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

Helena Oliveira [a,b,*], Camille Blocquel [c], Marta Santos [a,b], Murielle Fretigny [c], Tatiana Correia [a,b], Amparo Gonçalves [a,b], Ana G. Cabado [d], Lucía Blanco López [d], Birgitta Wäppling Raaholt [e], Francesca Ferraris [f], Francesca Iacoponi [f], Francesco Cubadda [f], Alberto Mantovani [f], Elisabeth Vallet [g], Geertrui Vlaemynck [h], Julio Fernández-Arribas [i], Ethel Eljarrat [i], Esther López [i], Miren López de Alda [i], Remigiusz Panicz [j], Małgorzata Sobczak [j], Piotr Eljasik [j], Sara Cunha [k], Ricardo Ferreira [k], José O. Fernandes [k], Sara Sousa [l], Valentina F. Domingues [l], Cristina Delerue-Matos [l], António Marques [a,b], Maria Leonor Nunes [a,**]

[a] CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos S/N, 4450-208, Matosinhos, Portugal
[b] IPMA, I.P, Portuguese Institute for the Sea and Atmosphere, I.P, Division of Aquaculture, Upgrading and Bioprospection, Av. Alfredo Magalhães Ramalho 6, 1495-165, Lisboa, Portugal
[c] IDmer, 2 rue batelière, 56100, Lorient, France
[d] ANFACO CECOPESCA - Ctra. Colexio Universitario, Pontevedra, 16, 36310, Vigo, Spain
[e] RISE Research Institutes of Sweden, Frans Perssons väg 6, SE-412 76, Göteborg, Sweden
[f] ISS, Istituto Superiore di Sanità – National Institute of Health, Department of Food Safety, Nutrition and Veterinary Public Health, Viale Regina Elena 299, 00161, Rome, Italy
[g] Ethic ocean, Tour ESSOR, 14 rue Scandicci, 93500, Pantin, France
[h] ILVO, Flanders Research Institute for Agriculture, Fisheries and Food, Department Technology and Food Science, Brusselsesteenweg 370, 9090, Melle, Belgium
[i] Water, Environment and Food Chemistry, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Jordi Girona 18-26, 08034, Barcelona, Spain
[j] West Pomeranian University of Technology in Szczecin, Faculty of Food Sciences and Fisheries, Department of Meat Science, Szczecin, Poland
[k] LAQV/Requimte-Faculty Pharmacy, University of Porto, Rua Jorge de Viterbo Ferreira, 228, 4050-313, Porto, Portugal
[l] REQUIMTE/LAQV-GRAQ, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, 4200-072, Porto, Portugal

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ABSTRACT

This study aimed to devise innovative, tailor-made, appealing, tasty and semi-industrialized dishes, using sustainable and under-utilized seafood species (bib, common dab, common carp, blue mussel and blue whiting), that can meet the specific nutritional and functional needs of children (8-10-years), pregnant women (20-40-years)

Abbreviations: Balls_S, Fish balls with vegetables and sauce for seniors with *Micromesistius poutassou*; DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; Fillet_P, Fish fillet with salad for pregnant women with *Limanda limanda*; Hg-T, Total mercury; I, Iodine; Na, sodium; *n*-3 PUFA, very-long chain polyunsaturated omega-3 fatty acids; *n*-6 PUFA, very-long chain polyunsaturated omega-6 fatty acids; PBDEs, Polybrominated diphenyl ethers; Roulade_P, Fish roulade for pregnant women with *Micromesistius poutassou*; Sausages_C, Fish sausages with vegetables for children with *Cyprinus carpio*; SFA, Saturated fatty acids; Soup_S, Mussel soup for seniors with *Mytilus edulis*; UNS, Unsaturated fatty acids.

