Semi-industrial development of nutritious and healthy seafood dishes from sustainable species

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Abstract

Considering the increasing demand towards healthy/nutritious, sustainable and economically accessible diets, this study aimed to devise innovative, tailor-made, appealing, tasty and semi-industrialized dishes, using sustainable and under-utilized seafood species, that can meet the specific nutritional and functional needs of children (8-10-years), pregnant women (20-40-years) and seniors (≥60-years). Hence, contests were organised among cooking schools from 6 European countries and the best recipes/dishes were selected, reformulated, semi-industrially produced and evaluated through chemical and microbiological analyses. The dishes intended for: (i) children and pregnant women had EPA+DHA and I levels that reached the target quantities, supporting the claim as "high in I"; and (ii) seniors were "high in protein" (24.8%-MusSou_S and 34.0%-FisBall_S of the energy was provided by proteins), "high in vitamin B12", and had Na contents (≤0.4%) below the defined limit. All dishes reached the vitamin D target value. FisSau_C, FisRou_P, FisFil_P and FisBall_S had a well-balanced protein/fat ratio. FisRou_P presented the highest *n*-3 PUFA/*n*-6 PUFA ratio (3.3), while FisSau_C the lowest SFA/UNS ratio (0.2). No health risks were identified in dishes. All represent dietary sources contributing to meet the reference intakes of target nutrients (33->100%), providing valuable options to overcome nutritional and functional imbalances of the three target population groups.

Abstract abbreviations: DHA - Docosahexaenoic acid; EPA - Eicosapentaenoic acid; FisBall_S - Fish balls with vegetables and sauce for seniors with *Micromesistius potassou*; FisFil_P - Fish fillet with salad for pregnant women with *Limanda limanda*; FisRou_P - Fish roulade for pregnant women with *Micromesistius poutassou*; FisSau_C

- Fish sausages with vegetables for children with *Cyprinus carpio*; I – Iodine; MusSou_S - Mussel soup for seniors with *Mytilus edulis*; Na – sodium; *n*-3 PUFA - Omega 3 long-chain polyunsaturated fatty acids; *n*-6 PUFA - Omega 6 long-chain polyunsaturated fatty acids; SFA - Saturated fatty acids; UNS - Unsaturated fatty acids.

Keywords: Seafood dishes; Sustainable and under-utilized species; Nutritional and functional criteria; Children; Pregnant women; Seniors

1. Introduction

Regular seafood consumption is strongly recommended by most dietary guidelines since it is an important source of essential nutrients such as omega-3 polyunsaturated fatty acids (*n*-3 PUFA), vitamins (e.g. A, D and B12), minerals (e.g. iodine (I) and selenium) and proteins with high biological value (Sioen et al., 2007; FAO, 2020). In addition, eating seafood provides non-monotonous meals due to the diversity of species available, being part of the food culture of many people.

An adequate intake of nutrients provided by seafood brings considerable public health benefits. Dietary intake of *n*-3 PUFA is associated to a decrease of plasma triglyceride concentrations, platelet aggregation, blood pressure and consequently, of cardiovascular morbidity and mortality. These fatty acids, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are also important for the normal development and functioning of the brain, retina and nervous system. Therefore, EPA and DHA suitable intakes are fundamental for all population groups, including women of childbearing age, pregnant and breastfeeding women, and children (EFSA, 2017a; Anses, 2019a). Vitamin D is indispensable for skeleton growth and maintenance thanks to its interaction with calcium and phosphorus; it is also important for the immune function. It can be synthesized endogenously in the skin following exposure to UV-B irradiation or obtained from food. Dietary intake of vitamin D is essential when endogenous synthesis is insufficient due to low UV-B exposure. Moreover, the capacity to synthetize vitamin D decreases with age (Anses, 2019b). Vitamin B12 is essential for brain and nervous system maintenance and is mainly found in animal products, including seafood (with amounts depending on species). The deficiency of this vitamin affects essentially seniors and can lead to serious neurological disturbances (Wlassoff, 2014). Iodine is vital for thyroid functioning and foetal neurological development. Thus, maternal I deficiency increases the risk of physical and mental retardation, accompanied by higher rates of stillbirths, abortions and congenital abnormalities; even a subclinical deficiency may lead to lower cognitive and motor performance in children. Meanwhile, an excess of I can cause adverse effects on thyroid, leading to hypothyroidism or hyperthyroidism, and increasing the incidence of autoimmune thyroiditis and of thyroid cancer. Hence, having an adequate I intake is particularly important for pregnant women (EFSA, 2017a). Finally, high-quality proteins are particularly important for seniors who often experience muscle and bone density losses (EFSA, 2012a).

The European Food Safety Authority (EFSA) recommends the consumption of 2 servings of seafood per week to ensure the provision of key nutrients (EFSA, 2014); however, many consumers do not meet this recommendation for several reasons. For example, seafood is not attractive for children because of its intrinsic smell and taste, as well as the presence of bones (in the case of fish portions) (McManus et al., 2007). Thus, there is a need to make seafood products more attractive for this population group. On the other hand, seniors undergo physiological changes, such as a reduction of taste and difficulties to chew and swallow, which can affect their food habits (Pilgrim et al., 2015). Additionally, the daily safe and adequate intake of sodium (Na) for this target group must be as low as possible because the sensitivity of blood pressure to sodium chloride (NaCl) intake increases with age, while renal and cardiovascular functions are less efficient (EFSA, 2019). The need of appealing and tasty (but with low Na levels) food, easy to chew (without bones) and shallow, is essential for this population group (Pilgrim et al., 2015; EFSA, 2019).

During the workweek days, a large portion of the population have meals outside home. Institutions and catering companies take care of providing food to students in school´s canteens or to the elderly living in retirement homes. For these institutions and companies, a low price of raw material is essential to ensure profitability. The preparation of meals based on low-commercial value and under-utilized species can be a good solution to overcome this problem, making seafood affordable for the whole population. Indeed, a high number of seafood species is under strong pressure due to overexploitation (EUMOFA, 2018; FAO, 2020). Therefore, it is crucial that processing, retailing and catering players, as well as consumers, purchase seafood products based on informed choices (e.g. selecting species caught or farmed in a sustainable way) in order to maintain the biodiversity, preserve aquatic ecosystems and consequently, ensure the availability of proteins and other nutrients for future generations (Ethic Ocean, 2020).

This work aimed to devise innovative, high quality, appealing, tasty, tailor-made and semi-industrialized dishes, using sustainable and under-utilized caught or farmed seafood species, that meet the specific nutritional and functional needs of three different population groups, namely children (8-10 years old), pregnant women (20-40 years old) and seniors (≥60 years old) without any unexpected safety issue. As far as the authors are aware, similar interdisciplinary works have never been published before.

2. Materials and Methods

2.1 Recipes/dishes selection

2.1.1 Criteria

Seafood species were selected based on their sustainability (stocks data or farming practices) and economic criteria (low or medium commercial value). Additionally, only those low or under-utilized and having insignificant levels of environmental contamination were considered. On the other hand, nutritional and functional criteria were defined based on specific needs of the three target groups according to European recommendations (Afssa, 2009; European Parliament, 2011; EC, 2012; GEM-RCN, 2015; PHE, 2015; EFSA, 2019). The criteria defined for each population group and complete dish (seafood and garniture) are shown in Table 1.

Table 1 – Nutritional and functional criteria for each population segment.

Target population	**Nutritional focus**[1,2]	**Target quantity per dish**	**Portions**[5,6]	**Other properties**
Children (8-10 years old)	Omega 3 Vitamin D	>80 mg of EPA+DHA/100 g and 100 kcal[2] >30% of 5 µg/100 g[2,3]	Seafood: 70 g (± 10%) Complete dish: 250 g	Protein/Lipid > 2 (whole dish)[5] No bones Appealing and tasty
Pregnant women (20-40 years old)	Omega 3 Vitamin D Iodine	>80 mg of EPA+DHA/100 g and 100 kcal[2] >30% of 5µg/100 g[2,3] >30% of 150 µg/100 g[3]	Seafood: 100-120 g Complete dish: 250-300 g	Protein/Lipid > 2 (whole dish)[5] Appealing and tasty
Seniors (≥ 60 years old)	Vitamin D Vitamin B12 Sodium/Salt High protein	>30% of 5 µg/100 g[2,3] >30% of 2.5 µg/100 g[2,3] <2 g of Na/day (<5.0 g of NaCl/day)[4] At least 20% of the energy provided by proteins[2,5]	Seafood: 70 g (± 10%) Complete dish: 250-300 g	Protein/Lipid > 2 (whole dish)[5] Easy to chew Easy to handle No bones Appealing and tasty

[1]Afssa, 2009; [2]EC, 2012; [3]European Parliament, 2011; [4]EFSA, 2019; [5]GEM-RCN, 2015; [6]PHE, 2015.

