following days. Therefore, it is necessary to conduct an appropriate shelf life assessment to properly determine the secondary shelf life (i.e. after opening of the product).

With respect to sensory attributes, changes in the quality changes of the fish pate were assessed at different storage periods as in the microbial assessment. **Table 10** shows the different attributes that are expected to decay in this product according to the literature (Man & Jones, 1994; Robertson, 2009). The product was evaluated after 30 days of its production when aroma/flavour characteristics became unbalanced and a significant diminution of the fresh garlic intensity was observed. In consequence, this storage period can be considered as the limit of acceptability.

Table 10. Sensory evaluation of the characteristic attributes which may change during the storage of the "*fish pate*" idea

Quality parameter	Attributes and score
Appearance	**0 no exudates/glossy/homogeneous colour**
	1 initial appearance of exudate
	2 exudates/rust-coloured (grey-brownish)
Odour	0 fresh fish/fresh garlic aroma
	1 unbalanced/decrease of fresh garlic intensity
	2 rancid fish/rotten/off-odour
Flavour	0 fresh fish/intense garlic flavour
	1 unbalanced/decrease of fresh garlic intensity
	2 rancid/off-flavour
Texture	**0 spreadable/juicy**
	1 less spreadable but juicy
	2 difficult to spread/less juicy

[1] Samples were stored at 4 °C during the first 10 days and the rest of the estimated period were stored at an abuse temperature of 8 °C. The attributes and scores reached at the end of the storage period are highlighted in bold.

Consumer handling/cooking specifications:

The product can be consumed directly from the fridge and served as appetizer or spread on sandwiches, toast, etc.

Suggested final presentation:

Two different presentations are suggested for this product idea (**Figs. 21** and **22**).

Figure 21. Suggested final presentation of the "*fish spreads/pate*" physical prototype.

Figure 22. Suggested final presentation of the "*fish spreads/pate*" physical prototype.

3.6. Idea 13: Frozen fish filet that is seasoned or marinated

Description of the product concept:

Frozen fish filet that is seasoned or marinated either traditional, Italian, Provence or Asian. The product is produced environmentally sustainable (containing ASC label). It is labelled as a premium product; the country of origin is EU. The product is in a sliding packaging, transparent vacuum-packed bag made of recyclable material, with clear pictures of the unfrozen product on the cardboard sleeve.

Reasons for its selection and existence of similar products in the market:

This idea concept was generated within D28.1 derived from focus groups and occupies the 16th position in the ranking of product concepts (D28.2; score 100.6000). Greater amberjack was the selected fish species for this idea.

This is a fast grower thus providing better yields (D28.2). The fat content of this fish is relatively high, which limits its applicability in the development of frozen products. Nonetheless, as stated in D28.3, fish of 1-2 kg are indicated for such products because they have relatively low fillet fats as opposed to larger fish (of 10-15 kg) that are fattier.

In the European market there are other similar products that can be used as references for this idea:

Description: Gourmet Provençal Wildlachsfilet mit Kräuterkruste (Provençal Wild Salmon Fillet with Herb Crust) is now available. This ready to cook product retails in a 700 g pack featuring the MSC logo and a QR code. Switzerland. Frozen.

Ingredients: Pacific wild salmon (96%) (*Oncorhynchus keta*), herbal mixture, salt, onions, sugar, garlic, spices.

Description: Delpierre au Four Filet de Morue Tomates & Herbes de Provence (Cod Fillet Tomatoes & Provence Herbs) has been reformulated. The microwaveable product comprises MSC certified, wild fish, Provence herbs, butter pieces, lemon and coriander. It retails in a 325 g pack. France. Stored under refrigeration.

Ingredients: salted cod and decoration (95.4%) (salted cod (96%) (cod (*Gadus macrocephalus*), water, salt (1.2%), preservatives (E326, E261, E202), acidifier (citric acid)), lemon slice, bay, butter, dried tomatoes (0.1%), Provence herbs (0.1%), red pepper), seasoning (4.6%) (butter (87.1%), lemon zest (5%), coriander (4%), salt, pink peppercorns, black pepper, cardamom, natural flavouring).

Description: Selection Odyssée Colin d'Alaska à la Bretonne (Brittany-Style Alaskan Hake) has been relaunched with a new brand name and a new packaging. It comprises bone-free, 100% fillet with vegetables and a cream and mushroom sauce and is said to be simple and rich. This product can be heated in a microwave or bain-marie, and retails in a newly designed 400 g partly recyclable pack containing two portions. France. Frozen.

Ingredients: bone-free Alaskan hake fillet (52.8%), water, button mushrooms (5.4%), leeks (4.4%), potatoes (4.2%), lactose (contains milk), milk proteins, tomatoes (2.4%), creme fraiche (2%), white wine concentrate, thickener (modified corn starch), shallots (shallots, salt), flavourings (contains fish, shellfish), dehydrated onions, salt, chive (0.2%), colouring (paprika extract), pepper.

Description: Almare Seafood Schlemmer-Pangasiusfilet Mediterran (Mediterranean Style Gourmet Shark Catfish Fillet) are skinned and de-boned, and feature a Mediterranean vegetable and shepherd's cheese topping on a decorative banana leaf. The product is ready to cook and retails in a 300 g pack. Also available are the varieties: Schlemmer-Pangasiusfilet India (Indian Style Gourmet Shark Catfish Fillet); and Schlemmer-Pangasiusfilet Asia (Asian Style Gourmet Shark Catfish Fillet). Germany. Frozen.

Ingredients: Shark catfish fillet (60%), Mediterranean vegetables (30%) (tomatoes, courgette, aubergine, onions, bell pepper, olives), cheese in brine from cow's milk (9%), dried tomatoes, spices, salt, sugar, hydrogenated vegetable fat.

Description: Lidl Pangasiusfilet Kerrie (Curry Pangasius Fillet) is now available. This ASC certified product features a soft curry marinade and retails in a 365 g pack containing two units weighing approximately 183 g each. The Netherlands. Stored under refrigeration.

Ingredients: Pangasius (*Pangasius hypophthalmus*) fillet (95%) (contains fish), marinade (5%) (vegetable oils (rapeseed oil, sunflower oil, linseed oil, palm oil, shea oil), vegetables (onion, bell pepper, garlic, leek), salt, curry (<2%) (turmeric, pepper, bell pepper, ginger, chilli pepper, cardamom, cumin), natural flavourings, herbs, colouring (curcumin))

Description: Laschinger Toscana-Lachs (Tuscan Style Salmon) consists of a fresh marinated salmon fillet with Mediterranean herbs cut in slices and a horseradish cream sauce in an added sachet. The product is delicate with buttered toast or fresh baguette as well as with crunchy green salads. It retails in a 170 g pack containing 150 g salmon and 20 g sauce. Austria. Stored under refrigeration.

Ingredients: Salmon: salmon from aquaculture in Norway, salt, sugar, Provencal herbs (rosemary, basil, thyme, savoury), carrots, red peppers, tomatoes, green peppers. Sauce: grated horseradish, rapeseed oil, cream (25%), spirit vinegar, sugar, whey product, thickeners (guar gum, xanthan), iodised salt, acidifier (citric acid), antioxidants (ascorbic acid, sodium metabisulphite).

Description: Friedrichs Biergarten Forelle (Beer Garden Trout) is a selected quality, traditionally smoked trout fillet, marinated with hops and malt. It is available with a sachet of sweet home-made mustard, ideal for the beer garden season. The product retails in a 125 g pack with 15 ml Händlmaier sweet mustard sauce. Austria. Stored under refrigeration.

Ingredients: Rainbow trout, sugar, salt, spices, dextrose, hydrogenated vegetable fat, barley malt extract, hops extract, smoke. Sweet homemade mustard: water, brown sugar, mustard seeds, spirit vinegar, spices.

Description: Laschinger Toscana-Lachs Marinierte Lachsspezialität in Schieben (Marinated Tuscany Style Salmon Slices) have received the Silver DLG Award 2011 and are said to be a real salmon delicacy. The fresh salmon fillet is marinated with salt and Mediterranean herbs such as basil, rosemary and thyme. During the maturing process, the herbs slowly penetrate the salmon and the fine flavour fully unfolds. After an adequate maturing time the salmon is sliced. The delicacy is complemented with an excellent horseradish cream sauce. The product is retailed in a 150 g pack with 20 g of sauce. Also available are Gravad Lachs (Gravad Salmon); and Schottischer Räucherlachs (Scottish Smoked Salmon) variants. Scottish Smoked Salmon is seasoned by hand with salt and then carefully smoked over beechwood. The top quality product is retailed in a 100 g pack. Germany. Stored under refrigeration.

Ingredients: Salmon from aquaculture in Norway, salt, sugar, herbs of the Provence (rosemary, basil, thyme, savoury), carrots, red bell pepper, tomatoes, green bell pepper), horseradish cream sauce (grated horseradish, rape seed oil, cream (25%), brandy wine vinegar, sugar, whey product, thickeners (guar gum, xanthan gum), iodised salt, acidulant (citric acid), antioxidants (ascorbic acid, sodium metabisulfate)).

Description: Queens Stoomfilet Panga Chili, Komijn & Koriander (Chilli, Cumin & Coriander Pangasius) is now available. The product is ASC certified and comprises seasoned Pangasius fillet. It is quick to prepare, can go straight from the freezer into the microwave and retails in a 125 g recyclable pack featuring the FSC Mix logo. Ireland. Frozen.

Ingredients: Pangasius fillet (92%) from Vietnamese aquaculture, acidifiers (E330, E332, E333), salt, vegetable oil (rapeseed oil, sunflower oil, linseed oil), dried vegetables (dried onion, dried bell pepper, dried garlic, dried leek), salt, spices (turmeric, pepper, paprika powder, chilli pepper, ginger, coriander, cardamom, cumin), yeast extract, lovage, colourings (E100).

To summarize, it is common to find different products from different fish species that are seasoned or marinated with different herbs and spices to provide the traditional, Italian, Provence or Asian flavour to the fish product. The proposed packaging is also common in this type of products.

Technical approach for the final prototype production:

As stated in the Deliverable 28.2, greater amberjack is a very suitable species for this product idea. There is demand for frozen products in Germany, as well as an established demand in the UK and Germany for pre-seasoned fish products.

For the elaboration of this prototype, the first step was to define the ingredients of the marinated sauce that could provide a different taste to greater amberjack fillet. Soya sauce and honey were selected to prepare the marinade (Asian marinade). Once the product was elaborated, it was immediately vacuum packed and frozen at -20ºC to have a ready to cook meal at any time. For this prototype it has been considered to use fish fillet without skin in order to get a more homogenous incorporation of the marinade into the flesh.

Ingredients:

Greater amberjack fish fillets without skin, honey, soya sauce, water, lemon juice and sesame seeds.

Manufacturing information:

The preparation of the marinade is based on the proportion of honey and soya, although water is included to moderate the strong taste of the soya sauce. The preparation of the marinade contains 200 ml of honey, 100 ml of soya sauce, 100 ml of water and 5-10 ml of lemon juice. Fillets are immersed in the marinade for 50 minutes to obtain a complete absorption into the fillet. Excess of marinade is removed. Fillets are then individually placed on the packaging tray and garnished with minced parsley and sesame seeds (**Fig. 23**). Finalized product is then vacuum packed and frozen (-20ºC).

Figure 23. Greater amberjack fish fillet marinated and seasoned ready for packaging (top); vacuum packed fillets ready for freezing (bottom).

Product packaging and retail market prototype:

Packaging system: vacuum skin packaging

Packaging equipment: vacuum packaging machine, EDESA VAC-40DT

Design: individually packed portion in bag sealed by thermo sealing.

Packaging material: 90 µm polyamide/polyethylene bag (Orved, Italy)

Size: 30 mm x 20 mm

Recyclable: yes

Physicochemical properties:

With respect to fish

Moisture (%): 69.01; pH: 6.05

Nutrition facts:

Expressed in 100 g of product:

Energy: 138 Kcal or 584 KJ

Protein (g): 16

Lipids (g): 2.7 of which saturated (g): not detected

Carbohydrates (g): not detected of which sugars (g): not detected

Salt (g): 0.43

Potential allergens:

Soya and sesame seeds.

Storage conditions:

The product should be stored frozen at -20ºC ± 2ºC

Microbiological and sensory shelf life assessment:

The content in histamine is quite often determined because it is not only an indicator of spoilage and microbial growth but also a foodborne chemical intoxication. Histamine fish poisoning results from the consumption of inadequately preserved and improperly refrigerated fish. It resembles an allergic reaction but is actually caused by bacterially-generated toxins in the fish's tissues (US Food and Drug Administration, 2011). The histamine levels were 7.2 ± 0.2 mg/kg and thus quite below those reported in the EU regulation 2073/2005 (European Commission 2005).