* Corresponding author. IPMA, I.P, Portuguese Institute for the Sea and Atmosphere, I.P, Division of Aquaculture, Upgrading and Bioprospection, Av. Alfredo Magalhães Ramalho 6, 1495-165, Lisboa, Portugal.
** Corresponding author.
E-mail addresses: helaoliveira@gmail.com (H. Oliveira), camille.blocquel@gmail.com (C. Blocquel), marta.santos@ipma.pt (M. Santos), murielle.fretigny@idmer.com (M. Fretigny), tatiana_correia@live.com.pt (T. Correia), amparo@ipma.pt (A. Gonçalves), agcabado@anfaco.es (A.G. Cabado), lucia@anfaco.es (L.B. López), birgitta.raaholt@ri.se (B.W. Raaholt), francesca.ferraris@iss.it (F. Ferraris), francesca.iacoponi@iss.it (F. Iacoponi), francesco.cubadda@iss.it (F. Cubadda), alberto.mantovani@iss.it (A. Mantovani), evallet@ethic-ocean.org (E. Vallet), geertrui.vlaemynck@ilvo.vlaanderen.be (G. Vlaemynck), jfaqam@cid.csic.es (J. Fernández-Arribas), eeeqam@cid.csic.es (E. Eljarrat), elgqam@cid.csic.es (E. López), mlaqam@cid.csic.es (M. López de Alda), rpanicz@zut.edu.pl (R. Panicz), malgorzata.sobczak@zut.edu.pl (M. Sobczak), peljasik@zut.edu.pl (P. Eljasik), sara.cunha@ff.up.pt (S. Cunha), ricardof@live.com.pt (R. Ferreira), josefer@ff.up.pt (J.O. Fernandes), sara_sousa_23@hotmail.com (S. Sousa), vfd@isep.ipp.pt (V.F. Domingues), cmm@isep.ipp.pt (C. Delerue-Matos), amarques@ipma.pt (A. Marques), nunes.leonor@gmail.com (M.L. Nunes).

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and seniors (≥60-years). Hence, contests were organised among cooking schools from 6 European countries and the best recipes/dishes were reformulated, semi-industrially produced and chemically and microbiologically evaluated. 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%-Soup_S and 34.0%-Balls_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. Sausages_C, Roulade_P, Fillet_P and Balls_S had a well-balanced protein/fat ratio. Roulade_P presented the highest *n*-3 PUFA/*n*-6 PUFA ratio (3.3), while Sausages_C the lowest SFA/UNS ratio (0.2). Dishes were considered safe based on different parameters (e.g. Hg-T, PBDEs, *Escherichia coli*). 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 groups.

1. Introduction

Regular seafood (e.g. fish and shellfish) consumption is strongly recommended by most dietary guidelines since it is a rich source of essential nutrients to human health, such as very-long chain polyunsaturated omega-3 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. Some of these nutrients cannot be acquired from other sources (Sioen et al., 2007; Hicks et al., 2019; 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.

The adequate intakes of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (both *n*-3 PUFA) are fundamental (e.g. for the normal development and functioning of the brain, retina and nervous system) in all population groups, including children, women of childbearing age, pregnant and breastfeeding women (250 mg/d; plus an additional supply of DHA (100–200 mg) in the last two cases) (EFSA, 2017a; Anses, 2019a). Dietary intake of vitamin D (15 μg/d) is essential (e.g. for skeleton growth and maintenance; immune function) in all population groups when endogenous synthesis in the skin is insufficient due to low UV-B irradiation exposure. The capacity to synthetize vitamin D decreases with age (EFSA, 2017a; Anses, 2019b). Moreover, the deficiency of vitamin B12 (intakes < 4.0 μg/d) affects essentially seniors and can lead to serious neurological disturbances (Wlassoff, 2014; EFSA, 2017a). 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. Hence, having an adequate I intake (200 μg/d) is particularly important for pregnant women (EFSA, 2017a). Finally, high-quality proteins are particularly important for seniors (population reference intake = 52.54 g/d) who often experience muscle and bone density losses (EFSA, 2012a; EFSA, 2017a).