Furthermore, industrial production feasibility was also considered as a key criterion since it was intended to semi-industrialize the recipes (see section 2.3). Finally, a maximum production cost per complete dish (for one person) (1.50 - 2.20 €) was defined following the requirements of the catering sector.

2.1.2 Contests

Contests were organised among cooking schools from 6 European countries (Belgium, France, Poland, Portugal, Spain and Sweden) to prepare recipes that met the criteria mentioned above. The best recipes for the three target groups selected in the participating countries (2 per group) competed at European level in Paris. They were assessed by a panel of international representatives of the seafood/food industry, distribution and catering companies, as well as chefs, nutritionists and researchers. The selection criteria considered by the jury in both national and European contests were: organoleptic properties (e.g. flavour), presentation, respect by the nutritional and functionality criteria, price per dish and potential for industrialization. The six winning recipes selected at European level are indicated in table 2.

Table 2 - Names and abbreviations of the six winning recipes.

Population groups	Full name of the recipe	Abbreviation
Children	Bib/Ocean pout fish balls with sweet potato and banana purée and crispy banana	FisBall_C
	Carp sausages with vegetables accompanied by salad and baked sweet potatoes	FisSau_C
Pregnant women	Blue whiting and cabbage roulade with boiled potatoes[1]	FisRou_P

	Sauteed common dab with wheat berries salad[1]	FisFil_P
Seniors	Hearty mussel soup with fish stock, root and tubber vegetables[1]	MusSou_S
	Blue whiting fish balls with vegetables and marinara sauce	FisBall_S

[1]Also in line with the Nordic Nutrition Recommendations (NCM, 2014.).

2.2 Final reformulation

The theoretical nutritional value of the selected dishes (250 g or 300 g for mussel soup) was calculated based on the amount of raw materials and ingredients used and their nutritional value information. Detailed information on energy value, contents of protein, dietary fibre, carbohydrates, fat, fatty acids, minerals and vitamins was obtained in Ciqual tables (Anses, 2017) and Kunachowicz et. al. (2017).

More specifically, the following equation was applied to establish the amount of a nutrient in each dish:

$$X = A \times \frac{B}{100}$$

where:

X is the amount of a nutrient in a dish (e.g. protein, vit. A) (g, mg or µg);

A is the amount of a nutrient in 100 g of the raw material/ingredient (g, mg or µg);

B is the amount of raw material/ingredient in the dish (g).

Subsequently, to establish the nutritional value of the complete dish, the total amount of nutrients was calculated based on their quantity in each raw material/ingredient used, as follows:

$$Y = \sum_{i=1}^{n} X_n$$

where:

Y is the total amount of a nutrient in a dish (e.g. protein, vit. A) (g, mg or µg);

X_n is the amount of a nutrient in each raw material/ingredient of the dish (g, mg or µg).

The nutritional composition for 100 g of dish (unit used for the nutritional claims) was calculated with the following formula:

$$Y\% = \frac{Y}{Q} \times 100$$

where:

Y is the amount of a nutrient for 100 g of a dish (g/100 g, mg/100 g or µg/100 g);

Q is the total weight of the dish (g).

The nutritional composition of the six winning recipes was calculated and deviations were identified. The composition was adjusted balancing the quantities and adding, replacing or removing ingredients to match the nutritional and functional criteria, and respecting the organoleptic properties of the initial recipe. It was decided to: (i) replace buttery sauces and mayonnaise by sauces made from milk and starch to reduce fat; (ii) substitute potatoes by lentils in the soup to increase the protein content and energy value, and reduce the glycaemic index; and (iii) not use additives. Moreover, it was taken into consideration the potential behaviour of dishes ingredients during frozen storage, defrosting and heating (e.g. microwave, oven) prior to consumption.

2.3 Semi-industrial production

2.3.1 Raw materials and ingredients

Seafood raw materials included fresh or frozen fillets of ocean pout/bib (*Trisopterus luscus*), common dab (*Limanda limanda*) and common carp (*Cyprinus carpio*); frozen and deshelled mussels (*Mytilus edulis*); and frozen whole blue whiting (*Micromesistius potassou*). Blue whiting was thawed in a defrosting room with an air flow at -4 °C, gutted and beheaded, washed, drained and mechanically deboned in a Baader separator 694 (Baader, Lübeck, Germany) to separate the flesh to be used in the recipe.

All other ingredients, such as frozen vegetables and fruits, frozen or dried herbs, dried powdered spices, fresh pasteurised cream, butter, liquid egg, etc. were obtained from food industrial suppliers or wholesalers. More information on the description of recipes/dishes composition in terms of ingredients (and their quantities) can be found in an e-book entitled "SEAFOOD^{TOMORROW}'S Recipes Challenge" that can be found in the site of SEAFOOD^{TOMORROW} project (https://seafoodtomorrow.eu/seafoodtomorrow-e-recipe-book-out-now/).

2.3.2 Processing

The six winning recipes at European level (Table 2) were semi-industrially developed at IDMer facilities by using classic culinary processes, widely used in food industry. For cooking, four types of equipment were used according to the recipe: (i) a 300 L steam fired vessel with or without mixer (Auriol, Marmande, France) for stirring vegetables, boiling, and cooking the soup, using a temperature set point between 70-90 °C according to the recipe; (ii) a 20 L stainless steel pot on a gas burner for the preparation of small amounts of sauces; (iii) a plancha grill KG2397 (SEVERIN, Sundern, Germany) set at maximum heat level (level 5; power: 2200 W) to sauté the fish fillets and fish roulades; and (iv) an oven Aircuiseur with steam generator and a 20 levels trolley (CAPIC, Quimper, France) for cooking the fish balls at 180 °C. To ensure safety, a core temperature of 63 °C was considered as the target in cooked products.

The ingredients were mixed using a Robotcoupe R10 (Robotcoupe, Vincennes, France) to obtain a fine texture. However, in some cases, the mixing (e.g. salad) was done manually.

The sausages were prepared with a sausage stuffer RL65 (AMB, Bologna, Italy) and shaping was done manually.

The conditioning of individual dishes in skin trays of 233x146.5x25 mm (Formplast 2325PPN, Coexpan, Madrid, Spain) was carried out manually. Then, they were cooled under refrigeration with an air flow at 0-2 °C, and frozen in an air-blast freezer tunnel at -30 °C. After 1 h, the trays were skin-packed in a Multivac Skin T200 (Multivac, Wolfertschweden, Germany) using a top web Cryovac VST936 of 150 µm (Sealed air, Charlotte, North Carolina, US).

2.4 Analytical methods

The semi-industrialized dishes (N=3) were analysed through the methods described below, at least in duplicate. The analytical quality control and/or optimized parameters/conditions are presented in supplementary tables (SM1, SM2, SM3 and SM4). All results are expressed in wet weight.

2.4.1 Proximate chemical composition and energy value

Moisture, ash and free fat were determined according to the Association of Official Analytical Chemists methods (AOAC, 2005). Briefly, moisture was determined by oven (ULE 500, Memmert, Schwabach, Germany) drying of sample overnight at 105±1 ºC, whereas ash was obtained by incineration of dry sample in a muffle furnace (TYP.MR170, Heraeus, Hanau, Germany) for 16 h at 500±25 ºC. Free fat was determined through the Soxhlet extraction method (in a Soxhlet apparatus, Behr Labor-Technik, Dusseldorf, Germany) using diethyl ether solvent (at approximately 40 ºC; 7h), and by weighing the fat residue after drying (105±1 ºC) in an air oven. Crude protein was calculated from total nitrogen using the conversion factor of 6.25 (FAO, 2003). Total nitrogen was analysed according to the Dumas method (Saint-Denis and Goupy, 2004) in an automatic nitrogen analyser (LECO FP-528, LECO Corp., St. Joseph, USA) calibrated with EDTA. Nitrogen was released by combustion at 850 ºC and detected by thermal conductivity. See Table SM1 for more details on the methods.

Total carbohydrates were determined by difference and the energy value was estimated using Food and Agriculture Organization factors (FAO, 2003).

2.4.2 Elements

Potassium (K) and sodium (Na) contents were determined by flame atomic absorption spectrophotometry (Spectr AA 55B spectrophotometer, Varian, Palo Alto, CA, USA) with a background deuterium correction, based on the method described by Jorhem (2000). The concentrations were calculated using linear calibration obtained from absorbance measurements of, at least, five different concentrations of standard solutions (KNO_3 and $NaNO_3$, dissolved in 0.5 M HNO_3).

The salt content was estimated through the Na levels according to the following formula:

$$X = \frac{K \times Y}{1000 \times Z}$$

where:

X is the salt content (g/100 g);

K is the NaCl molar mass (58.44 g/mol);

Y is the Na level (mg/100 g);

Z is the Na molar mass (22.99 g/mol).