For the shelf life assessment the first 1/3rd of the total shelf life, the product was stored at -18 °C which is the maximum recommended temperature and the rest of the estimated shelf life period was at an abuse temperature of 4 °C (Betts, Brown et al. 2004). **Table 11** shows the microbial stability of the product throughout the storage period whereas spoilage bacteria may be the determinants of the shelf life of this product.

Table 11. Shelf life assessment of the marinated greater amberjack frozen fillets: microbial counts over the shelf life assessment period[1].

	Day 0 Log (CFU/g)	Day 1 Log (CFU/g)	Day 4 Log (CFU/g)	Day 7 Log (CFU/g)
Mesophilic bacteria	<1.00*	<1.00*	<1.00*	3.81
Enterobacteriaceae	<1.00*	<1.00*	<1.00*	<1.00*
E.coli	<1.00*	<1.00*	<1.00*	<1.00*
Psycrophilic bacteria	<1.00*	<1.00*	<1.00*	2.00
Salmonella	A	A	A	A
Listeria monocytogenes	A	A	A	A
Listeria spp.	A	A	A	A
Shigella	A	A	A	A

[1]Samples were stored at -18ºC for one third of the corresponding estimated period (day 0, day 1, day 4) and the remaining period stored at an abused temperature of 4ºC (day 7). Results are averages of 3 different samples. The presence of microorganisms is determined in 25 g of sample. Letter "A" stands for absence and the asterisk indicates that is below the limit of detection. For more information see material and methods section.

The microorganisms *Salmonella, Listeria spp., Listeria monocytogenes* and *Shigella* were not detected in any sample tested. The mesophilic bacteria had significantly higher counts on day 7 (3.81 CFU/g) compared to the beginning of the shelf life assessment (<1.00 CFU/g). The same behaviour was observed in psychrophilic bacteria (2.00 CFU/g). In consequence, the limit of this product is 7 days of storage, taking into account that temperatures of use and abuse have been applied. After day 7, the number of CFU/g does not permit to continue the analysis.

With respect to sensory attributes, changes in the quality parameters of the marinated fillets were evaluated for the same time as in the microbial assessment. **Table 12** shows the different attributes that are expected to decay in this product according to the literature (Kilinc, 2009; Mariusz Szymczak, 2011; M. Szymczak et al., 2013). Following the obtained results, 7 days can be considered as the limit of acceptability for frozen marinated greater amberjack fillets, subjected to the use and abuse temperature preservation method.

Table 12. Sensory evaluation of the characteristic attributes which may change during the storage of the greater amberjack frozen fillets[1]

Quality parameter	Attributes and score	Day 0	Day 1	Day 4	Day 7
Appearance	0: totally homogeneous 1: appearance of some grey/oxidized areas 2: totally heterogeneous	0	0	1	**1**
Fluid retention capacity	0: no exudate 1: intermediate 2: intense exudate	0	0	1	**2**
Odour	0: fresh 1: mold 2: rotten taint	0	0	0	**1**

Texture	0: firm and not slimy 1: a little soft and slimy 2: very soft and slimy	0	0	0	**1**
Temperature	0: -20ºC < T < 0ºC 1: 0ºC < T < 4ºC 2: T > 4ºC	0	0	0	**1**
Freezer burn	0: no freezer burn 1: intermediate 2: intense freezer burn	0	0	0	**0**
Brightness	0: glossy 1: intermediate 2: matt	0	0	0	**0**

[1]Samples were stored at -18ºC for one third of the corresponding estimated period (day 0, day 1, day 4) and the remaining period stored at an abused temperature of 4ºC (day 7). The attributes and scores reached at the end of the storage period are highlighted in bold.

Consumer handling/cooking specifications:

The product should be kept frozen till the moment of consumption. It is advisable to thaw the product in the refrigerator. Once ready, the fillet can be grilled on the pan or prepared in the oven. Recipes can be included in the package or include the QR code to several Internet cooking sites to give more options to the consumer.

There are a wide number of recipes that can be used. Taking into account that the product is marinated with two very flavouring ingredients such as soya and honey, the recipes selected are based on simplicity of ingredients and healthiness. Marinade already provides the flavour and adds few calories to the dish. Different recipes were obtained from kitchen books, Internet and own recipes but only two simple preparations are selected here (**Table 13**).

Table 13. Description of recipes to prepare fish meals from the "*frozen fish fillet that is seasoned or marinated*" idea prepared with greater amberjack.

Recipe and Ingredients	Preparation
"**Amberjack with Purple Spring Onions & Haricot Verts**":	For 2 persons. Remove the fish from the freezer, rinse off frost or ice, dry the fish and prepare it.
2 fillets amberjack	Blanch the haricot verts in boiling water for a few minutes, until tender but not overcooked.
2 cups yellow haricot verts, blanched	Place in cool water. Once they have cooled, remove from heat and drizzle with a little olive and season with salt and pepper.
2 tablespoons grapeseed oil	Salt and pepper the amberjack fillets. In a pan, heat the grapeseed oil over medium high heat until almost smoking.
2 tablespoons olive oil, plus more for drizzling	Add the greater amberjack fillets, sautéing for a few minutes on each side, until cooked through. Remove

Recipe and Ingredients	Preparation
	from pan and set aside.
1 lemon juice	Add the olive oil to the same pan and then sauté the spring onions until tender. Finish with the lemon juice and season to taste with salt and pepper.
Salt and freshly ground black pepper	To plate, place some of the haricots verts in the bottom of a plate. Top with a greater amberjack fillet. Finish with the spring onion sauce.
"**Grilled greater amberjack**":	For 4 persons
4 great amberjack fillets marinated	Remove the fish from the freezer, rinse off frost or ice, dry the fish. In the meantime, prepare grill for medium heat, approximately 300-350 degrees.
Cracked pepper and salt to taste	Sprinkle with salt and pepper. Place fish on grill rack and cook 6 minutes on each side or until fish flakes easily with a fork.

Suggested final presentation:

The suggested final presentations of the different recipes are shown in **Fig. 24** and **25**.

Figure 24. Suggested final presentation for the "*frozen fish filet that is seasoned or marinated*" idea made with amberjack, purple spring onions and haricot verts.

Figure 25. Suggested final presentation of grilled greater amberjack for the "*frozen fish filet that is seasoned or marinated*" idea.

3.7. Idea 21: Fresh fish fillet with different 'healthy' seasoning and marinades (2 products)

Description of the product concept:

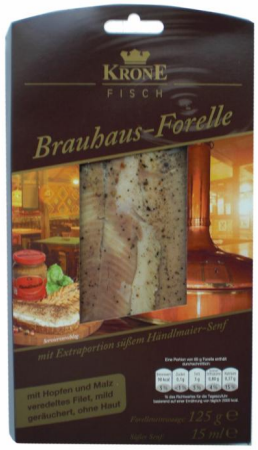
Fresh fish fillet with different 'healthy' seasoning and marinades separately packed that consumer can choose and vary depending on the occasion. This product is sold with recommendation for the appropriate vegetables and wine to accompany the dish. Product message: 'Not two same dishes in a row; 'You have it ready for you, healthy but still have the hectic lifestyle.' It is labelled as a premium product, the country of origin is EU.

Reasons for its selection and existence of similar products in the market:

The fresh fillet with different "healthy" seasoning and marinades was generated within D28.1 derived from focus groups and occupies the first position in the ranking of product concepts (D28.2; score 105.7833).

Grey mullet and pikeperch were selected for this idea, as they were suggested species in D28.2. It is important to bear in mind that these fish species are characterized by the presence of "earthy" odour and flavour (D28.3). However, the design of products that come with dressing spices or sauces can mask earthy characters that are mostly unwanted and, therefore, the development of such fish products can be suited for these fish species.

In the European market we can find the following, all refrigerated, related products:



Description: Krone Fisch Brauhaus Forellen-Filets (Brewery Trout Fillets) are now available. The lightly smoked product comes without the skin and is refined with pepper, hops and malt, and has an additional sweet mustard sauce. It is filleted by hand and retails in a pack containing 125 g fish and 15 ml mustard sauce. Germany.

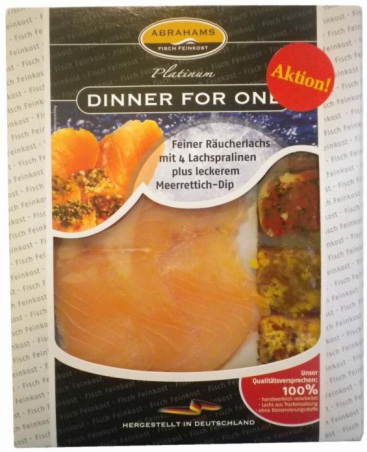
Ingredients: Rainbow trout, table salt, black pepper, white pepper, red pepper, barley malt extract, hops extract. Wood smoke sauce: water, brown sugar, mustard seeds, brandy vinegar, seasoning.

Description: Abrahams Fisch Feinkost Platinum Dinner for Two Salmon Selection with Sauce comprises fine Sockeye wild salmon and smoked salmon with four salmon pralines and two tasty sauces, including cranberry cream horseradish and orange mustard. This 100% handmade salmon from dry salting is free from preservatives, and retails in a 150 g pack. Germany.

Ingredients: Salmon (59%), red salmon (32%), seasoning, table salt, dried fruits, sugar, celery, dill, spices, dextrose, rice flour, flavour, paprika extract, smoke flavour. Cranberry sauce (grated horseradish, cream (22%), apple (12%), cranberries, palm seed oil, coconut fat (partly hardened), rapeseed oil, sunflower oil, dextrose, spirit vinegar, sugar, elderberry extract, whole hen's eggs, thickener (xanthan gum, guar flour, pectin), stabiliser (sorbitol), iodised table salt, acidifier (citric acid), emulsifier (soya lecithin), antioxidant (sodium metabisulphite)). Orange and mustard sauce (mustard (58%) (water, mustard seeds, spirit vinegar, table salt, sugar, seasoning), sugar, honey, rapeseed oil, orange juice concentrate (1.2%), natural orange flavour, seasoning).

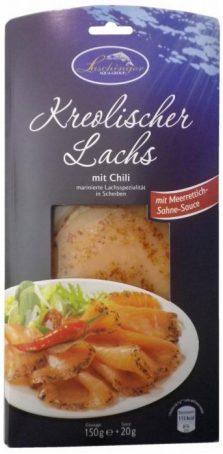
Description: Fini Selezione di Mare Carpaccio di Pesce Spada al Naturale (Swordfish Carpaccio) is now available. This product is said to be a source of omega-3 fat and is obtained from the finest fish fillets carefully selected, hand-salted, and dried to preserve its characteristic taste and tenderness. It is ideal as a quick and practical dish ideal for any occasion. The gluten free product retails in a 80g pack containing a 15 ml vinaigrette sachet for seasoning. Italy.

Ingredients: Swordfish (96%), salt, dextrose, spices. Vinaigrette: extra virgin olive oil, PGI balsamic vinegar of Modena (33%) (wine vinegar, concentrated grape must)



Description: Abrahams Fisch Feinkost Platinum Dinner for One Feiner Räucherlachs mit Lachspralinen und Meerrettich-Dip (Smoked Salmon & Salmon Pralines with Horseradish Dip) is an assortment of fine smoked salmon, which is dry cured by hand and smoked over local wood; four seasoned hot smoked salmon pralines; and a delicious cranberry-cream dip with horseradish in separate sachet. This premium product is free from preservatives and retails in a pack of 100 g fish and 20 g dip. Austria.

Ingredients: Salmon (88%), spices (contains celery), salt, dried fruit, sugar, seasoning, dextrose, rice flour, flavouring, paprika extract, smoke. Dip: ground horseradish (22%), cream, apples, cranberries (12%), partly hydrogenated vegetable fat, vegetable oil, dextrose, spirit vinegar, sugar, elder extract, whole egg, thickeners (xanthan, guar gum, pectin), stabiliser (sorbit), iodised salt, acidifier (citric acid), emulsifier (soya lecithin), antioxidant (sodium metabisulphite).



Description: Laschinger Kreolischer Lachs mit Chili (Creole-Style Salmon with Chili) is a fresh salmon fillet marinated with salt and chilli and left to ripen so that the seasoning slowly penetrates in the flesh. After the appropriate time, the fillet is carefully cut in slices and refined with an excellent horseradish and cream sauce. The product retails in a pack containing 150 g of salmon and 20 g of sauce in a separate sachet. Austria

Ingredients: Salmon, salt, sugar, chilli. Sauce: grated horseradish, rapeseed oil, cream (25%), spirit vinegar, sugar, whey product, thickeners (guar gum, xanthan), iodised salt, acidifier (citric acid), antioxidants (ascorbic acid, sodium metabisulphite).