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 intake of sodium (Na) for this target group must be as low as possible (safe and adequate intake = 2 g/d) 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

The species were selected by using a multi-criteria analysis, which included: (i) status at IUCN red list of threatened species (https://www.iucnredlist.org) and WWF seafood guides (https://wwf.panda.org/act/live_green/out_shopping/seafood_guides/methodology/); ii) harvesting from sustainable production sources; iii) high production volume/abundance; iv) low or medium production/catch commercial value; and v) local/regional relevance in terms of economy, employment, and cultural and traditional values. 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.

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, the maximum production cost per complete dish (for one person) (1.50–2.20 €, Table 1) 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

Table 1
Nutritional and functional criteria for each population segment as well as production cost defined per dish.

Target population	Nutritional focus[a,b]	Target quantity per dish	Portions[e,f]	Other properties/ price
Children (8–10 years old)	Omega 3	>80 mg of EPA + DHA/ 100 g and 100 kcal[b]	Seafood: 70 g (±10%) Complete dish: 250 g	Protein/Lipid > 2 (whole dish)[e] No bones Appealing and tasty Maximum production cost: 1.50–2.20 €/dish
	Vitamin D	>30% of 5 μg/ 100 g[b,c]		
Pregnant women (20–40 years old)	Omega 3	>80 mg of EPA + DHA/ 100 g and 100 kcal[b]	Seafood: 100–120 g Complete dish: 250–300 g	Protein/Lipid > 2 (whole dish)[e] Appealing and tasty Maximum production cost: 1.50–2.20 €/dish
	Vitamin D	>30% of 5 μg/ 100 g[b,c]		
	Iodine	>30% of 150 μg/100 g[c]		
Seniors (≥ 60 years old)	Vitamin D	>30% of 5 μg/ 100 g[b,c]	Seafood: 70 g (±10%) Complete dish: 250–300 g	Protein/Lipid > 2 (whole dish)[e] Easy to chew Easy to handle No bones Appealing and tasty Maximum production cost: 1.50–2.20 €/dish
	Vitamin B12	>30% of 2.5 μg/100 g[b,c]		
	Sodium/Salt	<2 g of Na/ day (<5.0 g of NaCl/day)[d]		
	High protein	At least 20% of the energy provided by proteins[b,e]		

[a] Afssa (2009).
[b] EC, 2012.
[c] European Parliament (2011).
[d] EFSA, 2019.
[e] GEM-RCN, 2015.
[f] PHE, 2015.

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	Balls_C
	Carp sausages with vegetables accompanied by salad and baked sweet potatoes	Sausages_C
Pregnant women	Blue whiting and cabbage roulade with boiled potatoes[a]	Roulade_P
	Sauteed common dab with wheat berries salad[a]	Fillet_P
Seniors	Hearty mussel soup with fish stock, root and tubber vegetables[a]	Soup_S
	Blue whiting fish balls with vegetables and marinara sauce	Balls_S

[a] 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 pout/bib (*Trisopterus luscus*), common dab (*Limanda limanda*) and common carp (*Cyprinus carpio*); frozen and deshelled blue mussel (*Mytilus edulis*); and frozen whole blue whiting (*Micromesistius poutassou*). 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 and 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 233 × 146.5 × 25 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 were evaluated through the methods described below, at least in duplicate. All analyses were performed in three dishes of each recipe that were randomly selected from the total production (200 units). 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 °C, whereas ash was obtained by incineration of dry sample in a muffle furnace (TYP.MR170, Heraeus, Hanau, Germany) for 16 h at 500 ± 25 °C. 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 °C; 7 h), and by weighing the fat residue after drying (105 ± 1 °C) 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 °C 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 55 B 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 (PerkinElmer, 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 × 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 × 4.60 mm (i.d.)) were used. The column was kept at 40 °C, the auto sampler maintained at ± 25 °C 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 °C; desolvation temperature, 350 °C; 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 °C and 260 °C, respectively, were used. Oven temperature program was as follows: initial temperature 100 °C for 5 min, increased at 1 °C/min to 215 °C 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 x C14:0) + C16:0)/(∑MUFA + ∑*n*-3 PUFA+ ∑*n*-6 PUFA); IT = (C14:0 + C16:0 + C18:0)/((0.5 x ∑MUFA) + (0.5 x ∑*n*-6 PUFA) + (3 x ∑*n*-3 PUFA) + (*n*-3 PUFA/*n*-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 °C 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-5973 N 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)