For iodine (I) and total mercury (Hg-T) analysis, samples were carefully homogenized and subsamples were placed in high-pressure Teflon containers with adequate volumes of ultrapure HNO_3 67-69% and H_2O_2 30%, and digested in a microwave system (UltraWAVE Single Reaction Chamber Microwave Digestion System, Milestone, Bergamo, Italy).

Hg-T was determined by an internally validated method using ICP-MS/MS with a triple quadrupole inductively coupled plasma mass spectrometer (Agilent 8800, Agilent Technologies Inc., Tokyo, Japan) and He as collision gas. I was determined by ICP-MS using a Nexion 350D ICP-MS (Perkin Elmer, Waltham, MA, USA). The analytical masses were ^{200}Hg, ^{201}Hg, and ^{202}Hg (with ^{103}Rh as internal standard), and ^{127}I (with ^{125}Te as internal standard), respectively. For Hg-T, quantitative determinations were carried out after forming an amalgam with Au (added to standards and samples) to eliminate memory effects. For I, standards and samples were prepared in 1.5% ammonia and 1% isopropanol, whereas 0.5% tetramethylammonium hydroxide TraceSelect was used to wash the quartz nebulizer and spray chamber and prevent memory effects. See Table SM1 for more details on the methods.

2.4.3 Vitamins

Vitamins D3 and E were analysed according to the method described in Byrdwell et al. (2013). Briefly, the samples (containing ascorbic acid, butylated hydroxytoluene (10 mg/mL) and tocol 100 µg/L as surrogate standard) were saponified with KOH 60% and extracted with hexane (2 ml x 3 times). After evaporation, adequate volumes of methanol with 0.1% of formic acid (eluent B) and vitamin D2[$^2H_3$] (100 µg/l, internal standard) were added. The separation and quantification of both vitamins was performed by high-performance liquid chromatography (HPLC) on a Waters 2695 system (Waters, Milford, MA, USA) coupled to a Micromass Quattro micro API™ triple quadrupole detector (Waters, Manchester, UK), equipped with the MassLynx 4.1 software for data processing. A pre-column from Phenomenex® (Torrance, CA, USA) and a C18 column from Kinetex® Phenomenex® (2.6 µm, 150 mm x 4.60 mm (i.d.)) were used. The column was kept at 40 ºC, the auto sampler maintained at ± 25 ºC and the gradient elution at a flow rate of 0.300 mL/min (mobile phase composed by water with 0.1% of formic acid (eluent A) and methanol with 0.1% of formic acid (eluent B)). The MS/MS acquisition was operated in positive-ion mode with multiple reaction monitoring (MRM). The optimized MS parameters were as follows: capillary voltage, 3.00 kV; source temperature, 150 ºC; desolvation temperature, 350 ºC; desolvation gas and cone gas flow, 350 and 50 L/h, respectively (Table SM2).

Vitamin B12 was determined by HPLC, as described by Campos-Giménez et al. (2008). Briefly, vitamin B12 was extracted from samples by sodium acetate buffer in the presence of sodium cyanide (100 °C, 30 min). After purification and concentration on an immunoaffinity column, vitamin B12 was determined by liquid chromatography on an UltiMate HPLC and Chromeleon software (Thermo Fisher Scientific, Waltham, Massachusetts) with UV detection (361 nm). The limit of quantitation (LOQ) of the method was 0.25 µg/100 g.

2.4.4 Fatty acids

Total lipids were extracted from dried samples with a dichloromethane-methanol mixture according to the method described in Folch et al. (1957). After vortex agitation, methanol was added and extracts were centrifugated and filtered by Whatman paper. The separation of lipid phase was improved with NaCl and the solvent was eliminated on a rotatory evaporator (Rotavapor® R-200, Buchi, Flawil, Switzerland). The lipid residue was re-dissolved in n-hexane and the internal standard C13:0 at 20 mg/mL was added. The fatty acids analysis was carried out according to Bondia-Pons et al. (2007), being the transesterification performed with sodium methylate solution and boron trifluoride-methanol. Then n-hexane with butylated hydroxytoluene (BHT) at 0.02% and NaCl were added. The organic phase was recovered, dried with anhydrous sodium sulphate, evaporated to dryness under nitrogen flow and finally re-dissolved in n-hexane.

Gas chromatography analyses were performed on Shimadzu (Kyoto, Japan) gas chromatograph (GC)-2010 (Agilent® J&W Cp-Sil 88 capillary column, 60 m × 0.25 mm I.D., 0.20 µm; Santa Clara, USA) equipped with a Shimadzu flame ionization detector (FID) and a Shimadzu AOC-20i auto injector. A split ratio of 1:50, injector and detector temperatures of 250 ºC and 260 ºC, respectively, were used. Oven temperature program was as follows: initial temperature 100 ºC for 5 min, increased at 1 ºC/min to 215 ºC and held at this temperature for 20 min. Fatty acid methyl esters (FAME) were identified by comparison with standard mixture (Sigma 47,885-U Supelco 37 Component FAME Mix, USA). The fatty acid composition in mg/100 g was estimated based on the total lipid content.

According to Chen and Liu (2020), the PUFA/SFA ratio is too general and unsuitable for assessing the atherogenicity of foods and they considered that the atherogenic (IA) and thrombogenic (IT) indexes calculated as proposed by Ulbricht and Southgate (1991) are good indicators. Thus, these indexes were determined as follows: $IA = (C12{:}0 + (4 \times C14{:}0) + C16{:}0)/(\sum MUFA + \sum n\text{-}3\ PUFA + \sum n\text{-}6\ PUFA)$; $IT = (C14{:}0 + C16{:}0 + C18{:}0)/((0.5 \times \sum MUFA) + (0.5 \times \sum n\text{-}6\ PUFA) + (3 \times \sum n\text{-}3\ PUFA) + (n\text{-}3\ PUFA/n\text{-}6\ PUFA))$, where MUFA = monounsaturated fatty acids.

2.4.5 Cholesterol

Cholesterol content was analysed according to Cunha et al. (2006). An accurate sample portion was extracted with n-hexane/ethyl acetate (90:10). Then, after evaporation of the solvent, a sodium methoxide solution and betulin as internal standard were added. The mixture was left at room temperature during 20 min and then adequate volumes of water and n-heptane were added. The aqueous phase was withdrawn and replaced by a 1% citric acid solution. The dried organic fraction was derivatized at 70 ºC for 20 min with N,O-bis(trimethylsilyl)trifluoroacetamide (with trimethylchlorosilane 1%) and pyridine. Each extract was analysed on an Agilent GC 6890 (DB-5MS fused silica capillary column, 30 m × 0.25 mm I.D., 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA) equipped with an electronically controlled split/splitless injection port and interfaced to a MSD-5973N mass selective detector (Agilent Technologies Inc., Little Falls, USA). The injection was performed at 270 °C in the split mode (ratio 15:1). The column and transfer line temperatures were 270 °C and 280 °C, respectively. Quantification was carried out in selective ion monitoring (SIM) mode (see Table SM2).

2.4.6 Polybrominated Diphenyl Ethers (PBDEs)

Homogenised and lyophilised samples were spiked with ^{13}C-labelled standards and extracted by pressurised liquid extraction (PLE) with hexane:dichloromethane (1:1) at 1500 psi and 100 ºC. Sulphuric acid treatment and solid phase extraction (SPE) using neutral alumina cartridges were done as purification steps. Extracts were analysed by gas chromatography (Agilent 7890 GC, Agilent Technologies Inc., Santa Clara, CA, USA) coupled to tandem mass spectrometry (Agilent 7000A MS triple quadrupole, Agilent Technologies Inc.) (GC-MS-MS). A DB-5ms capillary column (15 m × 0.1 mm i.d., 0.1 µm film thickness, Agilent Technologies Inc.) was used. For MS detection, MRM mode was applied (see Table SM3). More details on chromatographic and mass spectrometric conditions can be found in Barón et al. (2014).

2.4.7 Polycyclic aromatic hydrocarbons (PAHs)

Homogenised samples were spiked with a surrogate standard mixture of the 16 target compounds (see Table SM4), vortexed and stored during 12h at 4 ºC. Quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction of the samples was then done using acetone and the SALT KIT AC (magnesium sulphate ($MgSO_4$) and NaCl) from Bekolut GmbH & Co (Hauptstuhl, Germany) for extraction, and the PSA-KIT-04 (primary and secondary amine (PSA), C18e and $MgSO_4$) also from Bekolut GmbH & Co for subsequent clean-up. The so-obtained extracts were analysed by GC-MS-MS (Agilent 7890A GC coupled to an Agilent 7000B triple quadrupole mass spectrometer; Agilent Technologies Inc.) using an Agilent HP-5ms Ultra Inert column (30 m x 0.25 mm, 0.25 µm). MS analyses were performed using electron ionization (EI) and MS acquisition was done in the MRM mode (see Table SM4).