Description: Queen Mary Carpaccio al Naturale e Condimare Insieme (Natural Salmon Carpaccio and Seasoning) contains salmon carpaccio marinated with herbs and seasoning. The product retails in a 120 g pack including a 15 g packet of seasoning. Italy.

Ingredients: Salmon, salt, sugar, herbs (thyme, dill, chives, parsley), sunflower oil. Seasoning (extra virgin olive oil, lemon juice (from concentrate), white wine, ground pepper (black pepper, white pepper, pink pepper, green pepper in varying proportions)).

Description: M Fishmonger Salmon with Sweet Chilli, Lime & Ginger Sauce is ready in just 15 minutes with no mess and no fuss. It comprises farmed skin-on boneless Atlantic salmon portions with a sachet of sweet chilli, lime and ginger sauce. The product retails in a 280 g recyclable pack. UK.

Ingredients: Salmon (85%), sweet chilli, lime and ginger sauce (water, white sugar, red chilli, brown sugar, spirit vinegar, red wine vinegar, garlic purée, cornflour, lime zest, salt, ginger purée, coriander).

Description: Skandia Gourmet Japonés Salmón Rojo Estilo Sashimi (Sashimi Style Red Salmon) is now available. This salmon is ready to consume, has been MSC certified, and retails in an 80 g pack containing both chopsticks and a 15 ml sachet of naturally brewed Shoda branded soy sauce. Spain.

Ingredients: Sockeye salmon, salt, sugars, antioxidant (E316), preservatives (E326, E262), soy sauce (water, soy beans, wheat, salt).

Description: Carrefour Truite Fumée Façon Carpaccio (Carpaccio-Style Smoked Trout) has been marinated in Sichuan pepper, and includes a sachet of lemon flavoured extra virgin olive oil. This product has been smoked in beech wood, is said to be tender and to melt in the mouth, and is naturally rich in omega 3 fatty acids. It retails in a 125 g pack, containing 100 g of salmon, 25 g of lemon oil, and serves two to three.

Ingredients: Carpaccio rainbow trout (92%), marinade (5%) (dextrose, herbs (and) spices (onion, garlic, Sichuan pepper (0.1%), parsley, pepper, turmeric), salt, sugar, natural flavourings, pepper, rapeseed oil), salt (3%)). Lemon flavoured olive oil (olive oil (60%), rapeseed oil, water, lemon concentrate (4%) (lemon juice concentrate, lemon pulp, natural lemon essence, acidifier (citric acid)).

Description: Labeyrie Sardinenfilets mit Zitronen und Kräuter "Provençale" (Provençal Style Lemon & Herbs Flavoured Sardine Fillets) are now available in the range. They are said to be ideal for grilling and are ready in eight minutes. The product is retailed in a 375 g pack containing a separate marinade sachet.

Ingredients: Salted garnished sardines: salted anchovies (98%) (*Sardina pilchardus*), water, salt (2%), preservatives (E326, E261, E202), acidifier (citric acid); Provençal herbs (0.4%), lemon peel (0.4%), spices, flavourings. Marinade: rapeseed oil, virgin olive oil, natural lemon flavouring, herbs.

Description: BellaMare Pesto Lachs Geschnitten mit Senf-Orangen-Sauce (Salmon Slices with Pesto & Mustard-Orange Sauce) are now available. This premium quality salmon from Norwegian aquaculture has been freshly processed and marinated. The product retails in a 170 g pack containing 150 g of salmon and a 20 g sauce sachet. Austria.

Ingredients: Salmon, salt, sugar, dill extracts, pesto (0.1%). Mustard-orange sauce (mustard (39%), glucose-fructose syrup, vegetable oil, orange juice concentrate (contains natural flavourings), spices, thickeners (guar gum, xanthan gum)).

Description: Selection Capitaine Cook Carpaccio de Saumon Fumé (Smoked Salmon Carpaccio) is a limited edition product for the festive season. The dry salted salmon is smoked with beech wood and accompanied by a sachet of extra virgin olive oil marinade and a sachet of parmesan cheese. This product retails in a 260 g pack, which contains eight slices and features the Charte STF label. France.

Ingredients: Atlantic salmon (74.6%), salt, marinade (15.4%) (extra virgin olive oil (38%), rapeseed oil, water, lemon juice concentrate, salt, dill, pepper, seaweed, preservative (E202)). Parmesan (7.7%) (unpasteurised cow's milk, salt, rennet).

Description: Czas na Grill Pstrag Lososiowy Plat (Salmon Trout Steak) is now available. The fresh product retails in a 507 g pack containing an 8 g sachet of butter and lovage seasoning. Poland.

Ingredients: Rainbow trout. Spice sachet: lovage (5%), pepper, garlic, salt, sugar, maltodextrin, flavourings (including butter flavouring, milk protein (contains lactose)).

To summarize, the majority of the presentations are made of smoked salmon and skin packaged. However, we can also find other fish species (trout, sardines, swordfish). With respect to packaging we can find several presentations: vacuum sealed bags and trays in which skin packaging or MAP have been applied to the

product. In these products, fish weights normally range from 80 to 150 g whereas seasonings range from 15 to 25 g.

Technical approach for the final prototype production:

As stated before the success of this idea may depend on the seasoning or marinade to be incorporated. In this connection, a creamy yoghurt sauce may help to mask those undesirable attributes of the fish species. The same works for an olive oil sauce containing garlic and parsley. Virgin olive oil is well-known for its related beneficial effects on human health (Buckland & Gonzalez, 2015; Visioli & Bernardini, 2011). On the other hand, yoghurt has been reported to contribute to bone health due to its content in bioavailable calcium but also exert other positive effects including the reduction of cholesterol levels and cancer (Glanville, Brown, Shamir, Szajewska, & Eales, 2015; Shiby & Mishra, 2013).

For the preparation of the grey mullet seasoning, two very healthy seasonings were selected: extra virgin olive oil (first oil obtained only by mechanical process) and special marine salt with lower amount of ions (*flor de sal*, "salt flower", "ice salt"). The well-known qualities of the olive oil have been already mentioned. Ice salt is a very special type of salt formed on the surface of the crystalizing layer of the seawater (1-3 mm) produced in areas (salt marshes, wet lands) of special characteristics since they are associated to ecosystems of high ecological value. This type of salt has specific organoleptic and chemical properties such as a lower content of sodium chloride (maximum 93%) and a whiter and brilliant white colour. This preparation promotes a very healthy way of fish consumption based on the freshness of the product and the use of high quality dressing: extra virgin olive oil and a salt with lower amount of sodium chloride.

Ingredients:

There are two fish species (pikeperch and grey mullet) for this idea. With respect to pikeperch, two different accompanying dressings were designed:

- Pikeperch with yoghurt sauce: pikeperch (150 g) and yoghurt sauce (25 g): yoghurt, lemon juice and lemon zest, garlic, oregano, salt.
- Pikeperch with virgin olive oil: pikeperch (150 g) and virgin olive oil mix (25 g): virgin olive oil, garlic, parsley, salt.
- Grey mullet fillets with extra virgin olive oil and ice salt: grey mullet (150 g), 10 g extra virgin olive oil (obtained just by mechanical process) and 2 g ice salt.

Manufacturing information:

Pikeperch:

Manufacture of yoghurt sauce: Add 125 g plain yoghurt (86.9%), 15 g lemon juice (10.4%) plus 2 g grated zest (1.4%), 0.5 g of oregano leaves (0.3%), 1 g of garlic cloves (0.7%) already cut and a 0.3 g of salt (0.2%) into a blender (Baun minipimer MQ 5000) and mix until homogeneous. Afterwards, place 20 g the product into a sachet, seal and store under refrigeration.

Manufacture of olive oil sauce: Add 120 ml of extra virgin olive oil (94.1%), 6 g of garlic (4.7%), 1.5 g of parsley (1.2%) into the blender and mix until homogeneous. Afterwards, place 25 g of the product into a sachet, seal and store under refrigeration.

Pikeperch fresh fillets of approximately 100 g are laid on the tray together with the sachets containing 20 g of additional seasonings. The product is then packaged in skin pack.

Grey mullet:

Similarly to pikeperch, fresh grey mullet fillets of 150 g are placed on a tray together with the sachets containing the dressings. The product is then vacuum packed.

Product packaging and retail market prototype:

Pikeperch:

Packaging system: modified atmosphere packaging: 70% oxygen, 30% CO_2

Packaging equipment: thermosealing machine, SMART 500, ULMA Packaging, S. Coop (Oñati, Spain)

Packaging Materials: tray: EOST 1523-30, CRYOVAC (Sealed Air; Charlotte, USA). Sealing film: thickness 150 µm, VST 0280, CRYOVAC

Size: tray: 147 (width) x 132 mm (length) x 30 mm (height)

Recyclable: yes

Figure 26. Physical prototype of the "*fresh fillet with different 'healthy' seasoning and marinades*" product concept.

Grey mullet:

Packaging system: vacuum bag

Packaging equipment: vacuum packaging machine, EDESA VAC-40DT

Packaging Materials: 100 µm polyamide/polyethylene bag (Orved, Italy)

Size: 30 mm x 20 mm

Recyclable: yes

Physicochemical properties:

Pikeperch:

With respect to fish:

Moisture: 76.40%; water activity: 0.993; pH: 6.56

With respect to yoghurt sauce:

Moisture: 87.82%; water activity: 0.988; pH: 3.48

With respect to olive oil sauce:

Moisture: 9.75%; water activity: 0.980; pH: 5.79

Grey mullet:

With respect to fish:

Moisture (%): 69.01; pH: 6.05

Nutrition facts:

Pikeperch:

Expressed in 100 g of product (fish only):

Energy: 93 Kcal or 396 KJ

Protein (g): 21.77

Lipids (g): 0.70 of which saturated (g): traces

Carbohydrates (g): 0.01 of which sugars (g): not detected

Salt (g): 118.8

Expressed in 100 g of product (yoghurt):

Energy: 53 Kcal or 223 KJ

Protein (g): 2.84

Lipids (g): 2.27 of which saturated (g): 1.59

Carbohydrates (g): 5.34 of which sugars (g): 2.6

Salt (g): 1.11

Expressed in 100 g of product (olive oil):

Energy: 792 Kcal or 3.259 KJ

Protein (g): 1.05

Lipids (g): 86.37 of which saturated (g): 13.38

Carbohydrates (g): 2.68 of which sugars (g): not detected

Salt (mg): 7

Grey mullet:

Expressed in 100 g of product (fish only):

Energy: 114 Kcal or 485 KJ

Protein (g): 28.8

Lipids (g): 0.8 g of which saturated (g): not detected

Carbohydrates (g): not detected of which sugars (g): not detected

Salt (g): 0.14

Expressed in 100 g of product (extra virgin olive oil):

Energy: 899 Kcal or 3710 KJ

Protein (g): 0

Lipids (g): 99.9 of which saturated (g): 16.6

Carbohydrates (g): 0 of which sugars (g): 0

Potential allergens:

They will mainly depend on the sauce. Accordingly, yoghurt sauce is not for people with allergy to milk proteins.

With regard to the grey mullet preparations, the only possible allergen is the fish.

Storage conditions:

Store below 3 °C.

Microbiological analysis and sensory shelf life assessment:

This analysis as well as that of the different seasonings and marinades was determined by following the existing recommendations (EURL, 2014; European Commission). With respect to fish, the period of storage under the specified refrigeration conditions was estimated to be of 6-9 days due to risks associated with *C. botulinum* in vacuum and modified atmospheres (Peck et al., 2006) whereas that of the sauces was estimated to be 15 days. For the shelf life assessment the first 1/3rd of the total shelf life of the product was stored at 4 °C which is the maximum recommended temperature and the rest of the estimated shelf life period was at an abuse temperature of 8 °C (Betts et al., 2004).

Pikeperch:

It was not possible to obtain fresh pikeperch for the study and because of that fresh gilthead sea bream (*Sparus aurata*) was used for the shelf life assessment of the fish. **Table 14** shows the microbial stability of the sauces throughout the storage period whereas spoilage bacteria may be the main determinants of the shelf life of this product.

The sensory attributes defined for the different constituents of the product are shown in **Table 15**. In this particular case, a previously frozen pikeperch fish fillet was thawed and used to monitor quality changes during its further storage (10 days at 4 °C). The sensory properties were considered as acceptable by the panel in spite of the appearance of a slight mould aroma. As for the accompanying sauces, they were evaluated at 1, 7 and 15 days and only some minor quality changes were observed throughout the storage period.

Although we used sea bream instead of pikeperch it is reasonable to think that the shelf life of this product would be similar to the one reported in this deliverable and thus being of 6 days or higher. Despite that, it is wise not to extend the shelf life to more than 10 days because of the risk of growth and toxin formation by non-proteolytic *C. Botulinum* in this product at a potential abuse of temperature during its storage. In consequence, the accompanying sauces do not represent a problem in this product.