Homogenized 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 °C. 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 7000 A 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)

Homogenized samples were spiked with a surrogate standard mixture of the 16 target compounds (see Table SM4), vortexed and stored during 12 h at 4 °C. 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 7890 A GC coupled to an Agilent 7000 B triple quadrupole mass spectrometer; Agilent Technologies Inc.) using an Agilent HP-5ms Ultra Inert column (30 m × 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 × 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 Fillet_P. Regarding ash, Balls_C and Fillet_P presented values close to 1%, while the other dishes (Sausages_C, Roulade_P, Soup_S and Balls_S) had values between 1.6 and 1.8%. The protein content ranged from 5.8 to 6.7% in four dishes (Sausages_C, Balls_C, Roulade_P and Soup_S) and the remaining two showed values close to 9% (Fillet_P and Balls_S). In the case of dishes intended for seniors, 24.8% (Soup_S) and 34.0% (Balls_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 (Sausages_C, Roulade_P, Soup_S and Balls_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 and 4.1) in four dishes (Sausages_C, Roulade_P, Fillet_P and Balls_S) indicating that they were the best balanced in terms of protein and fat levels. The carbohydrates content varied from 11.0 to 13.0% in four dishes (Balls_C, Fillet_P, Soup_S and Balls_S) and was 8% in the other two (Sausages_C and Roulade_P). Finally, the energy value ranged between 70 and 80 kcal/100 g in two dishes (Sausages_C and Roulade_P), 90 and 110 kcal/100 g in three dishes (Balls_C, Soup_S and Balls_S) and was 124 kcal/100 g in Fillet_P. This higher energy value reflects the highest protein, fat and carbohydrates contents in such dish.

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 (Balls_C and Fillet_P) and ranged from 278.9 to 340.6 mg/100 g (i.e. salt: 0.70–0.85%) in the remaining four dishes (Sausages_C, Roulade_P, Soup_S and Balls_S) (Table 4). A 300 g dish of Soup_S and 250 g of Balls_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 Soup_S or Balls_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 Fillet_P (Table 4). Moreover, the Na/K ratio was 0.95 and 0.60 in Balls_C and Fillet_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 Soup_S or Balls_S, was slightly higher than 1 (between 1.2 and 1.5) (Table 4). Thus, some further refinement of these recipes may be envisaged.

The dishes intended for pregnant women - Roulade_P and Fillet_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

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	Sausages_C	291.2 ± 12.6	221.7 ± 13.0	1.31	0.73 ± 0.03	2.94 ± 0.38
	Balls_C	170.7 ± 1.3	180.2 ± 3.3	0.95	0.43 ± 0.00	0.71 ± 0.14
Pregnant women	Roulade_P	288.4 ± 22.7	240.2 ± 22.9	1.20	0.72 ± 0.06	1.02 ± 0.19
	Fillet_P	92.9 ± 16.7	155.3 ± 8.6	0.60	0.23 ± 0.04	0.46 ± 0.05
Seniors	Soup_S	278.9 ± 21.5	187.5 ± 14.8	1.49	0.70 ± 0.05	0.55 ± 0.15
	Balls_S	340.6 ± 25.5	228.0 ± 5.7	1.49	0.85 ± 0.06	0.77 ± 0.07