2.4.8 Histamine

The determination of histamine was carried out by HPLC according to an in-house accredited method (PEE/1/19; ANFACO-CECOPESCA, 2020) based on EN ISO 19343 (ISO, 2017). The previously homogenized sample (total dish content) was extracted with 0.4 M perchloric acid (1:5), homogenized with an Ultra-Turrax T-25 blender (Ika-Labortechnik, Germany) and centrifuged for 20 min (4000 rpm). The supernatant was filtered and derivatized with dansyl chloride. The equipment consisted of a Waters 2695 HPLC separations module, a Waters 2996 Photodiode Array Detector and Empower®3 Software (Waters, Milford, MA, USA). A Gemini C18 column (150 x 4.6 mm, 5 µm, 110

Å; Phenomenex®, Spain) was used. Mobile phase was composed by 0.1 M ammonium acetate (eluent A) and acetonitrile (eluent B) used in a gradient elution (flow rate of 1 mL/min), starting at 50% of eluent B and reaching 90% of this solvent over 24 min. Detection of histamine was carried out at 254 nm. The LOQ was 10 mg/kg.

2.4.9 Microbiological analysis

The enumeration of total aerobic microorganisms, coagulase-positive Staphylococci, *Escherichia coli* and *Bacillus cereus* were carried out by the Most Probable Number (MPN) using the fully automated system TEMPO® (bioMérieux, Inc., USA) certified by AOAC Research Institute (RI), AOAC OMA and Health Canada (Biomérieux, 2020).

Detection of *Salmonella spp.* and *Listeria monocytogenes* was performed by validated immunoassay (immunoconcentration and immunofluorescence (ELFA) detection), using the Biomérieux VIDAS® system (Biomérieux, 2021).

2.5 Nutritional Contribution (NC)

The NC of dishes was calculated based on (i) EPA+DHA and vitamin D3 for children; (ii) EPA+DHA, vitamin D3 and I for pregnant women; and (iii) vitamin D3, vitamin B12, salt and protein for seniors. To this end, a complete dish (250 g or 300 g for soup) and the dietary reference values (DRVs) recommended by EFSA (2017a, 2019) were considered, according to the following formula:

$$NC(\%) = \frac{C \times M}{DRV} \times 100$$

where:

C is the mean concentration of the nutrient (mg/kg);

M is the weight of the complete dish (g);

DRV is the PRI, *Population Reference Intake* (mg/day), in the case of proteins or the AI, *Adequate Intake* (mg/day), in the case of other specific nutrients.

3. Results and Discussion

3.1. Nutritional composition

3.1.1 Proximate chemical composition and energy value

The proximate chemical composition of the dishes prepared for the different population groups is shown in Table 3. The moisture content ranged from 73.0 to 82.1%, being the lowest value found for FisFil_P. Regarding ash, FisBall_C and FisFil_P presented values close to 1%, while the other dishes (FisSau_C, FisRou_P, MusSou_S and FisBall_S) had values between 1.6 to 1.8%. The protein content ranged from 5.8 to 6.7% in four dishes (FisSau_C, FisBall_C, FisRou_P and MusSou_S) and the remaining two showed values close to 9% (FisFil_P and FisBall_S). In the case of dishes intended for seniors, 24.8% (MusSou_S) and 34.0% (FisBall_S) of the energy value was provided by proteins. These values were above the defined minimum target quantity (20%) (Table 1), so such dishes meet the criterion to be claimed "high in protein" (European Parliament, 2006). In the case of fat, four dishes (FisSau_C, FisRou_P, MusSou_S and FisBall_S) presented values between 1.7 and 2.9% (i.e. <3%) meeting the criterion for "low in fat" claim (European Parliament, 2006). The protein/fat ratio (which is an indicator of nutritional quality of the product) was higher than 2 (between 2.4 – 4.1) in four dishes (FisSau_C, FisRou_P, FisFil_P and FisBall_S) indicating that they were the best balanced in terms of protein and fat levels. The carbohydrates content varied from 11 to 13% in four dishes (FisBall_C, FisFil_P, MusSou_S and FisBall_S) and was around 8% in the other two (FisSau_C and FisRou_P). Finally, the energy value ranged between 70-80 kcal/100 g in two dishes (FisSau_C and FisRou_P), 90-110 kcal/100 g in three dishes (FisBall_C, MusSou_S and FisBall_S) and was 124.5 kcal/100 g in FisFil_P. This higher energy value reflects the highest protein, fat and carbohydrates contents in such dish.

Table 3 - Proximate chemical composition of the dishes.

Target population	Dish	g/100 g					Energy value (kJ/kcal/100 g)
		Moisture	Ash	Protein	Fat	Carbohydrates*	
Children	FisSau_C	81.81±0.26	1.68±0.04	6.44±0.19	2.06±0.12	8.02±0.19	319.67±1.95 / 76.40±0.47
	FisBall_C	77.10±0.01	1.06±0.05	6.70±0.27	3.92±0.22	11.22±0.45	447.49±4.76 / 106.95±1.14
Pregnant women	FisRou_P	82.11±0.15	1.83±0.03	6.42±0.03	1.65±0.03	7.99±0.18	303.43±2.55 / 72.52±0.61
	FisFil_P	73.00±0.54	0.81±0.10	9.27±0.32	3.95±0.58	12.97±0.45	520.78±19.62 / 124.47±4.69
Seniors	MusSou_S	78.80±0.40	1.57±0.05	5.76±0.42	2.91±0.09	10.96±0.52	389.45±7.64 / 93.08±1.83
	FisBall_S	75.98±0.70	1.73±0.13	8.46±0.38	2.06±0.12	11.78±0.24	416.27±12.43 / 99.49±2.97

Results are given as average values ± standard deviations (N=3). FisSau_C: Fish sausages with vegetables for children with *Cyprinus carpio*; FisBall_C: Fish balls with a purée for children with *Trisopterus luscus*; FisRou_P: Fish roulade for pregnant women with *Micromesistius poutassou*; FisFil_P: Fish fillet with salad for pregnant women with *Limanda limanda*; MusSou_S: Mussel soup for seniors with *Mytilus edulis*; and FisBall_S: Fish balls with vegetables and sauce for seniors with *Micromesistius potassou*. *Calculated by difference.

The differences in the proximate chemical composition between dishes for the same or different population group(s) were expected since they were prepared with different raw materials, ingredients and culinary processes. It is well known that the cooking technique can affect the nutritional composition of seafood, depending on the intrinsic composition, temperature the product is submitted to, cooking time, as well as the method used (Oliveira et al., 2019).

3.1.2 Macro and microelements

The Na content was lower than 200.0 mg/100 g (i.e. salt: <0.5%) in two dishes (FisBall_C and FisFil_P) and ranged from 278.9 to 340.6 mg/100 g (i.e. salt: 0.70 to 0.85%) in the remaining four dishes (FisSau_C, FisRou_P, MusSou_S and FisBall_S) (Table 4). A 300 g dish of MusSou_S and 250 g of FisBall_S corresponded to 0.84 g and 0.85 g of Na (i.e.

salt amount: 2.09 g and 2.13 g), respectively. Hence, these two dishes intended for seniors presented values that were about 40% of the target quantity (i.e. <2.0 g of Na/day; <5.0 g of salt/day, NaCl, Table 1) defined to prevent cardiovascular disorders associated with increased blood pressure in this target group (EFSA, 2019). Within a balanced diet, it is therefore unlikely that either MusSou_S or FisBall_S will lead to any appreciable excess of Na. Additionally, the other dishes (250 g) also had Na (and NaCl) levels below the limit established for seniors.

On the other hand, K content ranged from 155.3 to 240.2 mg/100 g (around 200 mg/100 g), being the lowest value found for FisFil_P (Table 4). Moreover, the Na/K ratio was 0.95 and 0.60 in FisBall_C and FisFil_P, respectively, i.e., it was in the range recommended by WHO (<1) for maintaining a healthy cardiovascular condition (Whelton, 2014). The Na/K ratio of the other dishes, including MusSou_S or FisBall_S, was slightly higher than 1 (between 1.2-1.5) (Table 4). Thus, some further refinement of these recipes may be envisaged.

Table 4 - Na, K, salt and I concentrations of the dishes.

Target population	**Dish**	**mg/100 g**		**Na:K**	**Salt (%)***	**mg/kg**
		Na	**K**			**I**
Children	FisSau_C	291.23±12.55	221.66±12.99	1.31	0.73±0.03	2.94±0.38
	FisBall_C	170.66±1.34	180.21±3.30	0.95	0.43±0.00	0.71±0.14
Pregnant women	FisRou_P	288.43±22.69	240.20±22.93	1.20	0.72±0.06	1.02±0.19
	FisFil_P	92.89±16.65	155.32±8.60	0.60	0.23±0.04	0.46±0.05
Seniors	MusSou_S	278.92±21.52	187.46±14.83	1.49	0.70±0.05	0.55±0.15
	FisBall_S	340.57±25.52	228.02±5.66	1.49	0.85±0.06	0.77±0.07

Results are given as average values ± standard deviations (N=3). FisSau_C: Fish sausages with vegetables for children with *Cyprinus carpio*; FisBall_C: Fish balls with a purée for children with *Trisopterus luscus*; FisRou_P: Fish roulade for pregnant women with *Micromesistius poutassou*; FisFil_P: Fish fillet with salad for pregnant women with *Limanda limanda*; MusSou_S: Mussel soup for seniors with *Mytilus edulis*; and FisBall_S: Fish balls with vegetables and sauce for seniors with *Micromesistius potassou*. *Estimated through the Na levels.