Table 14. Microbiological shelf life assessment of the different components of the *"fresh fillet with different 'healthy' seasoning and marinades"* idea made with sea bream instead of pikeperch: microbial counts over the shelf life assessment periods. [1]

		Day 1 Log (ufc/g)	Day 3 Log (ufc/g)	Day 6 Log (ufc/g)
Sea bream	Lactic acid bacteria	<1.00*	<1.00*	<1.00*
	Mesophilic bacteria	3.89	4.18	3.95
	Enterobacteriaceae	2.36	2.42	2.84
	Psycrophilic bacteria	5.12	5.42	6.07
	Salmonella	A	A	A
	Listeria monocytogenes	A	A	A
		Day 1 Log (ufc/g)	Day 7 Log (ufc/g)	Day 15 Log (ufc/g)
Yoghurt sauce	Mesophilic bacteria	1.65	2.05	1.92
	Enterobacteriaceae	<1.00*	<1.00*	<1.00*
	Salmonella	A	A	A
	Listeria monocytogenes	A	A	A
	Lactic acid bacteria	0.26	<1.00*	<1.00*
Olive oil sauce	Mesophilic bacteria	5.40	5.09	4.43
	Enterobacteriaceae	3.78	3.42	2.87
	Salmonella	A	A	A
	Listeria monocytogenes	A	A	A

[1] Samples were stored at 4 °C for one third of the corresponding estimated period and the remaining period stored at an abuse temperature of 8 °C. Results are averages of 5 different samples. The presence of *Listeria* and *Salmonella* is determined in 25 g of sample and the rest in 10 g. Letter "A" stands for absence and the asterisk indicates that is below the limit of detection. For more information see material and methods section.

Table 15. Sensory evaluation of the characteristic attributes which may change during the storage of the different components of the "*fresh fillet with different 'healthy' seasoning and marinades*" idea[1]

Quality parameter			Attributes and score
Pikeperch fillet	**Appear.**	Colour	0: light and characteristic **1: slightly yellow** 2: grey/oxidized areas
		Brightness	0: bright **1: pale** 2: matte
	Odour	Overall	0: fresh and mild **1: slightly mold** 2: rotten taint
	Texture	Firmness	**0: firm** 1: intermediate 2: flaccid
		Cohesiveness	**0: cohesive** 1: not much cohesive but still 2: crumbly
Yoghurt sauce	**Appear.**	Overall	**0 fresh yoghurt with lemon zest and oregano showing bright colours/homogeneous mix** 1 initial syneresis 2: phase separation and appearance of lumps
	Odour	Overall	0: Fresh yoghurt, lemon and oregano **1: decrease of odour intensity** 2: sour/off-odour
	Flavour	Odour	0: Fresh yoghurt, lemon and oregano **1: decrease of flavour intensity** 2: sour/off-flavour
	Texture	Overall	**0: creamy** 1: less creamy 2: appearance of lumps
Olive oil sauce	**Appear.**	Overall	**0: white and opaque garlic/fresh green parsley** 1: yellowish garlic and parsley 2: transparent garlic/brown parsley
	Odour	Overall	**0: Intense fresh oil and garlic** 1: decrease of odour intensity 2 rancid/off-odour
	Flavour	Overall	**0: Intense fresh oil and garlic** 1: decrease of flavour intensity 2: rancid/off-flavour
	Texture	Overall	**0 oily/homogeneous**

1 some sprigs

2 not homogeneous/small pieces

[1] Fish was stored for 10 days at 4 °C whereas sauces were stored at 4 °C for the first 5 days and the remaining period stored at an abuse temperature of 8 °C. In both cases, the attributes and scores reached at the end of the storage period are highlighted in bold.

Grey mullet:

Table 16 shows the microbial stability of the grey mullet fillets during their storage. The microorganisms *Salmonella, Listeria spp., Listeria monocytogenes* and *Shigella* were not detected in any sample tested. The mesophilic bacteria had significantly higher counts on day 7 (2.80 CFU/g) compared to the beginning of the shelf life assessment (<1.00 CFU/g). In consequence, the limit of this product is before 7 days of storage.

Table 16. Shelf life assessment of fresh grey mullet fillet with different 'healthy' seasoning and marinades: microbial counts over the shelf life assessment period (7 days)[1].

	Day 0 Log (CFU/g)	Day 2 Log (CFU/g)	Day 5 Log (CFU/g)	Day 7 Log (CFU/g)
Mesophilic bacteria	<1.00*	<1.00*	<1.00*	2.30
Enterobacteriaceae	<1.00*	<1.00*	<1.00*	<1.00*
E.coli	<1.00*	<1.00*	<1.00*	<1.00*
Psycrophilic bacteria	<1.00*	<1.00*	<1.00*	<1.00*
Salmonella	A	A	A	A
Listeria monocytogenes	A	A	A	A
Listeria spp.	A	A	A	A
Shigella	A	A	A	A

[1]Samples were stored at 4ºC for one third of the corresponding estimated period (day 0, day 2, day 5) and the remaining period stored at an abused temperature of 8ºC (day 7). Results are averages of 3 different samples. The presence of *Listeria* and *Salmonella* is determined in 25 g of sample and the rest in 10 g. Letter "A" stands for absence and the asterisk indicates that is below the limit of detection. For more information see material and methods section.

The microorganisms *Salmonella, Listeria spp., Listeria monocytogenes* and *Shigella* were not detected in any sample tested. The mesophilic bacteria had significantly higher counts on day 7 (2.80 CFU/g) compared to the beginning of the shelf life assessment (<1.00 CFU/g). In consequence, the limit of this product is before 7 days of storage.

In order to determine the shelf life, initial characteristics and changes in the quality of fresh grey mullet with different 'healthy' seasoning and marinades were evaluated at different storage points (day 0, day 2, day 5 and day 7) as in the microbial assessment. Few minor changes were observed throughout the storage period (**Table 17**). In consequence, the limit of this product is before 7 days of storage.

Table 17. Sensory evaluation of the characteristic attributes which may change during the storage of the fresh grey mullet fillet with different 'healthy' seasoning and marinades[1]

Quality parameter	Attributes and score	Day 0	Day 2	Day 5	Day 7
Appearance	0: totally homogeneous	0	0	0	**1**
	1: appearance of some grey/oxidized areas				
	2: totally heterogeneous				
Fluid retention capacity	0: no exudate	0	0	1	**2**
	1: intermediate				
	2: intense exudate				
Odour	0: fresh	0	0	0	**1**
	1: mold				
	2: rotten taint				
Texture	0: firm and not slimy	0	0	0	**1**
	1: a little soft and slimy				
	2: very soft and slimy				
Temperature	0: 0ºC < T < 4ºC	0	0	0	**1**
	1: 4ºC < T < 8ºC				
	2: T > 8ºC				
Brightness	0: glossy	0	0	0	**1**
	1: intermediate				
	2: matt				

[1]Samples were stored at 4ºC for one third of the corresponding estimated period (day 0, day 2, day 5) and the remaining period stored at an abused temperature of 8ºC (day 7). The attributes and scores reached at the end of the storage period are highlighted in bold.

Consumer handling/cooking specifications:

It can be consumed pan fried until slightly roasted with a teaspoon of olive oil. Thereafter cover the cooked fillet with the seasoning of preference.

Suggested final presentation:

This product can be retailed with two seasonings in the package and the consumer decides which one to use. In **Fig. 27** a suggested final presentation of the fish prototype with the yoghurt sauce is shown. In **Fig. 28** fish fillet (grey mullet) baked in extra olive oil with ice salt is shown.

Figure 27. Suggested final presentation of the pikeperch "*fresh fillet with different 'healthy' seasoning and marinades*" with yoghurt sauce.

Figure 28. Suggested final presentation of the grey mullet "*fresh fillet with different 'healthy' seasoning and marinades*" with ice salt and extra virgin olive oil.

3.8. Idea 30: Ready-made fish tartar with additional soy sauce (2 products)

Description of the product concept:

Ready-made fish tartar with additional soy sauce for cold serving. Packaging is the golden tray that reflects the colours and physical appearance of the product and that could also be used for serving. Package contains information how the product was made. The product is produced environmentally sustainable (containing ASC label). It is labelled as a premium product; the country of origin is EU.

Reasons for its selection and existence of similar products in the market:

This fish product idea was generated within D28.1 derived from focus groups and occupies the 4th position in the ranking of product concepts (D28.2; score 103.3833). The selected fish species for this idea were pikeperch and greater amberjack. As indicated in D28.3, greater amberjack may be advantageous for raw products (e.g., carpaccio or tartar, ideas 24, 30 and 38 of D.28.2) because of its high fillet fat contents and distinct sensory characteristics (high acid flavour and juiciness). Conversely, pikeperch is characterized by the presence of "earthy" odour and flavour (D28.3) which may limit the success of the pikeperch tartar idea. Notwithstanding, in the same deliverable it is also mentioned that marinades can help masking undesirable attributes and, consequently, it is reasonable to think that this product containing pikeperch may be substantially improved by the addition of soy sauce.

A limited number of fish tartar presentations exists in Europe. Some examples of this idea and other similar to it are given as follows:

Description: iFish Tartar de Atún (Tuna Tartar) and iFish Tartar de Salmón (Salmon Tartar) are now available. The product retails in a 100 g pack that features the Dolphin Safe logo in the tuna version. Both products retail in a 100 g pack. Spain. Stored under refrigeration. Note that there are also frozen versions of the same products.

Ingredients: Tuna version contains *Thunnus albacares* (fished in Central-East Atlantic (FAO 34))), salt, natural flavour. Salmon version contains Atlantic salmon (raised in Norway), salt, natural flavour, sugar.

Description: Bio-verde Lachs-Tartar (Salmon Tartare) contains smoked salmon from organic aquaculture marinated in chili peppers, lemon and pink pepper. The product retails in a 125 g pack bearing the Naturland logo. Germany. Stored under refrigeration.

Ingredients: Smoked salmon from organic aquaculture (79%) and from organic farming: sunflower oil, chili peppers (2%), lemon juice (0.6%), pink pepper (0.2%), and spirit vinegar.

Description: Armoric Saumon Fumé Façon Sashimi (Sashimi Style Smoked Salmon) contains a selection of smoked salmon and nori wrapped salmon slices from fish that have been fed on a non-GMO diet. The ready to serve product retails in a 140 g recyclable pack, sufficient for two servings, including 10 g of Kikkoman soya sauce, 2.5 g of wasabi sauce and wooden sticks. Also available are the following variants: Tartare de Saumon Câpres-Tomate (Caper Tomato Salmon Tartar); and Tartare de Saumon Citron-Basilic (Lemon Basil Salmon Tartar). The pack includes a gravure printed plastic sachet of 2.5 g wasabi, a flexo printed plastic sachet of soya sauce and wooden sticks. France. Stored under refrigeration.

Ingredients: Sashimi (127.5 g): Atlantic salmon (95%) (from fish fed without GMO (<0.9%), salt (2.7%), nori seaweed (2.3%)). Soya sauce (10 g): water, soya beans, wheat, salt. Wasabi (2.5g): horseradish (31.7%), sweetener (E420), rice bran oil, salt, dextrin, *Wasabia japonica* (4.5%), potato starch, water, flavouring, colouring (curcumin), acidifier (E330), thickener (E415), colouring (E133).

Description: Casino Délices 2 Tartares de Saumon Fumé Ciboulette Citron (Smoked Salmon Tartar with Chives & Lemon) are made with beechwood smoked Atlantic salmon, chives and lemon zest. They are guaranteed never frozen, and retail in a 160 g pack containing 2 x 80 g units. France. Stored under refiregration.

Ingredients: Smoked Atlantic salmon (92.5%) (salmon (98%), salt), shallot (3.3%), chives (2.3%), lemon zest (1.7%), dehydrated dill.

Description: Dom Petroff Saumon Fumé en Tartare (Smoked Salmon Tartare) is new to the range. This salmon has been fished in Scotland and Norway and retails in a 140 g pack containing two individual units. France. Stored under refrigeration.
Ingredients: Smoked Atlantic salmon (90%) (salmon, salt), lemon juice, chive, shallot, dill, pepper.

Description: Palais des Mets Sélection Gourmet Saumon Fumé en Tartare (Smoked Salmon Tartare) has been repackaged and now retails in a newly designed 140 g pack containing two units. The gourmet product is fresh and light, ready to eat, and can be served with a sauce or a salad. It has been made with great care using Atlantic salmon from Norway or Scotland. France. Stored under refrigeration.
Ingredients: Smoked salmon (salmon, salt) (90%), lemon juice, chives, shallot, dill, pepper.

To summarize, salmon and tuna are typically used for this kind of product. Although there are other ingredients, this type of product mainly contains fish that is normally cut in cubes of relatively small size. The packaging usually consists in a thermoformed tray.