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

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	Sausages_C	81.8 ± 0.3	1.7 ± 0.0	6.4 ± 0.2	2.1 ± 0.1	8.0 ± 0.2	320 ± 2/76 ± 0
	Balls_C	77.1 ± 0.0	1.1 ± 0.1	6.7 ± 0.3	3.9 ± 0.2	11.2 ± 0.5	447 ± 5/107 ± 1
Pregnant women	Roulade_P	82.1 ± 0.1	1.8 ± 0.0	6.4 ± 0.0	1.7 ± 0.0	8.0 ± 0.2	303 ± 3/73 ± 1
	Fillet_P	73.0 ± 0.5	0.8 ± 0.1	9.3 ± 0.3	3.9 ± 0.6	13.0 ± 0.5	521 ± 20/124 ± 5
Seniors	Soup_S	78.8 ± 0.4	1.6 ± 0.1	5.8 ± 0.4	2.9 ± 0.1	11.0 ± 0.5	389 ± 8/93 ± 2
	Balls_S	76.0 ± 0.7	1.7 ± 0.1	8.5 ± 0.4	2.1 ± 0.1	11.8 ± 0.2	416 ± 12/99 ± 3

Results are given as average values ± standard deviations (N = 3). Sausages_C: Fish sausages with vegetables for children with *Cyprinus carpio*; Balls_C: Fish balls with a purée for children with *Trisopterus luscus*; Roulade_P: Fish roulade for pregnant women with *Micromesistius poutassou*; Fillet_P: Fish fillet with salad for pregnant women with *Limanda limanda*; Soup_S: Mussel soup for seniors with *Mytilus edulis*; and Balls_S: Fish balls with vegetables and sauce for seniors with *Micromesistius poutassou*. *Calculated by difference.

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, 2011). Thus, Roulade_P and Fillet_P can be valuable options for pregnant women, since this population group have special I needs for increased thyroid hormone production (to cover maternal and foetal needs) (EFSA, 2017a). The consumption of such dishes will also contribute to end with the I deficiency during pregnancy (that persists in Europe), which even at a mild-to-moderate level can have long-term adverse effects on child cognition (Zimmermann et al., 2015). Other authors also observed through a randomized controlled trial that the increased intake of other fish species (two weekly servings of cod for 16 weeks) during pregnancy improved the I status in women with mild-to-moderate I deficiency (Markhus et al., 2020).

It is noted that the consumption of one of the dishes intended for children, namely Sausages_C, would lead to an intake of 735 μg/day (without considering other dietary I sources such as milk and dairy products), which exceeds the Tolerable Upper Intake Level (UL) of 300 μg/day set for children aged 7–10 years (EFSA, 2006; Reijden et al., 2017). 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.

3.1.3.1. Vitamin D3. According to Table 5, vitamin D3 content ranged from 3.7 (Soup_S) to 11.6 μg/100 g (Fillet_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 intake of the six dishes may contribute to several health benefits such as the normal absorption/utilisation of calcium and phosphorus, function of the immune system, and the maintenance of normal bones, teeth and muscle function (EFSA, 2009, 2010a). 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 - Soup_S (1.9 μg/100 g) and Balls_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). Thus, Soup_S and Balls_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). Furthermore, the intake of these two dishes can also contribute to other health benefits such as the reduction of tiredness and fatigue (EFSA, 2010b). Moreover, one dish targeted for children and both for pregnant women also showed important amounts of vitamin B12: Sausages_C and Roulade_P had values > 0.75 μg/100 g, and hence they can be labelled as "high in vitamin B12"; Fillet_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).

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	Sausages_C	5.8 ± 0.6	1.5 ± 0.2	14.2 ± 3.0
	Balls_C	6.4 ± 0.4	0.3 ± 0.0	14.9 ± 3.4
Pregnant women	Roulade_P	5.1 ± 0.2	1.5 ± 0.0	14.1 ± 0.8
	Fillet_P	11.6 ± 2.2	0.5 ± 0.1	13.0 ± 0.4
Seniors	Soup_S	3.7 ± 0.8	1.9 ± 0.4	6.0 ± 0.4
	Balls_S	6.7 ± 0.5	1.2 ± 0.1	8.7 ± 1.6

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

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 (Fillet_P) to 14.9 mg/100 g (Balls_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 Soup_S and Balls_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, 2010c) could be stated.