The dishes intended for pregnant women - FisRou_P and FisFil_P - showed I contents of 102 and 46 µg/100 g, respectively (Table 4). These values reached the target quantity of I defined, i.e. > 30% of the nutrient reference value (NRV) (150 µg)/100 g (>45 µg/100 g, Table 1). The other dishes also contained at least 30% of this NRV. Hence, these data support the claim that all dishes are "high in iodine" (European Parliament, 2006; 2011). Iodine contributes to maintain normal skin, cognitive and neurological functions, energy-yielding metabolism, production of thyroid hormones and thyroid function of the general population (EFSA, 2009a, 2010a). Thus, FisRou_P and FisFil_P can be valuable options for pregnant women, since this population group have special I needs for increased maternal thyroid hormone production (EFSA, 2017a).
It is noted that the consumption of one of the dishes intended for children, namely FisSau_C, would lead to an intake of 735 µg/day (without considering other dietary I sources), which exceeds the Tolerable Upper Intake Level (UL) of 300 µg/day set for children aged 7-10 years (EFSA, 2006). Whereas the UL is defined as the maximum level of total daily intake of a nutrient that is unlikely to pose a risk of adverse health effects to humans, and although its occasional exceedance does not imply that there is a risk, a refinement of this recipe is envisaged to reduce the I concentration. Dishes targeted for the other two population segments had I values far below the UL (600 µg/day) defined for adults including pregnant and lactating women (EFSA, 2006).

3.1.3 Vitamins

The concentrations of vitamins (D3, B12 and E) in the six dishes are shown in Table 5.

Table 5 - Vitamins (D3, B12 and E) contents of the dishes.

Target population	Dish	Vitamin D3 (µg/100 g)[1]	Vitamin B12 (µg/100 g)[2]	Vitamin E (mg/100 g)[1]
Children	FisSau_C	5.79±0.65	1.52±0.20	14.21±2.97
	FisBall_C	6.36±0.43	0.27±0.02	14.92±3.44
Pregnant women	FisRou_P	5.12±0.19	1.47±0.03	14.05±0.81
	FisFil_P	11.62±2.15	0.45±0.06	13.00±0.40
Seniors	MusSou_S	3.68±0.75	1.90±0.36	5.96±0.44
	FisBall_S	6.74±0.46	1.24±0.07	8.72±1.56

Results are given as [1]average values or [2]means values ± standard deviations (N=3). FisSau_C: Fish sausages with vegetables for children with *Cyprinus carpio*; FisBall_C: Fish balls with a purée for children with *Trisopterus luscus*; FisRou_P: Fish roulade for pregnant women with *Micromesistius poutassou*; FisFil_P: Fish fillet with salad for pregnant women with *Limanda limanda*; MusSou_S: Mussel soup for seniors with *Mytilus edulis*; and FisBall_S: Fish balls with vegetables and sauce for seniors with *Micromesistius potassou*.

3.1.3.1 Vitamin D3

According to Table 5, vitamin D3 content ranged from 3.7 (MusSou_S) to 11.6 µg/100 g (FisFil_P). Hence, the six dishes reached the target quantity of vitamin D defined, i.e. > 30% of the NRV (5 µg)/100 g (> 1.5 µg; Table 1). These results support the inclusion of the claim "high in vitamin D" (European Parliament, 2006; 2011). Thus, the claims approved by EFSA (2009b; 2010b) stating that vitamin D contributes i) to normal absorption/utilisation of calcium and phosphorus, blood calcium levels, cell division, function of the immune system and healthy inflammatory response; and ii) to the maintenance of normal bones, teeth and muscle function can be applied to the six dishes. Furthermore, all values were well below the UL/day defined for children aged 7-10 years (50 µg) and adults including pregnant women (100 µg) (EFSA, 2012b).

3.1.3.2 Vitamin B12

The vitamin B12 values found in dishes intended for seniors - MusSou_S (1.9 µg/100 g) and FisBall_S (1.2 µg/100 g) (Table 5) - were higher than 0.75 µg/100 g (the target content set in Table 1). So, the data support the claim "high in vitamin B12" (European Parliament, 2006; 2011). According to EFSA (2009c; 2010c), vitamin B12 i) contributes to normal energy-yielding metabolism, homocysteine metabolism, red blood cell formation, function of the immune system, cell division, neurological and psychological functions, and ii) can contribute to the reduction of tiredness and fatigue. Thus, MusSou_S and FisBall_S are good options for seniors because they need to avoid inadequate vitamin intake from the diet, specifically of vitamin B12, once its deficiency is responsible for cognitive impairment (Kennedy, 2016). One dish targeted for children and both for pregnant women also showed important amounts of vitamin B12: FisSau_C and FisRou_P had values > 0.75 μg/100 g, and hence they can be labelled as "high in vitamin B12"; FisFil_P presented values > 0.38 μg/100 g (15% of the NRV) and thus it can be labelled as a "source of vitamin B12" (European Parliament, 2006; 2011) (Table 5).

3.1.3.3 Vitamin E

Although no target amount for vitamin E was defined in this study (it was not a nutritional focus), its levels were quantified due to its importance to human health. The vitamin E concentration ranged from 13.0 (FisFil_P) to 14.9 mg/100 g (FisBall_C) in dishes intended for children and pregnant women, while it was lower in those targeting seniors (6.0 and 8.7 mg/100 g in MusSou_S and FisBall_S, respectively) (Table 5). All dishes had values well below the UL (EFSA, 2006) for children aged 7-10 years (160 mg) and adults, including pregnant women (300 mg). Additionally, the six dishes contained at least 30% of the NRV (> 3.6 mg), so a claim that they are "high in vitamin E" (European Parliament, 2006) and consequently can contribute to the protection of cells constituents from oxidative damage (EFSA, 2010d) could be stated.

3.1.4 Fatty acids and cholesterol

The MUFA were the predominant fatty acids in five dishes, ranging from 40.7 (FisFil-P) to 59.9% (FisBall-C) of the total fatty acids. They were followed by SFA, amounting to between 25.6 (FisBall-C) and 39% (MuSou-S), and PUFA, which ranged from 14.5 (FisBall-C) to 22.6% (FisFil-P) in four dishes; finally, PUFA and SFA in FisSau-C were 27.2 and 19.0%, respectively. In the case of FisRou-P, SFA were the predominant followed by MUFA and PUFA (51.2, 27.6 and 21.3%, respectively) (Table 6). Therefore, this last dish was the only one that presented a saturated content similar to that of unsaturated (SFA/UNS = 1.1). The remaining five dishes showed a total amount of unsaturated higher than that of saturated (0.2 (FisSau_C) ≤ SFA/UNS ≤ 0.6 (MusSou_S)). It is generally recognized that intakes of MUFA and PUFA are associated with a lower risk of cardiovascular disease (CVD) and death, whereas SFA intake with a higher risk of CVD (Guasch-Ferré et al., 2015). Hence, FisRou-P and FisSau_C seemed to be the less and most balanced dishes, respectively, as to their capacity to provide amounts of fatty acids, intended as main groups, in line with a generally healthy diet.

Table 6. Fatty acids and cholesterol concentrations of the dishes.