Technical approach for the final prototype production:

In relation to the fish tartar made with pikeperch, the main concern was to extend the shelf life of the product that is cut in small dices and, if possible, combined with other food components to make it more appealing to consumers. In this regard, the combination of fatty fish species with avocado is common in many dishes on Internet. Therefore, the first step was to examine the shelf life of these ingredients separately.

The fish marinated in soy sauce was within microbiological criteria after 6 days of storage at 4 °C (European Commission). The avocado was stabilized with citric acid at different concentrations (0.12; 0.24, 0.48 and 0.7 mg/g product). In this product, enzymatic browning occurred below 0.48 mg/g after 5 days of storage at 4 °C whereas this did not occur at higher concentrations. However, this product presented other sensory drawbacks such as soft texture and tangy flavour. Therefore, it is possible to elaborate a fish tartar with the soy sauce but without the avocado for a limited period of time.

However, we examined the application of high hydrostatic pressure processing (HPP) on the fish product combined with avocado to improve the shelf life of both ingredients as this was considered a limiting factor. This technology led to an increased hardness and lightness (less dark, more brownish) of the fish. The harder texture is not considered to be a problem at moderate pressures and, as for this, 450 MPa was the selected pressure. Lower pressures were found to be inefficient in the reduction of microbial counts. Colourings and other ingredients were used to improve the flavour of the fish and overcome the unwanted light brown colour. However, colourings were the most efficient in reducing the detrimental effect of HPP on colour.

Many tartars presented a cylinder shape which makes its packaging in a skin pack difficult. This is mainly due to the temperature of the film and the applied vacuum during the packaging of the product. As for this, we employed different amounts of fish gelatine to provide a solid gel at low temperatures and liquid above 18 °C. In addition, the product is cooled down to 0 °C just before packaging in skin in order to maintain the desired shape during the packaging process.

In the case of greater amberjack, no avocado has been used. Fish was marinated shortly in a soya sauce and then prepared as tartar with fresh ingredients. Several tests were done to find out the appropriate container of the tartar without the need of adding extra binding ingredients to keep the cylindrical shape of the preparation. A plastic cylindrical container (food grade polystyrene) was selected for this purpose. Packaging was done with this especially resistant container that could stand the vacuum packing process. Once opened tartar can be consumed directly from the container.

Ingredients:

Pikeperch with avocado: Pikeperch, avocado (optional), onions, soy sauce (water, glucose-fructose syrup, sugar, 12% soya sauce (water, soy sauce, wheat, salt) molasses, salt yeast extract, colouring, plant and spices extracts, lactic acid), olive oil, water, Worcestershire sauce (malt vinegar, alcohol vinegar, molasses, sugar, salt, anchovies, tamarind extract, onion, garlic, spices flavourings), lemon juice (in case that avocado is added) ginger, sesame seeds, gelatine, citric acid (in case that avocado is used; E-330) and colourings (Tartrazine (E102), ponceau 4R (E124), patent Blue (E131), salt).

Greater amberjack: greater amberjack fillet, fresh onions, lime, salt, pepper, olive oil, soya sauce (Water, wheat, salt, sugar), Sherry vinegar (Sherry vinegar, colour (E-150d), antioxidant (E-224), mustard (water, spirit vinegar, mustard, glucose and fructose syrup, sugar, salt, modified starch, stabilisers (guar gum,

xanthan gum), flavourings, turmeric extract, preservative (potassium sorbate) and antioxidant (tocopherol-rich extract).

Manufacturing information:

With respect to tartar made with pikeperch fish:

In a bowl, add 100 g of pikeperch (51.25%) already cut in cubes (1.5 cm approximately), 50 g chopped onions in small cubes (25.63%), 30 g of soy sauce (15.4%), 7.5 g of olive oil (3.84%), 2 g of English Worcestershire sauce (1%), 0.6 g of dried ginger (0.31%) and allow to macerate the whole mixture for 30 min at 4 °C. After that, mix the fish with 0.02 g of brown colouring (Sosa Ingredients, S.L.; Moià, Spain) (0.01%) plus 5 ml of fish gelatine (Trades; Barcelona, Spain) solution (5% w/v) dissolved in warm water (2.56 and 0.13%, for water and fish gelatine, respectively). Note that the brown colouring can be omitted in the formulation. Allow to cool at 4 °C till starting to gel.

In case that we want to elaborate the fish tartar with avocado the preparation continues as follows: Prepare 60 g of avocado (90%) cut in cubes (1.5 x 1.5 cm), add 5 ml of an aqueous solution containing citric acid (0.60 g/5 ml; meaning 0.9% and 1,5% for water and citric acid, respectively) immediately plus 1 ml of lemon juice (1.5%), mix to favour an even distribution and allow to drain at 4 °C for a few minutes.

On the packaging tray place a cylinder mould (7 cm diameter) and, if required, fill with the avocado and press a little. Subsequently, fill the mould with the marinated fish mixture and press again to structure the tartar into the mould. The moulding process of the tartar on the tray is illustrated in **Fig. 29**.

Figure 29. Moulding of the fish tartar.

Decorate the top with sesame seeds and refrigerate until reaching 0-2 °C, remove the cylinder mould and immediately package as described below. Keep the product between 2-4 °C until its processing at a hydrostatic pressure of 450 MPa for 5 min at 7 °C within the same day.

With respect to tartar made with greater amberjack:

Cut the fish fillet (100 g; 65.66%) in cubes of 1-1.5 cm approx. Sprinkle the cubes with ground peel of half lime (1.25 g; 0.82%) and add 0.28 g of salt (0.18 %) and 0.05 g freshly ground black pepper (0.03 %). Allow to macerate for 5 minutes while preserving in the refrigerator (4ºC).

Mix in a bowl 6.25 g of extra virgin olive oil (4.10%), 2.17 g of soya sauce (1.42%), 1.54 g of Sherry vinegar (1.01%) and 3.26 g of mustard (2.14%). Make a homogenous sauce and drizzle it over the amberjack. Toss it to ensure the mixture is well distributed. Allow 5 minutes resting in the fridge (4ºC) while draining excess of liquid. Add 37.50 g of fresh chopped onions (24.62%). Construct the tartar within the appropriate recipient, adding the marinated fish and onions and pressing to have an even distribution in the

recipient. Ground parsley and sesame seeds can be added for garnishment. Place the container (cylinder of 6.5 cm diameter) on the packaging tray.

Product packaging and retail market prototype:

Packaging system:	vacuum skin packaging
Packaging equipment:	thermosealing machine, SMART 500, ULMA Packaging, S. Coop (Oñati, Spain). Settings: Program 10, 210 °C.
Packaging Materials:	black tray: EOST 1523-30. Sealing film: 150 µm, VST 0280, Cryovac (Sealed Air; Charlotte, USA)
Size:	tray: 147 (width) x 132 mm (length) x 30 mm (height)
Recyclable:	yes

Figure 30. Physical prototype of the "*ready-made fish tartar with additional soy sauce*" made with pikeperch and avocado after processing at high hydrostatic pressures (450 MPa, 5 min, 7 °C).

Figure 31. Physical prototype of the "*ready-made fish tartar with additional soy sauce*" made with greater amberjack after packing under vacuum.

Physicochemical properties:

With respect to pikeperch with avocado:

Moisture: 73.90%; water activity: 0.979; pH: 5.49

With respect to greater amberjack:

Moisture (%): 69.01; pH: 6.05

Nutrition facts:

Expressed in 100 g of product (pikeperch fish plus avocado):

Energy: 193 Kcal or 510 KJ

Protein (g): 11.70

Lipids (g): 5.30 of which saturated (g): 1.16

Carbohydrates (g): 6.76 of which sugars (g): 4.8

Salt (g): 1.54

Expressed in 100 g of product (greater amberjack):

Energy: 193 Kcal or 804 KJ

Protein (g): 21.20

Lipids (g): 12 of which saturated (g): no detected

Carbohydrates (g): no detected of which sugars (g): not detected

Salt (g): 1.34

Potential allergens:

Soya sauce, sesame seeds, mustard and Sherry vinegar (may contain sulphites).

Storage conditions:

Pikeperch:

Store between 0-2°C.

Greater amberjack:

Store between 0-4°C.

Microbiological and sensory shelf life assessment:

In this report we considered two fish species (greater amberjack and pikeperch).

Pikeperch:

As commented before, two different prototypes of this fish species were prepared: the marinated pikeperch and the marinated pikeperch with avocado, which undergoes further high hydrostatic pressure processing (HPP). The shelf life assessment of the fish and the different seasonings and marinades were carried out according to the recommendations (EURL, 2014; European Commission). With respect to pikeperch alone, this period of storage under refrigeration conditions (<3 °C) was estimated to be of 6 days whereas the fish plus avocado processed by means of HPP was estimated to be of 15 days. Accordingly, in each case the first one third of the estimated shelf life was stored at 4 °C and the rest of the estimated period was at an abuse temperature of 8 °C. Results shown in **Table 18** indicate that the pikeperch tartar with additional soy sauce and not submitted to HPP met microbial requirements after 6 days of cold storage and suggest that longer shelf life may be given to this product. This fact can be due to the presence of organic acids that come from soy sauce and thus efficiently contribute to the product stability.

Despite that, it is important to remember the recommendations given on the safe production of chilled foods packaged under vacuum and MAP with respect to *C. botulinum* (Peck et al., 2006). Therefore, the refrigeration temperature below 3 °C should be guaranteed. It is recommended to not extend its shelf life to more than 10 days as temperature abuse may occur during retail, purchase and home storage. Regarding this issue, a pH reduction of the product below 5 is also advisable to reduce this risk especially in the pikeperch tartar with the avocado that is stored for 15 days. In this particular case, a challenge test or the use of predictive microbiology is recommended to establish the safety of this product idea as spores may resist the proposed HPP treatment. **Table 19** shows the microbial counts of the pikeperch tartar that received a hydrostatic pressure of 450 MPa for 5 min (7 °C). The effect of this treatment on microbial counts can be observed through the comparison of these results with those of the previous table.

With respect to sensory characteristics, the characteristic attributes of the fish tartar made with pikeperch that can vary during the storage of the pikeperch tartar product that is not submitted to HPP are reported in **Table 20**. These attributes were monitored during the storage (days 1 and 6) of this product idea to determine the shelf life of the product. At the end of the studied storage time, an important loss of the global quality was observed in this fresh tartar (without the application of HHP) especially in terms of fish texture as well as aroma and flavour balance. The flavour of cooked/pungent onion appears and was dominant. Taking these results into consideration the shelf life of this product should be limited to a maximum of 6 days, which is therefore quite limiting for retailers and producers.

Table 18. Shelf life assessment of the pikeperch tartar with additional soy sauce: microbial counts over the shelf life assessment periods. [1]

	Day 1 Log (ufc/g)	Day 6 Log (ufc/g)
Lactic acid bacteria	2.24	2.06
Mesophilic bacteria	4.23	3.98
Enterobacteriaceae	2.22	1.92
Psycrophilic bacteria	3.53	3.10
Listeria monocytogenes	A	A

[1] Samples were stored at 4 °C for one third of the corresponding estimated period and the remaining period stored at an abuse temperature of 8 °C. Results are averages of 5 different samples. The presence of *Listeria* and *Salmonella* is determined in 25 g of sample and the rest in 10 g. Letter "A" stands for absence and the asterisk indicates that is below the limit of detection. For more information see material and methods section.

Table 19. Shelf life assessment of the pikeperch tartar with additional soy sauce submitted to high hydrostatic pressure processing (HPP): microbial counts over the shelf life assessment periods. [1]

	Day 1 Log (ufc/g)	Day 7 Log (ufc/g)	Day 15 Log (ufc/g)
Lactic acid bacteria	0.76	0.20	1.22
Mesophilic bacteria	2.48	2.30	2.56
Enterobacteriaceae	<1.00*	<1.00*	<1.00*
Psycrophilic bacteria	0.40	<1.00*	<1.00*
Listeria monocytogenes	A	A	A

[1] Samples were stored at 4 °C for one third of the corresponding estimated period and the remaining period stored at an abuse temperature of 8 °C. Results are averages of 5 different samples. The presence of *Listeria* and *Salmonella* is determined in 25 g of sample and the rest in 10 g. Letter "A" stands for absence and the asterisk indicates that is below the limit of detection. For more information see material and methods section.

As for the fish tartar made with pikeperch plus avocado and submitted to HPP, the different components of the product were evaluated separately at different storage periods and up to 14 days. **Table 21** shows the characteristic attributes that were monitored during the storage of this product idea as they were considered to be susceptible to change during the shelf life of the product. After 14 days, some detrimental attributes regarding the vegetal component were recorded. As shown in the table, the avocado became brown, sour as well as soft and pasty. In addition, the flavour of the fish component turned out to be unbalanced.