3.1.4. Fatty acids and cholesterol

In most dishes, the fatty acids profile was MUFA, SFA and PUFA, as showed in Table 6. In Sausages_C, the profile was MUFA, PUFA and SFA. Only in the case of Roulade_P, SFA were the predominant followed by MUFA and PUFA. 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 (Sausages_C) ≤ SFA/UNS ≤ 0.6 (Soup_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, Roulade_P and Sausages_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.

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 Sausages_C and between 65.0 (Fillet_P) and 79.6% (Balls_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 Roulade_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 Fillet_P and Soup_S (ratio = 1.2), being also well-balanced dishes. Finally, Balls_S, Sausages_C and Balls_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 Fillet_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
Fatty acids and cholesterol concentrations of the dishes.

Target population	Children		Pregnant women		Seniors	
Dish	Sausages_C	Balls_C	Roulade_P	Fillet_P	Soup_S	Balls_S
Fatty acids (mg/100 g)[a]						
ΣSFA[b]	531.3 ± 14.6	1233.5 ± 142.4	1150.9 ± 31.4	1875.2 ± 224.7	1317.7 ± 137.7	906.0 ± 63.3
ΣMUFA[c]	1504.3 ± 23.4	2881.7 ± 43.6	620.1 ± 16.4	2072.6 ± 172.9	1515.3 ± 162.7	2054.5 ± 168.6
ΣPUFA[d]	761.2 ± 13.6	697.1 ± 27.0	479.0 ± 16.5	1149.8 ± 132.1	545.9 ± 110.9	545.6 ± 68.8
SFA/UNS[e]	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[f]	81.8 ± 3.6	120.3 ± 0.8	278.1 ± 12.7	405.5 ± 102.6	208.1 ± 58.4	159.8 ± 28.1
Σ*n*-3 PUFA[g]	201.0 ± 1.9	181.1 ± 1.4	368.3 ± 14.8	624.1 ± 139.7	297.6 ± 84.6	200.8 ± 42.9
Σ*n*-6 PUFA[h]	560.2 ± 12.1	516.0 ± 25.8	110.6 ± 3.1	525.7 ± 38.8	248.3 ± 26.9	344.7 ± 26.1
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[i]	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[j]	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]	34.7 ± 5.1	119.8 ± 10.0	98.4 ± 10.7	175.9 ± 13.8	73.3 ± 5.7	61.7 ± 8.0

Sausages_C: Fish sausages with vegetables for children with Cyprinus carpio; Balls_C: Fish balls with a purée for children with Trisopterus luscus; Roulade_P: Fish roulade for pregnant women with Micromesistius poutassou; Fillet_P: Fish fillet with salad for pregnant women with Limanda limanda; Soup_S: Mussel soup for seniors with Mytilus edulis; and Balls_S: Fish balls with vegetables and sauce for seniors with Micromesistius poutassou.

[a] Results are given as average values ± standard deviations (N = 3).
[b] Total saturated.
[c] Total monounsaturated.
[d] Total polyunsaturated.
[e] Total saturated/Total unsaturated.
[f] Eicosapentaenoic + Docosahexaenoic.
[g] Total omega 3.
[h] Total omega 6.
[i] Thrombogenic index.
[j] Atherogenic index.

Table 6), according to Ulbricht and Southgate (1991). On the other hand, Roulade_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 Sausages_C (34.7 mg/100 g) and Fillet_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 (Roulade_P and Balls_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 (2010d) 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 Balls_C, 0.008 ± 0.001 mg/kg in Roulade_P, 0.015 ± 0.004 mg/kg in Fillet_P and 0.009 mg/kg in Balls_S) and in some cases below the LOQ (≤0.006 mg/kg in Sausages_C and Soup_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 Soup_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 "Fillet_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.