Target population	Children		Pregnant women		Seniors	
Dish	FisSau_C	FisBall_C	FisRou_P	FisFil_P	MusSou_S	FisBall_S
Fatty acids (mg/100 g)[a]						
ΣSFA[1]	531.34±14.56	1233.49±142.41	1150.94±31.40	1875.22±224.75	1317.72±137.67	905.99±63.35
ΣMUFA[2]	1504.30±23.37	2881.66±43.58	620.09±16.40	2072.65±172.93	1515.30±162.72	2054.50±168.55
ΣPUFA[3]	761.19±13.60	697.15±27.02	478.96±16.50	1149.77±132.05	545.88±110.90	545.55±68.78
SFA/UNS[4]	0.23±0.00	0.35±0.05	1.05±0.01	0.58±0.02	0.64±0.02	0.35±0.01
EPA+DHA[5]	81.80±3.55	120.30±0.78	278.07±12.68	405.50±102.55	208.13±58.39	159.82±28.12

Σ*n*-3 PUFA[6]	201.03±1.94	181.14±1.40	368.32±14.81	624.07±139.68	297.56±84.59	200.85±42.90
Σ*n*-6 PUFA[7]	560.16±12.13	516.01±25.83	110.63±3.06	525.70±38.79	248.32±26.89	344.71±26.06
n-3 PUFA/ *n*-6 PUFA	0.36±0.01	0.35±0.02	3.33±0.12	1.20±0.32	1.18±0.23	0.58±0.08
IT[8]	0.30±0.00	0.44±0.03	0.70±0.01	0.50±0.01	0.68±0.07	0.47±0.03
IA[9]	0.23±0.00	0.37±0.07	1.34±0.02	0.72±0.04	0.79±0.02	0.35±0.01
Cholesterol (mg/100 g)[a]	34.70±5.12	119.77±10.04	98.38±10.67	175.91±13.76	73.31±5.68	61.66±7.98

[a]Results are given as average values ± standard deviations (N=3). [1]Total saturated; [2]Total monounsaturated; [3]Total polyunsaturated; [4]Total saturated/Total unsaturated; [5]Eicosapentaenoic + Docosahexaenoic; [6]Total omega 3; [7]Total omega 6; [8]Thrombogenic index; [9]Atherogenic index. FisSau_C: Fish sausages with vegetables for children with *Cyprinus carpio*; FisBall_C: Fish balls with a purée for children with *Trisopterus luscus*; FisRou_P: Fish roulade for pregnant women with *Micromesistius poutassou*; FisFil_P: Fish fillet with salad for pregnant women with *Limanda limanda*; MusSou_S: Mussel soup for seniors with *Mytilus edulis*; and FisBall_S: Fish balls with vegetables and sauce for seniors with *Micromesistius potassou*.

Among PUFA, EPA and DHA, which are of importance to human health particularly to prevent CVD diseases (Sheppard and Cheatham, 2018), showed a considerable percentage of the total *n*-3 fatty acids, i.e. 40.7% in FisSau_C and between 65.0 (FisFil_P) and 79.6% (FisBall_S) in the other dishes (Table 6). All values obtained in the six dishes reached the target quantity of EPA+DHA defined for children and pregnant women, i.e. >80 mg of EPA+DHA/100 g and 100 kcal (Table 1). Furthermore, the dishes targeting pregnant women were those with the highest levels of *n*-3 PUFAs, followed by dishes for seniors and children (Table 6). In the case of FisRou_P, the *n*-3 PUFA/*n*-6 PUFA ratio (3.3) also indicated that the amount of *n*-3 was about three times higher than that of *n*-6. Hence, the composition of this dish appears beneficial in the light of the lower ratio of *n*-6/*n*-3 fatty acids, which is a desirable feature in terms of reduction of the risk of many diseases (Simopoulos, 2002; Candela et al., 2011). The amounts of *n*-3 PUFA were also higher than those of *n*-6 PUFA in FisFil_P and MusSou_S (ratio = 1.2), being also well-balanced dishes. Finally, FisBall_S, FisSau_C and FisBall_C presented levels of *n*-6 PUFA 2-3 times higher than those of *n*-3 PUFA (ratio ≤ 0.58), suggesting that these dishes are the less well-balanced in terms of composition of these fatty acids (Candela et al., 2011; Sheppard and Cheatham, 2018).

The dishes targeted for children and seniors and FisFil_P for pregnant women appear effective in contributing to the goal of reducing the atherogenicity of the diet in these population segments (0.23≤IA≤0.79, Table 6), according to Ulbricht and Southgate (1991). On the other hand, FisRou_P seems to have little effect in such reduction, since IA was higher than 1. It has to be noted that the IT values observed in all dishes (≤0.70, Table 6) were similar or only slightly higher than those found by the same authors in products considered as the most antithrombogenic. Thus, in general, the IA and IT values suggest that the dishes are adequate in the perspective of the prevention of coronary heart diseases (Ulbricht and Southgate, 1991).

The lowest and highest cholesterol levels were found in FisSau_C (34.7 mg/100 g) and FisFil_P (175.9 mg/100 g) (Table 6), respectively, as observed for SFA, suggesting that these dishes were the most and less healthy (in terms of cholesterol) (Carson et al., 2019). In both dishes for seniors, the cholesterol levels were in the range of 60-75 mg/100 g, whereas in the remaining dishes (FisRou_P and FisBall_C) the range was between 98 and 120 mg/100 g. Therefore, all dishes had cholesterol values <300 mg, the maximum daily value recommended by WHO/FAO (2003). However, it is to be noted that EFSA (2010e) did not propose a reference value for cholesterol intake and rather focused on the intake of SFA.

3.2. Potential risks

3.2.1 Chemical contaminants

3.2.1.1 Toxic elements

Hg-T was quantified in all dishes since it is well known that methylmercury, the most toxic among the mercury compounds, usually represent more than 90% of the Hg-T found in fish and a significant proportion in other seafood (CEC, 2006). However, Hg-T values found in all dishes were exceedingly low (0.036±0.009 mg/kg in FisBall_C, 0.008±0.001 mg/kg in FisRou_P, 0.015±0.004 mg/kg in FisFil_P and 0.009 mg/kg in FisBall_S) and in some cases below the LOQ (≤0.006 mg/kg in FisSau_C and MusSou_S). Overall, they were well below the maximum limit (0.5 mg/kg) for fishery products in Europe, taking into account the proportion of seafood in the recipes (CEC, 2006). These data are supported by the Hg-T and methylmercury contents of the fish species used in the recipes (Ferraris et al. 2021).

Cadmium and lead were also quantified and presented very low levels in all dishes; the same holds true for total and inorganic arsenic in MusSou_S notwithstanding mussels, which may be relatively rich in inorganic arsenic (Cubadda et al., 2017), were used as ingredients. Hence, these low values indicate that the consumption of all dishes represents a negligible source of exposure to these toxic elements (Ferraris et al. 2021).

3.2.1.2 PBDEs

PBDEs analysis showed non-detected to very low levels in all dishes (Table 7). BDE-47 was found more than other PBDEs, as it was quantifiable in some dishes, with “FisFil_P” showing the highest value (4.8 ng/100 g). This dish was the only one where also BDE-209 was quantified (37 ng/100 g; though with a great variability observed between samples) and had the highest ∑PBDEs value (50 ng/100 g). The levels of the two indicator congeners, BDE-47 and BDE-209, are fully in the range of the average intake in the EU assessed by the CONTAM Panel, who concluded that the dietary exposure to these two

PBDEs does not raise a health concern (EFSA, 2011). Therefore, the levels of PBDEs detected in some of the products do not represent a safety issue.

Table 7. PBDEs concentrations of the dishes.

Target population	**Dish**	**ng/100 g**								
		BDE-28	**BDE-47**	**BDE-100**	**BDE-99**	**BDE-154**	**BDE-153**	**BDE-183**	**BDE-209**	**ΣPBDEs**
Children	FisSau_C	<0.55**	2.0±0.57	<0.91*	<1.3*	<1.9*	<2.9*	<15*	<0.36*	2.0±0.57
	FisBall_C	<0.17*	<0.81**	<0.91*	<1.3*	<1.9*	<2.9*	<15*	<0.36*	0.17±0.06
Pregnant women	FisRou_P	<0.17*	0.92±0.29	<0.91*	<1.3*	<1.9*	<2.9*	<15*	<0.36*	0.92±0.29
	FisFil_P	1.5±2.3	4.8±4.5	2.0±2.6**	2.2±2.6**	3.0±3.4**	2.8±2.2**	<15*	37±63	50±82
Seniors	MusSou_S	<0.17*	0.47±0.46**	<0.91*	<1.3*	<1.9*	<2.9*	<15*	<0.36*	0.47±0.46
	FisBall_S	<0.17*	0.43±0.39**	<0.91*	<1.3*	<1.9*	<2.9*	<15*	<0.36*	0.43±0.39

Results are given as mean values ± standard deviations (n=3). Mean values *≤LOD; or **<LOQ. Mean values were calculated considering non-detected values as the 50% of LOD value. When the compound was detected (>LOD) but cannot be quantified (<LOQ), concentration values were assumed to be the LOD value. FisSau_C: Fish sausages with vegetables for children with *Cyprinus carpio*; FisBall_C: Fish balls with a purée for children with *Trisopterus luscus*; FisRou_P: Fish roulade for pregnant women with *Micromesistius poutassou*; FisFil_P: Fish fillet with salad for pregnant women with *Limanda limanda*; MusSou_S: Mussel soup for seniors with *Mytilus edulis*; and FisBall_S: Fish balls with vegetables and sauce for seniors with *Micromesistius potassou*.

3.2.1.3 PAHs

Only three low molecular weight PAHs, namely, naphthalene, fluorene and phenanthrene, were quantified in some dishes (Table 8). Naphthalene was the most ubiquitous PAH, but a high variability was detected between samples. Naphthalene, fluorene and phenanthrene concentrations ranged from 1964 to 6968 ng/100 g, 149 to 306 ng/100g, and 144 to 278 ng/100 g, respectively. The total PAHs levels ranged between 2000 and 7217 ng/100 g, and once again a high variability between samples was observed. Neither benzo[a]pyrene nor any of the other 3 PAHs included in the group of PAH4 (benz[a]anthracene, chrysene, and benzo[b]fluoranthene), regulated in terms of maximum levels in food by the European Commission (CEC, 2006), were detected. While the content of phenanthrene indicates that PAHs should be monitored along the industrial production process, the absence of these four target compounds suggests that the six dishes do not raise safety concerns in terms of PAHs presence.