Table 20. Sensory evaluation of the characteristic attributes which may change during the storage of the "*ready-made fish tartar with additional soy sauce*" idea made with pikeperch fish and not submitted to high hydrostatic pressure processing.

Quality parameter by ingredients		**Score**
Appearance	Fish colour	**0: light brown colour**
		1: intermediate
		2: oxidized colour
	Onion	0: white and opaque
		1: a little coloured and transparent
		2: totally coloured and transparent
	Fluid retention capacity	0: no exudate
		1: intermediate
		2: intense yellowish exudate
	Soya sauce penetration	0: superficial
		1: intermediate
		2: total penetration
Odour	Fish	0: fresh
		1: initial appearance of off-flavours
		2: rotten taint
	Onion	0:fresh and intense

		1: cooked onion
		2: pungent (sulphurous)
	Overall	0: balanced
		1: intermediate
		2: unbalanced
Flavour	Fish	0: fresh
		1: mold
		2: rotten taint
	Onion	0:fresh and intense
		1: cooked onion
		2: pungent (sulphurous)
	Overall	0: balanced
		1: intermediate
		2: unbalanced
Texture	Fish	0: firm and juicy
		1:firm but less juicy
		2: soft and not juicy
	Onion	**0: firm and crunchy**
		1: intermediate
		2: soft and not crunchy

[1] Fish was stored for 2 days at 4 °C and the remaining period (up to 6 days) stored at an abuse temperature of 8 °C. In both cases, the attributes and scores reached at the end of the storage period are highlighted in bold.

Table 21. Sensory evaluation of the characteristic attributes which may change during the storage of the "*ready-made fish tartar with additional soy sauce*" idea made with pikeperch fish plus avocado and submitted to high hydrostatic pressure processing.

Quality parameter by ingredients		Attributes and score
Appearance	Fish colour	0: light brown colour
		1: intermediate
		2: oxidized colour
	Onion	0: white and opaque
		1: a little coloured and transparent
		2: totally coloured and transparent
	Fluid retention capacity of fish	0: no exudate
		1: intermediate
		2: yellowish exudate
	Soya sauce penetration	0: superficial
		1: intermediate
		2: total penetration
	Avocado	0: bright green and yellow colour
		1: some brownish areas

		2: totally brown
Odour	Fish	0: fresh **1: initial appearance of off-flavours** 2: rotten taint
	Onion	0: fresh and intense **1: cooked onion** 2: pungent (sulphurous)
	Overall	0: balanced 1: intermediate **2: unbalanced**
	Avocado	0: fresh/grassy 1: odourless **2: sour/off-odours**
Flavour	Fish	0: fresh **1: mold** 2: rotten taint
	Onion	0:fresh and intense **1: cooked onion** 2: pungent (sulphurous)
	Overall	0: balanced 1: intermediate **2: unbalanced**
	Avocado	0: fresh/grassy 1: odourless **2: sour/off-flavours**
Texture	Fish	0: firm and juicy **1:firm but less juicy** 2: soft and not juicy
	Onion	0: firm and crunchy **1: intermediate** 2: soft and not crunchy
	Avocado	0: firm and creamy 1: creamy and little soft **2: soft/doughy**

[1] Fish was stored for 5 days at 4 °C and the remaining period (up to 14 days) stored at an abuse temperature of 8 °C. In both cases, the attributes and scores reached at the end of the storage period are highlighted in bold.

As commented before, the application of high hydrostatic pressure processing (HPP) leads to an increased texture in fish which can be an interesting feature up to certain level since this fish species was found to have a low chewiness compared to others (D28.3). Despite that, the shelf life of this product can be set between 7 and 15 days as various sensory attributes were found to decay to unacceptable limits (highlighted in bold)

within this storage period. The decrease in the hardness and browning of the avocado can be attributed to the residual enzyme activity. The changes that were recorded in the fish were considered of less importance. Therefore, it is suggested to increase the pressure in the HPP or not to use the avocado in the elaboration of this product idea.

Greater amberjack:

For the shelf life assessment and similarly to the pikeperch prototypes, the product was stored at 4 °C which is the maximum recommended temperature (Betts et al., 2004). **Table 22** shows the microbial stability of the product throughout the storage period whereas spoilage bacteria may be the main determinants of the shelf life of this product.

Table 22. Shelf life assessment of the ready-made greater amberjack tartar with additional soy sauce: microbial counts over the shelf life assessment period[1].

	Day 0 Log (CFU/g)	Day 1 Log (CFU/g)	Day 2 Log (CFU/g)
Mesophilic bacteria	2.00	3.93	4.40
Enterobacteriaceae	2.79	3.24	3.62
E.coli	<1.00*	<1.00*	<1.00*
Psycrophilic bacteria	2.20	3.19	3.19
Salmonella	A	A	A
Listeria monocytogenes	A	A	A
Listeria spp.	A	A	A
Shigella	A	A	A

[1]Samples were stored at 4ºC. Results are averages of 3 different samples. The presence of microorganisms is determined in 25 g of sample. Letter "A" stands for absence and the asterisk indicates that is below the limit of detection. For more information see material and methods section.

The microorganisms *Salmonella, Listeria spp., Listeria monocytogenes* and *Shigella* were not detected in any sample tested. The mesophilic bacteria had significantly higher counts on day 2 (4.40 CFU/g) compared to the beginning of the shelf life assessment (2.00 CFU/g). The same behaviour was observed in psychrophilic bacteria and enterobacteriaceae. In consequence, the limit of this product is before 1 day of storage.

With respect to sensory attributes, changes in the quality parameters of the greater amberjack tartar with additional soy sauce were evaluated for the same time as in the microbial assessment. **Table 23** shows the different attributes that are expected to decay in this product according to the literature (Betts et al., 2004; Man & Jones, 1994; Robertson, 2009). According to the results, 2 days can be considered as the limit of acceptability at sensory level.

Table 23. Sensory evaluation of the characteristic attributes which may change during the storage of the ready-made greater amberjack tartar with additional soy sauce [1]

Quality parameter	Attributes and score	Day 0	Day 1	Day 2
Appearance	0: totally homogeneous	0	0	1
	1: appearance of some grey/oxidized areas			
	2: totally heterogeneous			
Fluid retention capacity	0: no exudate	0	0	0
	1: intermediate			
	2: intense exudate			
Odour	0: fresh	0	0	1
	1: mold			
	2: rotten taint			
Texture	0: firm and not slimy	0	0	1
	1: a little soft and slimy			
	2: very soft and slimy			
Temperature	0: 0ºC < T < 4ºC	0	0	0
	1: 4ºC < T < 8ºC			
	2: T > 8ºC			
Brightness	0: glossy	0	0	0
	1: intermediate			
	2: matt			
Sauce	0: good	0	0	0
	1: poor sauce			
	2: excess			

[1]Samples were stored at 4ºC. The attributes and scores reached at the end of the storage period are highlighted in bold.

Consumer handling/cooking specifications:

These products need to be stored under refrigeration until its consumption. The product can be consumed in the same tray or alternatively serve it in a plate when still cold as it will maintain its original form.

Suggested final presentation:

With respect to the fish tartar made with pikeperch a suggested final presentation is shown in **Fig. 32.** Note that the avocado can be added by the consumer in case that this is not provided in the retailed product. Greater amberjack tartar is shown in **Fig. 33**.

Figure 32. Suggested final presentation of the "*ready-made fish tartar with additional soy sauce*" physical prototype made with pikeperch.

Figure 33. Suggested final presentation of the "*ready-made fish tartar with additional soy sauce*" physical prototype made great amberjack.

3.9. Idea 33: Ready-made fish fillets in olive oil

Description of the product concept:

Ready-made fish fillets stored in olive oil with visible glass packaging. Product message: 'Tradition'. It is labelled as a premium product; the country of origin is EU.

Reasons for its selection and existence of similar products in the market:

This fish product idea was generated within D28.1 derived from focus groups and occupies the 25th position in the ranking of product concepts (D28.2; score 97.10000). Grey mullet was suggested for this idea due to its technical compatibility in D28.2. This fish species was found to have a bitter flavour and hard texture (D28.3). These characteristics can be therefore in line with the characteristic bitterness of some virgin olive oils and the hard texture of tuna, which is commonly sold in that kind of packaging.

In this regard it is important to note that in Europe the number of filleted tuna species (albacore, yellowfin, bonito) and anchovies stored in olive and packaged in glass containers is huge. Some examples of these products and other are shown as follows:

Description: Bonpreu Tonyina Clara en Oli d'Oliva (White Tuna in Olive Oil) has been repackaged with a new look. The product retails in a 190 g pack. Spain. Stored at room temperature.

Ingredients: White tuna, olive oil, salt.

Description: Rewe Feine Welt Weißer Thun in Öl (White Tuna Fish in Oil) is made from tuna of certified fishery and with Spanish olive oil. The light colour, firm meat structure and tender flavour are said to be the characteristics of this valuable tuna from the Pacific, which has been canned by hand. The MSC certified and dolphin safe product retails in a 190 g jar. Germany. Stored at room temperature.
Ingredients: White tuna fish (66%) (*Thunnus alalunga*), olive oil (33%), table salt

Description: Marque Repère Ronde des Mers Filets d'Anchois Allongés a l'Huile d'Olive (Anchovy Fillets in Olive Oil) are now available. The product retails in a 105 g jar. France. Stored at room temperature.
Ingredients: Salted anchovy fillets (62%) (anchovy, salt), olive oil (38%).

Description: Bontà del Pescatore Filetti di Tonno all'Olio di Oliva (Tuna Fillets in Olive Oil) comprise yellowfin tuna fillets that have a typical pink colour, and are described to be tender and tasty. The product retails in a 190 g jar. Italy. Stored at room temperature.
Ingredients: Yellowfin tuna (*Thunnus albacares*), olive oil, salt.

Description: Emperatriz Filetti di Sgombro in Olio di Oliva (Mackerel Fillets in Olive Oil) are now available. The product retails in a 250 g jar. Italy. Stored at room temperature.
Ingredients: Mackerel (72%), olive oil, salt.

Description: Sebastiano Drago Filetti di Pesce Spada all'Olio di oliva (Swordfish Fillets in Olive Oil) are produced in Sicily. The preservative-free product retails in a 200 g jar. Italy. Stored at room temperature.
Ingredients: Swordfish, olive oil, salt

Description: Eroski Bonito del Norte en Aceite de Oliva (Albacore Tuna in Olive Oil) has been repackaged. The product is high in protein, and retails in a 220 g pack that features the Dolphin Safe logo. Spain. Stored at room temperature.
Ingredients: Albaroce tuna (*Thunnus alalunga*), olive oil, salt

Description: Frantoio di Sant'Agata d'Oneglia Sapori del Mare Baccalà Grigliato (Grilled Codfish) is now available. This product is described as an excellent main course, or can be served in salads, as a starter or aperitif. It retails in a 280 g pack. Italy. Stored at room temperature.
Ingredients: Codfish, extra virgin olive oil (in origin), parsley, garlic, sea salt.

Description: OR.PA.GU Lomos de Pez Espada en Aceite de Oliva (Swordfish Fillets in Olive Oil) are now available. The product is a source of omega 3, and retails in a 230 g pack. Spain. Stored at room temperature.
Ingredients: Swordfish, olive oil, salt

Description: To.Eat [Da Mangiare] Filetti di Salmone in Olio d'Oliva (Salmon Fillets in Olive Oil) are now available. These hand crafted, steamed fillets retail in a 200 g pack. Italy. Stored at room temperature.
Ingredients: Salmon fillets, olive oil (30%), sea salt

Description: Costa Vasca Lomos de Bacalao en Aceite de Oliva (Codfish Fillets in Olive Oil) are now available. The premium product retails in a 200 g pack. Spain. Stored at room temperature.
Ingredients: Codfish, olive oil, salt.

Description: Ortiz El Velero Sardines in Organic Olive Oil have been elaborated from fresh fish which has been hand cleaned and fried in olive oil in an old-fashioned way so that it becomes more tender and creamier. The product retails in a 190 g pack. Spain. Stored at room temperature.
Ingredients: Sardines (*Sardina pilchardus*), organic extra virgin olive oil (26%), salt.

Description: Delicius Rolled Anchovy Fillets in Olive Oil with Pantelleria Capers are now available. The product retails in a 90 g jar. UK. Stored at room temperature.
Ingredients: Anchovies, olive oil, capers (12%), salt

Overall, the majority of these products are of higher quality and mainly sold in Spain and Italy. Although tuna and anchovy are mainly used in this type of product, other fish species are also available in the European market. The basic ingredients are fish, olive oil and salt, however other ingredients and flavouring can be also present in the formulation.