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/100 g, 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 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	Sausages_C	<0.55**	2.0 ± 0.57	<0.91*	<1.3*	<1.9*	<2.9*	<15*	<0.36*	2.0 ± 0.57
	Balls_C	<0.17*	<0.81**	<0.91*	<1.3*	<1.9*	<2.9*	<15*	<0.36*	0.17 ± 0.06
Pregnant women	Roulade_P	<0.17*	0.92 ± 0.29	<0.91*	<1.3*	<1.9*	<2.9*	<15*	<0.36*	0.92 ± 0.29
	Fillet_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	Soup_S	<0.17*	0.47 ± 0.46**	<0.91*	<1.3*	<1.9*	<2.9*	<15*	<0.36*	0.47 ± 0.46
	Balls_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. Sausages_C: Fish sausages with vegetables for children with *Cyprinus carpio*; Balls_C: Fish balls with a purée for children with *Trisopterus luscus*; Roulade_P: Fish roulade for pregnant women with *Micromesistius poutassou*; Fillet_P: Fish fillet with salad for pregnant women with *Limanda limanda*; Soup_S: Mussel soup for seniors with *Mytilus edulis*; and Balls_S: Fish balls with vegetables and sauce for seniors with *Micromesistius poutassou*.

Table 8
PAHs concentrations of the dishes.

Target population	Children		Pregnant women		Seniors	
Dish	Sausages_C	Balls_C	Roulade_P	Fillet_P	Soup_S	Balls_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. Sausages_C: Fish sausages with vegetables for children with *Cyprinus carpio*; Balls_C: Fish balls with a purée for children with *Trisopterus luscus*; Roulade_P: Fish roulade for pregnant women with *Micromesistius poutassou*; Fillet_P: Fish fillet with salad for pregnant women with *Limanda limanda*; Soup_S: Mussel soup for seniors with *Mytilus edulis*; and Balls_S: Fish balls with vegetables and sauce for seniors with *Micromesistius poutassou*.

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* spp. 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 (5.4×10^4 cfu/g) detected were low.

3.3. *Nutritional contribution*

The dishes (250 g) for children (Sausages_C and Balls_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 Roulade_P and Fillet_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 Soup_S and Balls_S (300 g and 250 g, respectively) contributes with 33–40% for protein, 42–43% for Na, 74% (Soup_S)-112% (Balls_S) for vitamin D3, and 77% (Balls_S)-143% (Soup_S) for vitamin B12, of the DRVs (PRI or AI) for adults, including seniors (Table 9).

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

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 (%)					
			Sausages_C	Balls_C	Roulade_P	Fillet_P	Soup_S	Balls_S
Proximate composition								
Protein	Seniors	**52539**[2]	–	–	–	–	33 ± 2	40 ± 2
Macro and micro elements								
I	Pregnant Women	0.20[2]	–	–	128 ± 23	58 ± 7	–	–
Na	Seniors	2000[2,3]	–	–	–	–	42 ± 3	43 ± 3
Vitamins								
D3	Children		97 ± 11	106 ± 7	–	–	–	–
	Pregnant Women	0.015[2,4]	–	–	85 ± 3	194 ± 36	–	–
	Seniors		–	–	–	–	74 ± 15	112 ± 8
B12	Seniors	0.004[2]	–	–	–	–	143 ± 27	77 ± 4
n-3 fatty acids								
EPA + DHA	Children	250[2]	82 ± 4	120 ± 1	–	–	–	–
	Pregnant Women	450[2,5]	–	–	155 ± 7	225 ± 57	–	–

Values are means ± standard deviations (n = 3). Sausages_C: Fish sausages with vegetables for children with *Cyprinus carpio*; Balls_C: Fish balls with a purée for children with *Trisopterus luscus*; Roulade_P: Fish roulade for pregnant women with *Micromesistius poutassou*; Fillet_P: Fish fillet with salad for pregnant women with *Limanda limanda*; Soup_S: Mussel soup for seniors with *Mytilus edulis*; and Balls_S: Fish balls with vegetables and sauce for seniors with *Micromesistius poutassou*. [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 foetus.