Table 8. PAHs concentrations of the dishes.

Target population	Children		Pregnant women		Seniors	
Dish	FisSau_C	FisBall_C	FisRou_P	FisFil_P	MusSou_S	FisBall_S
PAHs (ng/100 g)						
Naphthalene	5956±571	6968±1743	5823±2717	6173±1072	3789±2993	1964±2608
Acenaphtylene	<600*	<600*	<600*	<600*	<600*	<600*
Acenaphthene	<500**	<500**	<500**	<500**	<500**	<500**
Fluorene	<40*	149±830	299±125	306±15	225±55	240±30
Phenanthrene	231±16**	160±166**	<64*	278±24	<64*	144±139**
Anthracene	<70*	<70*	<70*	<70*	<70*	<70*
Fluoranthene	<20*	<20*	<20*	<20*	<20*	<20*
Pyrene	<28*	<28*	<28*	<28*	<28*	<28*
Benzo[a]anthracene	<7*	<7*	<7*	<7*	<7*	<7*
Chrysene	<14*	<14*	<14*	<14*	<14*	<14*

Benzo[b]fluoranthene	<30*	<30*	<30*	<30*	<30*	<30*
Benzo[k]fluoranthene	<30*	<30*	<30*	<30*	<30*	<30*
Benzo[a]pyrene	<10*	<10*	<10*	<10*	<10*	<10*
Indeno[1,2,3-cd]pyrene	<20*	<20*	<20*	<20*	<20*	<20*
Dibenzo[a,h]anthracene	<20*	<20*	<20*	<20*	<20*	<20*
Benzo[g,h,i]perylene	<20*	<20*	<20*	<20*	<20*	<20*
ΣPAHs	6187±558	7217±1686	6122±2827	6758±1085	3862±3194	2000±2761

Results are given as mean values ± standard deviations (n=3). Mean values *≤LOD; or **<LOQ. Mean values were calculated considering non-detected values as the 50% of LOD value. When the compound was detected (>LOD) but cannot be quantified (<LOQ), concentration values were assumed to be the LOD value. FisSau_C: Fish sausages with vegetables for children with *Cyprinus carpio*; FisBall_C: Fish balls with a purée for children with *Trisopterus luscus*; FisRou_P: Fish roulade for pregnant women with *Micromesistius poutassou*; FisFil_P: Fish fillet with salad for pregnant women with *Limanda limanda*; MusSou_S: Mussel soup for seniors with *Mytilus edulis*; and FisBall_S: Fish balls with vegetables and sauce for seniors with *Micromesistius potassou*.

3.2.2 Histamine

Although the seafood species used in the dishes are not in the list of those revealing histidine concerns (CEC, 2005), the presence of other ingredients, such as dairy products, cereals and vegetables led to consider histamine as a potential hazard (Comas-Basté et al., 2020). In addition, recent publications reported histamine poisoning from other fish species not considered in the EU legislation (Colombo et al., 2018). The control of this parameter was also considered essential since its ingestion may be responsible for cases of food intolerance, which may include allergies-like symptoms such as sneezing, flushing, headache, diarrhea, and even shortness of breath (Comas-Basté et al., 2020). However, histamine was not quantified in any dish (<10 mg/kg, LOQ), which means that all were compliant with the current EU regulations (CEC, 2005) and prepared according to the current recommendations, i.e. adequate chilling conditions and hygienic measures (EFSA, 2017b).

3.2.3 Microbiological aspects

The most spoilage bacteria and pathogens were assessed in the six dishes. The counts of total aerobic microorganisms, *Bacillus cereus*, coagulase positive *Staphylococcus*, *E. coli*, *Salmonella* and *Listeria monocytogenes* were lower than the LODs or below the legal or recommended limits (CEC, 2005), taking into account both food quality/safety and process hygiene criteria. Even the highest levels of total aerobic microorganisms ($5.4x10^4$ cfu/g) detected were low.

3.3 Nutritional contribution

The dishes (250 g) for children (FisSau_C and FisBall_C) almost fulfil or fulfil the daily AIs of vitamin D3 (97% and >100%, respectively) and EPA+DHA (82% and >100%, respectively). Regarding dishes for pregnant women, the consumption of FisRou_P and FisFil_P (250 g) contributes with >100% and 58% respectively, of the daily AI of I for this target group. For vitamin D3, the contribution is of 85% and >100%, respectively, whereas for EPA+DHA is >100%. Finally, the consumption of MusSou_S and FisBall_S (300 g and 250 g, respectively) contributes with 33-40% for protein, 42-43% for Na, 74% (MusSou_S)-112% (FisBall_S) for vitamin D3, and 77% (FisBall_S)-143% (MusSou_S) for vitamin B12, of the DRVs (PRI or AI) for adults, including seniors (Table 9).

Table 9 - Nutritional contribution of the dishes in terms of target nutrients, taking into account a complete dish of 250 g or 300 g (in the case of mussel soup).

Target nutrient	**Target population**	**DRVs: AI or PRI (mg/d)[1]**	**Nutritional contribution (%)**					
			FisSau_C	FisBall_C	FisRou_P	FisFil_P	MusSou_S	FisBall_S
Proximate composition								
Protein	Seniors	**52539**[2]	---	---	---	---	32.9±2.4	40.2±1.8
Macro and micro elements								
I	Pregnant Women	0.20[2]	---	---	127.9±23.2	57.9±6.8	---	---

Na	Seniors	2000[2,3]	---	---	---	---	41.8±3.2	42.6±3.2
Vitamins								
D3	Children		96.5±10.8	105.9±7.2	---	---	---	---
	Pregnant Women	0.015[2,4]	---	---	85.3±3.2	193.6±35.9	---	---
	Seniors		---	---	---	---	73.6±15.0	112.4±7.6
B12	Seniors	0.004[2]	---	---	---	---	142.8±26.7	77.3±4.4
n-3 fatty acids								
EPA+DHA	Children	250[2]	81.8±3.6	120.3±0.8	---	---	---	---
	Pregnant Women	450[2,5]	---	---	154.5±7.0	225.3±57.0	---	---

Values are means ± standard deviations (n=3). FisSau_C: Fish sausages with vegetables for children with *Cyprinus carpio*; FisBall_C: Fish balls with a purée for children with *Trisopterus luscus*; FisRou_P: Fish roulade for pregnant women with *Micromesistius poutassou*; FisFil_P: Fish fillet with salad for pregnant women with *Limanda limanda*; MusSou_S: Mussel soup for seniors with *Mytilus edulis*; and FisBall_S: Fish balls with vegetables and sauce for seniors with *Micromesistius potassou*. [1]DRVs (Dietary Reference Values): AIs (Adequate Intakes) are presented in ordinary type and PRIs (Population Reference Intakes) in **bold type**; [2]EFSA (2017a); [3]EFSA (2019); [4]Under conditions of assumed minimal cutaneous vitamin D synthesis. In the presence of endogenous cutaneous vitamin D synthesis, the requirement for dietary vitamin D is lower or may be even zero. [5]AI of DHA and EPA for other adults (250 mg) + additional supply of DHA (200 mg) to compensate for oxidative losses and accumulation in the fetus.

Therefore, the consumption of the six innovative dishes can bring benefits to human health associated to their optimum levels of target nutrients. For instance, it can contribute, in the context of an overall balanced diet, to (i) prevent cardiovascular diseases in children and pregnant women (due to the EPA and DHA levels); (ii) supply DHA compensating for oxidative losses and accumulation in the foetus in pregnant women; (iii) musculoskeletal health (due to the vitamin D levels) in all population segments considered; (iv) prevent goitre and to increased maternal thyroid hormone production (due to the I levels) and I uptake by the foetus (needs of pregnant women); (v) prevent neurological dysfunction in seniors (due to the vitamin B12 levels); and (vi) achieve a higher protein to energy ratio in sedentary elderly people (that have lower energy requirements), which is essential for tissue maintenance (EFSA, 2017a).