Technical approach for the final prototype production:

Preservation of fish in olive oil is a very popular method of preserving fish for human consumption and provides a shelf life that can range from one to five years. Fish is usually processed (filleted), sealed in airtight container (glass container in this case) and heated for sterilization of the product. The use of olive oil as filling media has an important impact on the final quality of the product. A protective effect of extra virgin olive oil against lipid oxidation in canned tuna has been found (Naseri, Rezaei, Moieni, Hosseini, & Eskandari, 2011). Extra virgin olive oil is reach in tocopherols, which have a protective effect against lipid oxidation.

A side effect of canning/bottling can be a change in the nutritional value of the fish, when canned/bottled with added oil (Sampels, 2015a). In general, there is a decrease of the n-3/n -6 ratio when different plant oils are used.

Ingredients:

Grey mullet fillet, extra virgin olive oil.

Manufacturing information:

For the preparation of the ready-made grey mullet fillet in olive oil, the fish has been processed in order to obtain clean fillets that have been deboned and salted (2 g of sea salt per 225 g of fish fillet). Fillets are then cut in adequate portions to guarantee correct sterilization in the complete piece of fish during the sterilization process (**Fig. 34**). Glass containers of 250 ml with metallic covers (with ceramic lining) specifically designed for sterilization by heat, have been used. The containers were filled with adequate portions of grey mullet fillet. A total of 220 to 225 g of grey mullet fillet was used per bottle. Once the glass container has been filled with fish pieces, extra virgin olive oil is added till all the fish is covered with oil. Containers are closed and sterilized during 40 minutes in a pressure cooker. For the prototype preparation this method was considered adequate for the amount of product to be prepared. A pilot size sterilizing equipment will be used for the preparation of bigger amount of product needed for the deliverable D29.3.

Figure 34. Portions of grey mullet clean fillets that have been deboned ready for the sterilization process.

Product packaging and retail market prototype:

Packaging Materials: transparent glass jars with lid.

Size: 250 ml volume

Recyclable: yes

The product is packed in a transparent glass bottle, which allows seeing the piece of fish and the olive oil inside. The product message is a traditional product, premium quality.

Physicochemical properties:

Determined in fish only:

Moisture = 80.11%; pH = 5.90

Nutrition facts:

Expressed in 100 g of product:

Energy: 1054 Kcal or 253 KJ

Protein (g): 24

Lipids (g): 18 of which saturated (g): traces

Carbohydrates (g): not detected of which sugars (g): not detected

Salt (g): 0.16

Potential allergens:

Fish is the only ingredient

Storage conditions:

Store at room temperature.

Microbiological and sensory shelf life assessment:

The shelf life assessment of the ready-made fish fillets in olive oil was carried out according to the given recommendations (EURL, 2014; European Commission). The product was stored at room temperature till the end of the study period (12 days). Results are shown in **Table 24**.

Table 24. Shelf life assessment of ready-made fish fillets in olive oil: microbial counts over the shelf life assessment period (12 days). [1]

	Day 0 Log (CFU/g)	Day 5 Log (CFU/g)	Day 12 Log (CFU/g)
Mesophilic bacteria	<1.00*	<1.00*	<1.00*
Enterobacteriaceae	<1.00*	<1.00*	<1.00*
E.coli	<1.00*	<1.00*	<1.00*
Psycrophilic bacteria	<1.00*	<1.00*	<1.00*
Salmonella	A	A	A
Listeria monocytogenes	A	A	A
Listeria spp.	A	A	A
Shigella	A	A	A

[1] Samples were stored at room temperature during 12 days. Results are averages of 3 different samples. The presence of *Listeria* and *Salmonella* is determined in 25 g of sample and the rest in 10 g. Letter "A" stands for absence and the asterisk indicates that is below the limit of detection. For more information see material and methods section.

In order to determine the shelf life, initial characteristics and changes in the quality of ready-made fish fillets in olive oil were evaluated at different storage points (day 1, day 5 and day 12). The analysis was carried out for only 12 days (**Table 25**), but the typical shelf life of this product ranges from one to five years (Sampels, 2015a).

Table 25. Sensory evaluation of the characteristic attributes which may change during the storage of the ready-made fish fillets in olive oil[1]

Quality parameter	Attributes and score	Day 0	Day 5	Day 12
Appearance	0: totally homogeneous	0	0	**0**
	1: appearance of some grey/oxidized areas			
	2: totally heterogeneous			
Oil odour	0: fresh	1	1	**1**
	1: intermediate			
	2: intense			
Texture	0: firm and not slimy	1	1	**1**
	1: a little soft and slimy			
	2: very soft and slimy			
Brightness	0: glossy	0	0	**0**
	1: intermediate			
	2: matt			

[1] Samples were stored at room temperature during 12 days. The attributes and scores reached at the end of the storage period are highlighted in bold.

Consumer handling/cooking specifications:

No further cooking is needed for this product. It can be used directly in a salad or any other natural (no cooked) preparation.

Suggested final presentation:

Final retail market and serving presentation are depicted in **Figs. 35** and **36**.

Figure 35. Prototype of ready-made grey mullet fillet in olive oil.

Figure 36. Suggested presentation for ready-made grey mullet fillet in olive oil with fresh tomato and salad.

3.10. Idea 34: Fresh fish steak for grilling in the pan

Description of the product concept:

Fresh fish steak for grilling in the pan. Transparent packaging. The product is produced environmentally sustainable (containing ASC label). It is labelled as a premium product; the country of origin is EU.

Reasons for its selection and existence of similar products in the market:

This fish product idea was generated within D28.1 derived from focus groups and occupies the 33rd position in the ranking of product concepts (D28.2; score 95.5000). Greater amberjack is a fast grower and thus suggested for this idea. In addition, it has distinct sensory characteristics (high acid flavour and juiciness) as reported in D28.3 and high fillet fat contents (Rodriguez-Barreto et al., 2012; Thakur, Morioka, Itoh, Wada, & Itoh, 2009). These particular traits make it very interesting for grilling.

In the European market there are a considerable number of fish products that are sold in transparent packaging and stated to be ideal for grilling. Some examples of this and related kind of product are the following:

Description: Open BBQ Season Grill-Lachsfilet mit Haut Kräuter der Provence (Grill Salmon Fillet with Skin and Herbs de Provence) is made using salmon from aquaculture in Norway and can be cooked in the pan or on the grill. This product retails in a 250 g pack featuring a QR code. Germany. Stored under refrigeration.

Ingredients: salmon fillet (99%) with skin, rosemary, thyme, basil, marjoram, parsley, savory, oregano, chervil, lovage.

Description: BellaMare Grill Lachs Venezia (Venetian Style Grilling Salmon) is marinated with Italian herbs, and can be prepared in the frying pan. The premium quality product retails in a 125 g pack. Austria. Stored under refrigeration.

Ingredients: Salmon, salt, herb-marinade (0.35%) (tomatoes, garlic, parsley, basil, oregano, chilli), acidity regulators (E261, E326).

Description: BBQ Zeit Zum Grillen Garnelen Spiesse Provence (Provençal Style Prawn Skewers) have been repackaged and are now available in a newly designed 0.185 kg pack featuring the Check Your Product logo and a QR code. These precooked prawns from aquaculture in Vietnam feature a fine herb marinade are ready for frying and grilling. Austria. Stored under refrigeration.

Ingredients: Peeled prawns (*Panaeus vannamei*) (from Vienamese aquaculture), vegetable oil (rapeseed oil, palm fat), spices, salt, seasoning, spice extracts, flavouring, dextrose, spinach powder, stabilisers (triphosphate, polyphosphate), acidifiers (sodium lactate, sodium acetate).

Description: Delpierre Barbecue Rouget Barbet Cinnabare & Marinade Herbes de Provence (Striped Mullet & Provence Herb Marinade) has been prepared in France and is bone-free. This ready-to-cook product can be barbecued or oven cooked in 10 minutes and retails in a 270 g pack that includes an aluminium grilling dish for clean and easy cooking. France. Stored under refrigeration.

Ingredients: Salted striped mullet with decoration (salted striped mullet (90%) (striped mullet (contains *Parupeneus heptacanthus*), water, salt (1.5% on average), preservatives (E326, E261, E202), acidifiers (citric acid, sodium citrate)), lemon slices (8%), parsley (0.8%), red pepper (0.4%), thyme, basil, chive (0.2%), garlic (contains sulphites)), marinade (rapeseed oil, virgin olive oil, natural lemon flavouring (1%), Provence herbs (1%)).

Description: Labeyrie Sardinenfilets mit Zitronen und Kräuter "Provençale" (Provençal Style Lemon & Herbs Flavoured Sardine Fillets) are now available in the range. They are said to be ideal for grilling and are ready in eight minutes. The product is retailed in a 375 g pack containing a separate marinade sachet. Austria. Stored under refrigeration.

Ingredients: Salted garnished sardines: salted anchovies (98%) (sardines, water, salt (2%), preservatives (E326, E261, E202), acidifier (citric acid)), Provençal herbs (0.4%), lemon peel (0.4%), spices, flavourings. Marinade: rapeseed oil, virgin olive oil, natural lemon flavouring, herbs.

Description: Minna Laks Side (Salmon Side) comes from Norwegian aquaculture with high requirements for the feed and environment. The product is suitable for pan frying, oven baking and grilling, and retails in a 700 g pack featuring the Green Keyhole logo. Denmark. Stored under refrigeration.

Ingredients: Salmon (with skin, no bones)

Description: Royal Bacalao Solomillo en Rodajas al Punto de Sal (Salted Cod Fillet Slices) are now available. These boneless slices are said to be ideal for grilling or breading, have been hook-caught in the Northwest Atlantic, and retail in a 300 g pack containing 12 x slices and featuring the Facebook, Twitter, and YouTube logos. Spain. Frozen

Ingredients: Codfish (*Gadus morhua*), salt

Description: Grillmeister Grill-Lachs Pfeffer-Knoblauch (Grilling Salmon with Pepper & Garlic) is a salmon fillet with skin and spiced with pepper, garlic and paprika. The salmon originates from the intensive aquaculture in Norway. The product retails in a 250 g pack. Germany. Stored under refrigeration.

Ingredients: Salmon fillet with skin (98%), table salt, pepper (red pepper, white pepper, black pepper), paprika, garlic.

Description: Edeka Selection Wolfsbarschfilets (Sea Bass Fillets) are a premium product described as lean meat that is finely aromatic in taste. According to the company, this fish is perfect in a Mediterranean cuisine. It has been caught and hand picked from the Ionian Sea in Greece. They come glazed, with skin and without bones. This vacuum packed product is said to be ideal for frying or grilling but it can also be oven baked. It retails in a 310 g pack containing four fillets. Germany. Frozen.

Ingredients: Sea bass, water (protective glazing)

Description: Apetit Grillivalmis Kirjolohifilee Yrtti-Valkosipuli (Barbecue Ready Rainbow Trout Fillet with Herb & Garlic) is produced from rainbow trout farmed in Sweden, and seasoned with a selection of herbs and garlic. This product is gluten-free, rich in protein, and suitable for baking or grilling. It is retailed in a 580 g pack. Finland. Stored under refrigeration.

Ingredients: Rainbow trout (94%), spices (herbs (0.1%) (dill, chives, marjoram), onion, garlic (0.2%), leek, black pepper), salt, acidity regulator (E326, E262), glucose, flavouring

Overall, salmon is the most common for grilling although it is also normal to find other fish species such as trout, sardine and striped mullet. Typical packagings in fresh fish are skin pack and trays. They are commonly sold with herbs and spices to give the desired flavour in the final product.

Technical approach for the final prototype production:

Greater amberjack has been appointed in D28.3 *Report on product and process solutions* as a very suitable species to be used for raw preparations products due to its high fillet fat contents and distinct sensory characteristics.

Fish is often considered to be a difficult culinary object due to the fact that it is easily spoiled, prone to oxidation and it may develop off-flavours due to wrong handling or incorrect storage. It is very important to

preserve the freshness and the high nutritional value of fish to keep the product on temperature slightly above 0ºC from harvest till processing or consumption (Sampels, 2015b). Cooling should start as soon as possible after killing the fish. Ice flakes are normally used in the farms and more recently, ice slurry is also in use. This water-ice system reaches temperatures below zero faster and provides to the fish additional protection against oxidation. Also the fact that the ice particles are smaller than flakes decreases the physical damage on the fish.

For the preparation of the product, greater amberjack of average body weight of 1.8 to 2 kg were selected for filleting and obtaining a clean, de boned portion of fish fillet ready for the pan.

Ingredients:

Greater amberjack fillets

Manufacturing information:

For the preparation of the fresh fish steak for grilling in the pan, fresh greater amberjack were descaled and eviscerated. Fillets are obtained and cleaned thoroughly to eliminate any traces of blood. Belly flaps are removed and a nice fillet piece is ready to be cut in portions and packed. Different number of portions can be included per package. The product is then vacuum packed.

Figure 37. Fresh greater amberjack fillet ready to be cooked on the pan.