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).

Furthermore, current data reinforce consumers to focus on healthy dietary patterns, with an increased proportion of foods rich in essential nutrients such as vitamins and minerals (e.g. seafood dishes), rather than on the supplements use (Jenkins et al., 2018).

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. More specifically, dishes for children and pregnant women presented adequate levels of EPA + DHA and can be claimed as “high in I”. Those for seniors had low Na/salt contents and also can be claimed as “high in protein” and “high in vitamin B12”. All dishes can be claimed as “high in vitamin D″ and “high in vitamin E”. Sausages_C, Roulade_P, Fillet_P and Balls_S exhibited a well-balanced protein/fat ratio. Sausages_C was considered 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 Roulade_P, suggesting that its consumption is particularly beneficial in terms of *n*-3 supply. The contents of Hg-T, Cd, Pb, As, PBDEs, regulated PAHs, histamine, total aerobic microorganisms, *Bacillus cereus*, coagulase positive *Staphylococcus*, *E. coli*, *Salmonella* spp. and *Listeria monocytogenes* indicated that all dishes can be considered safe products. It is also important to highlight that the consumption of the new dishes with 250 or 300 g (in Soup_S) contributes to the supply of the daily requirements of the target nutrients as well as the functional needs of children, pregnant women and seniors. However, some minor adjustments of the recipes may be necessary before the scaling up. In future, it will be important to evaluate the dishes stability under frozen storage to establish the adequate shelf life.

CRediT authorship contribution statement

Helena Oliveira: Conceptualization, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Supervision, Project administration. **Camille Blocquel:** Conceptualization, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Project administration. **Marta Santos:** Investigation, Formal analysis. **Murielle Fretigny:** Investigation, Writing – review & editing. **Tatiana Correia:** Investigation. **Amparo Gonçalves:** Validation, Formal analysis, Investigation, Writing – review & editing. **Ana G. Cabado:** Conceptualization, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Lucía Blanco López:** Investigation, Writing – original draft, Writing – review & editing. **Birgitta Wäppling Raaholt:** Conceptualization, Investigation, Writing – review & editing. **Francesca Ferraris:** Investigation. **Francesca Iacoponi:** Investigation, Formal analysis. **Francesco Cubadda:** Validation, Formal analysis, Investigation, Writing – review & editing. **Alberto Mantovani:** Validation, Investigation, Writing – review & editing. **Elisabeth Vallet:** Conceptualization, Investigation, Writing – original draft. **Geertrui Vlaemynck:** Conceptualization, Investigation, Writing – original draft. **Julio Fernández-Arribas:** Investigation. **Ethel Eljarrat:** Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Esther López:** Investigation, Writing – review & editing. **Miren López de Alda:** Investigation, Writing – review & editing. **Remigiusz Panicz:** Conceptualization, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Małgorzata Sobczak:** Conceptualization, Validation, Formal analysis, Investigation, Writing – original draft. **Piotr Eljasik:** Investigation. **Sara Cunha:** Validation, Investigation, Writing – review & editing. **Ricardo Ferreira:** Investigation. **José O. Fernandes:** Investigation, Writing – review & editing. **Sara Sousa:** Investigation. **Valentina F. Domingues:** Validation, Investigation, Writing – review & editing. **Cristina Delerue-Matos:** Investigation. **António Marques:** Conceptualization, Investigation, Writing – review & editing, Funding acquisition, All authors read and agreed with the published version of the manuscript. **Maria Leonor Nunes:** Conceptualization, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Supervision, Project administration.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2021.112431.

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