4. Conclusions

The results obtained in this work show that it is possible to devise high quality, healthy, appealing, tasty, affordable, sustainable, tailor-made and industrialized seafood dishes for (i) children and pregnant women with essential EPA+DHA and I levels, that can be claimed as "high in I"; (ii) seniors with low Na/salt and that can be claimed as "high in protein" and "high in vitamin B12"; (iii) the three above-mentioned target populations that can be claimed as "high in vitamin D" and "high in vitamin E" (all dishes), as well as with a well-balanced protein/fat ratio (FisSau_C, FisRou_P, FisFil_P and FisBall_S). FisSau_C may be considered as the most well-balanced dish in the perspective of a healthy and adequate diet in terms of amounts of fatty acids (lowest SFA/UNS ratio=0.2) and cholesterol (lowest level). The highest *n*-3 PUFA/*n*-6 PUFA ratio (3.3) was found in FisRou-P and suggested that the consumption of this dish can be particularly beneficial in terms of *n*-3 supply. All dishes can be considered safe products as far as the contents of Hg-T, Cd, Pb, As, PBDEs, regulated PAHs, histamine, total aerobic microorganisms, *Bacillus cereus*, coagulase positive *Staphylococcus*, *E. coli*, *Salmonella* and *Listeria monocytogenes* are concerned. It is also important to highlight that the consumption of dishes with 250 or 300 g (in MusSou_S) can contribute to essential daily intake of target nutrients and can be an excellent option to meet the nutritional and functional needs of children, pregnant women and seniors. However, some minor adjustments of the recipes are still necessary before an industrial production. In future studies it will be important to evaluate the dishes stability once frozen with the aim to establish the adequate shelf life for each.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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(ID 164), maintenance of normal skin (ID 164), maintenance of normal nails (ID 164), maintenance of normal cardiac function (ID 166), maintenance of normal vision by protection of the lens of the eye (ID 167), contribution to normal cognitive function (ID 182, 183), regeneration of the reduced form of vitamin C (ID 203), maintenance of normal blood circulation (ID 216) and maintenance of a normal scalp (ID 2873) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA J. 8(10), 1816. 30 p. https://doi.org/10.2903/j.efsa.2010.1816.

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Supplementary material

Table SM1. Analytical quality control of proximate composition and macro, micro and toxic elements analyses.

Elements	Technique	LOD	LOQ	Proficiency Test or CRM	Reference[1]	Observed[2]
Proximate composition (g/100 g)						
Moisture	Drying	n.a.	n.a.	FAPAS Test 0133	59.89±0.42	60.35±0.16
Ash	Incineration	0.16	0.38		2.14±0.08	2.17±0.01
Nitrogen	Combustion	0.01	0.02		1.15±0.02	1.13±0.00
Free fat	Soxhlet extraction	0.1	0.3		12.61±0.40	12.29±0.31
Elements						
Potassium (mg/kg)	FAAS	0.01	0.07	Dorm-4	15500±1000	14500±495
Sodium (mg/kg)	FAAS	0.09	0.3	FAPAS Test 01120	0.60±0.03	0.55±0.02
Iodine (µg/g)	ICP-MS	0.057*	0.113*	ERM-BB422	1.40±0.40	1.33±0.10
				BCR-063R	0.81±0.05	0.81±0.05
Total mercury (µg/g)	ICP-MS/MS	0.003*	0.006*	ERM-CE278K	0.071±0.007	0.073±0.004
				ERM-BB422	0.601±0.030	0.598±0.031
				NIST 1946	0.433±0.009	0.441±0.011
				NIST 1566b	0.037±0.001	0.039±0.012

Values are presented as [1]average±uncertainty or [2]average±standard deviation. Abbreviations: LOD - Detection Limit; LOQ – Quantification limit; CRM - Certified reference material; n.a. - not applicable; FAAS - Flame Atomic Absorption Spectrometry; ICP-MS - Inductively Coupled Plasma Mass Spectrometry; ICP-MS/MS - Inductively Coupled Plasma - Tandem Mass Spectrometry;

FAPAS Test 0133 and Test 01120 - Nutritional Components in Canned Meat, January 2020 and January-March 2018, respectively (Fera Science Ltd, York, UK); Dorm-4 - Fish protein CRM (National Research Council of Canada, Canada); ERM-BB422 – Fish muscle CRM (Joint Research Centre (JRC), Brussels); BCR-063R – Skim milk powder CRM (JRC); ERM-CE278K – Muscle tissue CRM (JRC); NIST 1946 –Lake superior fish tissue CRM (National Institute of Standards and Technology, United States); NIST 1566b – Oyster Tissue CRM (National Institute of Standards and Technology). *LODs (LOQs) achieved based on the 3σ (6 σ) criterion, where σ is the standard deviation of the measurement of 15 method blanks.

Table SM2. Optimized MS/MS parameters in positive-ion mode with multiple reaction monitoring (MRM) for vitamins quantification and MS parameters with selective ion monitoring (SIM) for cholesterol quantification and respective limits of detection (LOD) and quantification (LOQ).

Analyte	MRM transition quantifier (quantification)	MRM transition qualifier (confirmation)	LOD (µg/kg)	LOQ (µg/kg)
Vitamin D3	385>259	385>367	10	31
Vitamin D2 [$^{2}H_{3}$](IS)	399>69.1	-	-	-
Vitamin E	430>165	430>205	10	31
Tocol (IS)	388>123	388>163	-	-
Analyte	SIM quantifier (quantification)	SIM qualifier (confirmation)	LOD (µg/mg)	LOQ (µg/mg)
Betulin (IS)	189	496, 493	-	-
Cholesterol	329	129, 73, 368, 458	2	6

IS – Internal Standard.

Table SM3. GC-EI-MS-MS conditions used for analysis of the 16 target PBDEs and their corresponding isotopically labelled analogues, and method limits of detection and quantification.

Analyte	Retention time (min)	MRM transition (quantification)	MRM transition (confirmation)	LOD (ng/100 g ww)	LOQ (ng/100 g ww)
Tri-BDE-28	10.8	408 >246	408 >248	0.17	0.55
^{13}C-BDE-28		418>258			
Tetra-BDE-47	12.8	486 >326	488 >328	0.24	0.81
^{13}C-BDE-47		498>338			
Penta-BDE-100	14.1	406 >297	564 >404	0.91	3.0
^{13}C-BDE-100		576>416			
Penta-BDE-99	14.6	406 >297	564 >404	1.3	4.4
^{13}C-BDE-99		576>416			
Hexa-BDE-154	15.6	486 >377	644 >484	1.9	6.5
^{13}C-BDE-154		656>496			
Hexa-BDE-153	16.2	486 >377	644 >484	2.9	9.7
^{13}C-BDE-153		656>496			
Octa-BDE-183	17.7	721 >562	721 >564	15	48
^{13}C-BDE-183		733>574			
Deca-BDE-209	23.2	298 >220	361 >280	0.36	1.4
^{13}C-BDE-209		406>326			

Table SM4. GC-EI-MS-MS conditions used for analysis of the 16 target PAHs and their corresponding isotopically labelled analogues, and method limits of detection (LOD) and quantification (LOQ).

Analyte	Retention time (min)	MRM transition (quantification)	MRM transition (confirmation)	LOD (ng/100 g)	LOQ (ng/100 g)
		4 - 7.3 min			
Naphthalene	5.4	128 >102	128 >77	900	3100
Naphthalene-d_8		136 >108			
Acenaphthylene	6.8	152 >150	152 >126	600	1900
Acenaphthylene-d_8		160 >158			
Acenaphthene	7.0	154 >153	154 >152	150	500
Acenaphthene-d_{10}		164 >162			
		7.3 – 11 min			
Fluorene	7.6	166 >165	166 >163	40	140
Fluorene-d_{10}		176 >174			
Phenanthrene	9.2	178 >152	178 >176	64	250
Phenanthrene-d_{10}		188 >184			
Anthracene	9.3	178 >176	178 >152	70	230
Anthracene-d_{10}		188 >184			
		11 – 22 min			
Fluoranthene	13.3	202 >200	202 >150	20	80
Fluoranthene-d_{10}		212 >208			
Pyrene	14.2	202 >200	202 >150	28	90
Pyrene-d_{10}		212 >208			
Benz[a]anthracene	19.9	228 >226	228 >224	7	50
Benz[a]anthracene-d_{12}		240 >236			
Chrysene	20.0	228 >226	228 >224	14	60
Chrysene-d_{12}		240 >236			
		22 – 28 min			
Benzo[b]fluoranthene	24.7	252 >250	250 >248	30	90
Benzo[b]fluoranthene-d_{12}		264 >260			
Benzo[k]fluoranthene	24.5	252 >250	250 >248	30	100
Benzo[k]fluoranthene-d_{12}		264 >260			
Benzo[a]pyrene	25.9	252 >250	250 >248	10	40
Benzo[a]pyrene-d_{12}		264 >260			
		28 – 33 min			
Indeno[1,2,3-cd]pyrene	30.1	276 >274	276 >272	20	80
Indeno[1,2,3-cd]pyrene-d_{12}		288 >284			
Dibenz[a,h]anthracene	30.3	278 >276	278 >274	20	70
Dibenz[a,h] anthracene-d_{14}		292 >288			

Benzo[g,h,i]perylene	30.9	276 >274	268 >272	20	70
Benzo[g,h,i]perylene-d_{12}		288 >284			