Product packaging and retail market prototype:

Packaging system:	vacuum packaging
Packaging equipment:	vacuum packaging machine, EDESA VAC-40DT
Design:	individually packed portion in bag sealed by thermo sealing.
Packaging material:	90 µm polyamide/polyethylene bag (Orved, Italy)
Size:	30 mm x 20 mm

Recyclable: yes

Physicochemical properties:

Moisture = 80.11%; pH = 5.90

Nutrition facts:

Expressed in 100 g of product:

Energy: 127 Kcal or 538 KJ

Protein (g): 25.2

Lipids (g): 2.5 of which saturated (g): not detected

Carbohydrates (g): not detected of which sugars (g): not detected

Salt (g): 0.29

Potential allergens:

Fish is the only ingredient.

Storage conditions:

Store refrigerated at 2-3ºC.

Microbiological and sensory shelf life assessment:

The microorganisms *Salmonella, Listeria spp., Listeria monocytogenes* and *Shigella* were not detected in any sample tested (**Table 26**). The mesophilic bacteria had significantly higher counts on day 5 (3.10 CFU/g) compared to the beginning of the shelf life assessment (<1.00 CFU/g). According to the results shown in the table, the product had good microbiological qualities from the first day to 5 days of storage.

In order to determine the shelf life, initial characteristics and changes in the quality of fresh greater amberjack steak were evaluated at different storage points (day 0, day 2, day 5 and day 7) as in the microbial assessment. As shown in Table 27, various attributes as odour and appearance decayed after 7 days of storage and thus modified the sensory properties of the product.

Table 26. Shelf life assessment of fresh greater amberjack steak for grilling in the pan: microbial counts over the shelf life assessment period (7 days)[1].

	Day 0 Log (CFU/g)	Day 2 Log (CFU/g)	Day 5 Log (CFU/g)	Day 7 Log (CFU/g)
Mesophilic bacteria	<1.00*	2.30	3.10	3.80
Enterobacteriaceae	1.50	2.00	2.30	3.00
E.coli	<1.00*	<1.00*	<1.00*	<1.00*
Psycrophilic bacteria	<1.00*	<1.00*	<1.00*	<1.00*

Salmonella	A	A	A	A
Listeria monocytogenes	A	A	A	A
Listeria spp.	A	A	A	A
Shigella	A	A	A	A

[1]Samples were stored at 4ºC for one third of the corresponding estimated period (day 0, day 2, day 5) and the remaining period stored at an abused temperature of 8ºC (day 7). The attributes and scores reached at the end of the storage period are highlighted in bold.

Table 27. Sensory evaluation of the characteristic attributes which may change during the storage of the fresh greater amberjack steak for grilling in the pan [1]

Quality parameter	Attributes and score	Day 0	Day 2	Day 5	Day 7
Appearance	0: totally homogeneous	0	0	0	**1**
	1: appearance of some grey/oxidized areas				
	2: totally heterogeneous				
Fluid retention capacity	0: no exudate	0	1	1	**2**
	1: intermediate				
	2: intense exudate				
Odour	0: fresh	0	0	1	**2**
	1: mold				
	2: rotten taint				
Texture	0: firm and not slimy	0	0	0	**1**
	1: a little soft and slimy				
	2: very soft and slimy				
Temperature	0: 0ºC < T < 4ºC	0	0	0	**1**
	1: 4ºC < T < 8ºC				
	2: T > 8ºC				
Brightness	0: glossy	0	0	0	**1**
	1: intermediate				
	2: matt				

[1]Samples were stored at 4ºC for one third of the corresponding estimated period (day 0, day 2, day 5) and the remaining period stored at an abused temperature of 8ºC (day 7). The attributes and scores reached at the end of the storage period are highlighted in bold.

Consumer handling/cooking specifications:

Greater amberjack fillets should be prepared as soon as possible since the product has a very limited shelf life.

Open the vacuum package and place the fish on a pan with olive oil. Grilling time will depend on the thickness of the fillet and personal taste. Some recommendations indicate that fish can be cooked to preference or pink in the middle since microorganisms are located in the outer part of the fish but not inside. In the case of this product, the processing of the fish includes handling for filleting, which it has to be done following the HACCP protocol in order to provide a safe product. Nevertheless, it is advisable to grill enough time so the whole fillet is cooked.

Suggested final presentation:

Two preparations have been selected here from kitchen books, Internet and own recipes (**Table 28**). A greater amberjack fillet ready for cooking is shown in **Fig. 38**.

Table 28. Description of recipes to prepare fish meals from fresh greater amberjack steak for grilling in the pan

Recipe and Ingredients	Preparation
"Grilled greater amberjack with country-style Dijon Cream Sauce":	For 4 persons
4 greater amberjack fillets (about ¾ inch thick)	Combine steak seasoning and tarragon in a small bowl; set aside.
1 lemon	Heat a grill pan over medium-high heat. Coat pan with cooking spray. Coat fillets with cooking spray, and rub with seasoning mixture. Add fish to pan.
Cream sauce (Country-Style Dijon)	Cook 3 to 4 minutes on each side or until fillets flake easily when tested with a fork.
1 ½ tablespoon fresh tarragon (chopped)	While amberjack cooks, grate 1 teaspoon rind from lemon; squeeze juice to measure 1 tablespoon. Reserve lemon rind for Country-Style Dijon Cream Sauce.
Cooking spray	Place 1 fillet on each of 4 serving plates. Drizzle fillets evenly with lemon juice, and top a dollop of cream sauce.
2 tsps salt-free steak grilling blend	Serve with green asparagus lightly sautéed
"Lemon butter Amberjack Fish":	For 6 persons
6 fillets amberjack	Lightly salt and pepper six greater amberjack fillets. Bring a large
1 ½ tablespoon unsalted butter	Non-stick pan to medium-high heat and add butter (add enough to heavily coat the bottom of the pan). When hot lay fish down (skin

Recipe and Ingredients	Preparation
	side first if it still has skin). Squeeze a wedge of lemon over each
1 lemon, wedged into 6 pieces	Piece and toss in six thyme sprigs (reserve the other 6 for garnish).
12 thyme sprigs	Cook 3-4 minutes uncovered and flip. The fish should have a nice brown buttery colour to it before you flip it. Add more butter if necessary. Cook an additional 3-4 minutes or until fish is flaky.
Sea salt	Add sea salt to taste and garnish with six fresh thyme sprigs.
Black pepper	Serve with rattlesnake green beans and halved cherry tomatoes tossed with mint pesto.

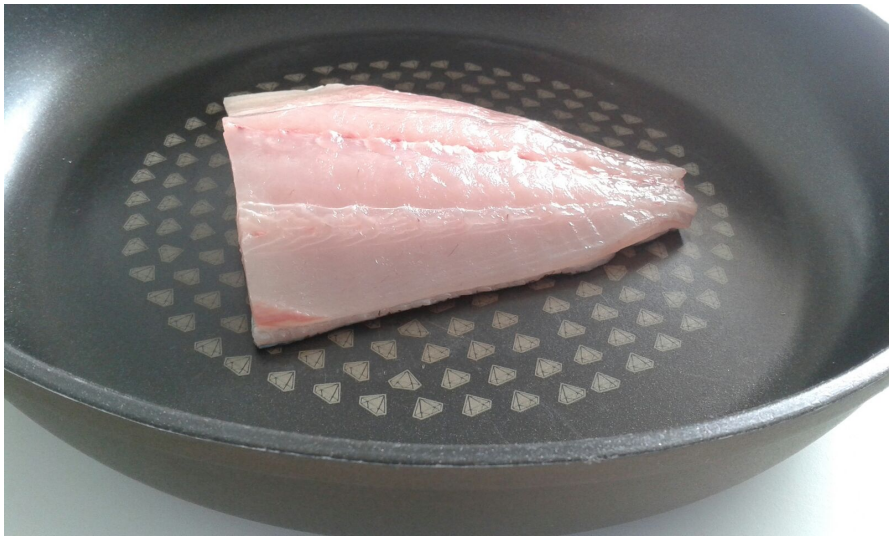


Figure 38. Greater amberjack fresh fillet ready for grilling in the pan

4. Conclusions

Twelve different prototypes have been elaborated based on ten selected ideas (idea numbers 1, 2, 4, 6, 9, 13, 21, 33 and 34) and using 4 fish species. Meagre fish was used for the development of the following ideas "*frozen fish fillets with different recipes*" (idea 1), "*fish burgers shaped as fish*" (idea 6) and "*ready to eat meal: salad with fish*" (idea 4). Pikeperch was used for the development of "*fresh fish fillet with different 'healthy' seasoning and marinades*" (idea 21), "*ready-made fish tartar with additional soy sauce*" (idea 30) and "*fish spreads/pate*" (idea 9). Grey mullet was used for the development of "*thin smoked fillets*" (idea 2), "*ready-made fish fillets in olive oil*" (idea 33) and "*fresh fish fillet with different 'healthy' seasoning and marinades*" (idea 21). Finally, greater amberjack was used for the development of "*frozen fish fillet that is seasoned or marinated*" (idea 13), "*ready-made fish tartar with additional soy sauce*" (idea 30) and "*fresh fish steak for grilling in the pan*" (idea 34).

The necessary information to obtain these new products has been provided in this deliverable as well as a number of guidelines, processing conditions, technical specifications and troubleshooting. In addition, basic information regarding the food products packaging, conservation conditions, preliminary product shelf life and consumer handling/cooking specifications have been also reported. Regarding the different processing conditions, these new product ideas are of different degree of complexity but, in all cases, it was possible to elaborate different prototypes with the selected fish species. The technical feasibility suggests that it is possible to produce these products at an industrial scale, which is corroborated by the presence of other similar products in the market. These aspects have been summarized in Table 29.

Since these prototypes have potential as fish product diversification, they can be used in further tasks of the project, including their consumer acceptability evaluation.

Table 29. Summary of the most relevant characteristics of the new product physical prototypes.

Idea number	Product	Fish species	Main characteristics	Feasibility	Specifications	Guidelines
1	Frozen fish fillets with different recipes	Meagre (*Argyrosomus regius*)	Fish only, vacuum packed	Minor difficulties	Store at -20ºC	Rapid freezing
2	Thin smoked fillets	Grey mullet (*Mugil cephalus*)	Hot smoking, dry salting	Minor difficulties	Preserve refrigerated	Smoke at 60ºC during 40 minutes
4	Ready to eat meal: salad with fish	Meagre (*Argyrosomus regius*)	The fish is marinated and separately packed. The salad components determine the shelf life of the product	Minor difficulties	Store at refrigeration temperature. Use previously frozen fish to kill *Anisakis spp.*	Apple cider is recommended to minimize tangy taste. The pH of the fish has to be ≤ 4.4
6	Fish burgers shaped as fish	Meagre (*Argyrosomus regius*)	It is possible to use trimmings, skin packaging	Relatively complex	Store at -20°C	Induce protein crosslinking by means of transglutaminase
9	Fish spreads / pate	Pikeperch (*Sanders lucioperca*)	It is possible to use trimmings. Needs to be pasteurized or sterilized. Special machinery is required	Complex	Store at refrigeration temperature if pasteurized	Texture is improved by the addition of a thermally stable emulsion. Viscosity can be controlled by the level of fish grinding
13	Frozen fish filet that is seasoned or marinated	Greater amberjack (*Seriola dumerili*)	Marinade is based on honey and soya; additional sesame seeds were used	Minor difficulties	Store at -20°C	Marinade can be tailor made in function of the target consumer
21	Fresh fish fillet with different 'healthy' seasoning and marinades	Grey mullet (*Mugil cephalus*) and pikeperch (*Sanders*	MAP was used to store pikeperch. The seasonings and marinades should not limit the shelf life of the	Minor difficulties	Store at refrigeration temperature	Seasoning and marinades should promote freshness of

		lucioperca)	product			the product
30	Ready-made fish tartar with additional soy sauce	Pikeperch (*Sanders lucioperca*) and greater amberjack (*Seriola dumerili*)	Pikeperch tartar was skin packaged. HPP allows extending shelf life and the use of avocado as ingredient. Greater amberjack tartar did not include avocado.	Relatively complex	Store at refrigeration temperature	In order to maintain the structure during skin packing fish gelatine and low temperatures are desired. The use of citric acid is recommended. Overall, the product has a very limited shelf-life
33	Ready-made fish fillets in olive oil	Grey mullet (*Mugil cephalus*)	Fish fillets preserved (bottled) in olive oil. Traditional fish preservation method.	Minor difficulties	Store at room temperature	High quality olive oil should be used
34	Fresh fish steak for grilling in the pan	Greater amberjack (*Seriola dumerili*)	Fresh deboned greater amberjack fillets skin on	Minor difficulties	Store refrigerated	Preparation highlights freshness of the product

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